Genes Governing the Fermentation of Maltose and Flocculation in a Brewer's Yeast[1]

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ABSTRACT

The genetics of maltose fermentation and flocculation of a brewer's yeast were studied using diploid fusion hybrids obtained previously (11). Genetic analyses demonstrated that each fusion hybrid possessed one maltose gene, *MAL1*, which was the same in each hybrid. Flocculence segregated 4:0 in all fusion hybrids studied. Analyses revealed that hybrid F was homozygous for a single *FLO* gene, which mapped 41.8 centimorgans from *ADE1* on chromosome I. Tests for allelism identified this gene as *FLO1*.

Key words: *Brewer's yeast, Flocculence, Genetic analysis, Maltose fermentation, Spheroplast fusion.*

Fusions of polyploid brewing strains with haploid *Saccharomyces cerevisiae* strains were recently described (10–12). Fusion rendered the brewing strains amenable to classic methods of genetic analysis. In our previous study (11), hybrids isolated from fusion of a brewing strain with an α-mating type haploid sporulated. Hybrids isolated from fusion of the brewing strain with an a-mating type haploid did not sporulate. Quantitative DNA analyses, confirmed by genetic analyses, indicated that sporulating hybrids were diploid.

A benefit produced by fusion was the capacity to readily identify and map genes of a brewer's yeast strain. Genes of interest to us were those that govern maltose fermentation and flocculence. These characteristics influence the rate of fermentation and the size of the harvested yeast crop. Because of their practical significance, controlling their expression would be advantageous. In *Saccharomyces*, expression of each of these properties may be controlled by more than one genetic locus (7,9,14). Studies concerned with the existing genetic composition of a brewer's yeast with regard to maltose fermentation and flocculence aid in the genetic modification of these properties. In this article, the use of fusion hybrids to identify the genes governing maltose fermentation and flocculence in a brewer's yeast is described.

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TABLE I
Yeast Strains Used in Identifying the *MAL* and *FLO* Genes of a Brewer's Yeast

Number (MBC1-1) Strain	Source	Genotype
558	Yeast Genetics Stock Center (SS12A)	α*SUC4,mal,his4,flo*
587	Mutagenesis of AJ2329[a]	*ade2,met1,MAL, FLO*
610	Fusion hybrid E[a]	*MAL,FLO*
652	Fusion hybrid F[a]	*MAL,FLO*
653	Fusion hybrid G	*MAL,FLO*
625	D. Mowshowitz, Columbia University (179)	α,*MAL1,ade1,trp1,lys1*
563	Yeast Genetics Stock Center (1453-3a)	a,*MAL2,suc,his4,leu2*
561	Yeast Genetics Stock Center (1412-4d)	a,*MAL3,ade2*
621	D. Mowshowitz, Columbia University (1)	α,*MAL4,trp1,ura3,ade2,ade8*
804	D. Mowshowitz, Columbia University (71)	a,*MAL5*
803	Yeast Genetics Stock Center (C9)	a,*MAL6*
892	G. G. Stewart, Labbott Breweries (1209)	*FLO1*
872	Our laboratory	α,*flo, ade1,met1*
873	Our laboratory	a,*flo,ade1,trp1,his4*
866	9a from hybrid F	a,*FLO,MAL*
867	9b from hybrid F	α,*FLO,mal,ade2,met1,his4*
868	9c from hybrid F	a,*FLO,mal,ade2,met1*
869	9d from hybrid F	α,*FLO,his4,MAL*

[a]Yeast strain described by Skatrud et al (11).

EXPERIMENTAL

Yeast Strains

Yeast strains used are described in Table I.

Media

Cultures were maintained on MYGP (0.3% malt extract, 0.3% yeast extract, 2% glucose, 0.5% Bacto peptone, and 2% agar) agar slants or plates.

Floc broth was used for the determination of flocculence. This medium consisted of 0.5% yeast extract, 1% peptone, 2% glucose, and 0.12% calcium chloride (pH 4.5). When auxotrophic strains were analyzed, nutrient requirements were added (0.4 g/L).

Determination of Flocculence

Flocculence was determined in 16 × 125-mm culture tubes, each containing 8 ml of floc broth. These tubes were inoculated with 24-hr cultures grown on MYGP agar plates and incubated statically for 48 hr at 20° C. Each tube was vortexed for 3 sec and allowed to settle for approximately 2 min. Flocculation was determined visually and scored as either flocculent or nonflocculent.

Maltose Fermentation Test

Yeast strains were tested for the ability to ferment maltose in Bacto Purple Broth Base containing 2% maltose.

Genetic Analysis

Standard techniques were used for mating, sporulation, tetrad analysis, and determination of auxotrophic requirements (4).

RESULTS AND DISCUSSION

Fusion hybrids described previously (11) were used to study the genes governing maltose fermentation and flocculence in a brewer's yeast strain. At that time, the ability to ferment maltose segregated 2:2 (*MAL:mal*) in fusion hybrids E, F, and G. This segregation pattern indicated that each hybrid possessed one maltose gene. However, we did not determine whether the hybrids possessed the same or different maltose genes. Each hybrid may have possessed a different maltose gene; published reports indicated seven polymeric maltose genes for *Saccharomyces* (14). To examine this possibility, we mated maltose-fermenting ascospore cultures from hybrid F to those from hybrids E and G. Results of tetrad analysis are shown in Table II. In each cross, all of the ascospores fermented maltose (4:0 segregation). These results indicated that each hybrid possessed the same maltose gene, yet its identity remained unknown.

TABLE II
Segregation of Maltose Genes in Crosses Between Maltose-Fermenting Ascospores of Fusion Hybrids E, F, and G

Cross	MAL:*mal* Segregation 2:2	3:1	4:0
MAL?-Hybrid F × *MAL*? = Hybrid G	0	0	18
MAL?-Hybrid F × *MAL*? = Hybrid E	0	0	23

TABLE III
Genetic Analyses of Crosses Between Maltose Fermenting Ascospores from Fusion Hybrids with Maltose Tester Strains

Parental Strains	Tetrad Analysis *MAL:mal* Segregation[a] 2:2	3:1	4:0	Total Spore Analysis Total Ascospores	*MAL* (%)	*mal* (%)
MAL1 × *MAL*?	0	0	19	103	100	0
MAL2 × *MAL*?	9	12	1	135	67	33
MAL3 × *MAL*?	3	11	3	119	77	23
MAL4 × *MAL*?	5	19	6	120	76	24
MAL5 × *MAL*?	2	3	1	144	66	34
MAL6 × *MAL*?	4	9	3	162	69	31

[a]All other genetic markers segregated 2:2.

To determine which of the six polymeric maltose genes these hybrids possessed, we made crosses to tester strains carrying known *MAL* genes (*MAL7* was not available.) The results of tetrad analyses of hybrids obtained from these crosses are presented in Table III. In crosses involving the *MAL1* tester strain, only 4:0 segregation for maltose fermentation was observed. In crosses with the other five maltose tester strains, the maltose genes segregated 2:2, 3:1, and 4:0. Data from total spore analysis (Table III) was used to support tetrad data for crosses in which ascospore viability was poor (ie, in *MAL5* and *MAL6* crosses). These results identified the maltose gene in fusion hybrids and, hence, the brewing strain, as *MAL1*.

The second part of the present study was concerned with the genetic control of flocculence in hybrid F. In the original fusion, no attempt was made to select for flocculent hybrids. However, all

TABLE IV
Segregation of Flocculence in Crosses of Ascospores From Tetrad Nine of Hybrid F with Nonflocculent Strains[a]

Cross[1]	*FLO:flo* Segregation[2] 0:4	1:3	2:2	3:1	4:0
9a × *flo*⁻	0	2	38	0	0
9b × *flo*⁻	0	1	57	0	0
9c × *flo*⁻	1	1	71	0	0
9d × *flo*⁻	0	1	64	0	0

[a]Nonflocculent tester strains were 872 and 873.
[b]All other genetic markers segregated 2:2.

TABLE V
Segregation of Genes Controlling Flocculence in Crosses Between Ascospores from Tetrad Nine of Hybrid F

Cross	*FLO:flo* Segregation[a] 0:4	1:3	2:2	3:1	4:0
9a × 9b	0	0	0	0	13
9a × 9d	0	0	0	1	26
9b × 9c	0	0	0	0	30
9c × 9d	0	0	0	2	60

[a]All other genetic markers segregated 2:2.

hybrids recovered were flocculent. Furthermore, dissection of these hybrids produced only flocculent progeny. These results suggested that flocculence could be under the control of one or more *FLO* genes. To determine the number of *FLO* genes in hybrid F, each ascospore from tetrad nine was mated to powdery tester strains. The resulting diploids were sporulated and dissected in the usual manner. In each cross, flocculence segregated primarily 2:2 (Table IV). The tetrads that did not segregate 2:2 were considered to be influenced by genetic markers, by the composition of the medium, or by a combination of these factors. These results indicated that each member of the tetrad possessed one gene for flocculence. To test whether different *FLO* genes were present in tetrad nine, we tested all possible matings within this tetrad. The results of this analysis are given in Table V. Flocculence segregated 4:0 in all crosses, indicating that each ascospore possessed the same *FLO* gene. Therefore, hybrid F was homozygous for a single *FLO* gene.

To determine the location of this *FLO* gene on the genetic map of *Saccharomyces*, ascospore cultures of tetrad nine from hybrid F were mated with powdery tester strains possessing other genetic markers. The resulting diploids were analyzed for the segregation of flocculence with respect to these markers. The results of these analyses are presented in Table VI. The data indicated that this *FLO* gene was on chromosome I, linked to *ADE*1 at a distance of 41.8 cM. These results were consistent with those presented by Russell et al (9) for *FLO*1. To verify our results, we mated tetrad nine ascospores with *FLO*1 tester strains. Genetic analysis of these crosses (Table VII) confirmed the identity of this gene as *FLO*1.

The results demonstrate a practical application of spheroplast fusion on an industrial yeast strain. Using fusion hybrids, we identified two genes found in a brewer's yeast that play significant roles in fermentation. With this basic knowledge concerning maltose fermentation and flocculence, genetic manipulation of these properties may be performed in a precise manner. For example, a brewer's yeast with multiple copies of *MAL*1 and/or other maltose genes could be constructed to maximize the rate of maltose fermentation (5,8). The incorporation of constitutive *MAL* mutants may further enhance the fermentation rate. Altering the degree of flocculence may also provide desirable effects in fermentation; this property may be entirely eliminated if centrifugation is to be used for harvesting the yeast crop.

The events that occur at the nuclear level during spheroplast fusion are not totally understood, particularly in fusions involving polyploid yeast. When haploids are fused, diploid fusion products are normally recovered (1,2,3,13). Stable diploids may also be formed in polyploid fusions as demonstrated in the present work. We have also observed a wide range of aneuploids from other polyploid fusions (12). Fusion of polyploid yeasts appears to be a random event. However, certain genetic characteristics may be studied by using strains possessing genetic lesions at appropriate sites.

TABLE VI
Estimation of Distance Between *FLO* Gene and *ADE*1

Cross	Genetic Markers	Ascus Class PD:NPD:T[a]	Ratio PD:NPD	Distance[b] (cM)
9c × *flo*⁻	*FLO-ADE*1	23:2:44	11.5[c]	40.5
	*FLO-TRP*1	15:9:46	1.7[d]	
	*FLO-HIS*4	10:13:41	0.8	
	*FLO-MET*1	11:15:45	0.7	
9d × *flo*⁻	*FLO-ADE*1	21:0:42	21.0[e]	33.3
	*FLO-TRP*1	5:10:48	0.5	
9a × *flo*⁻	*FLO-ADE*1	14:4:20	3.5[e]	57.9
Total	*FLO-ADE*1	58:6:106	9.7[e]	41.8

[a]PD = parental ditype, NPD = nonparental ditype, T = tetratype.
[b]cM = 100 (T+6 (NPD)/2 (PD+NPD+T) (6).
[c]Linkage *FLO* to *ADE*1, 99% significant (7).
[d]Linkage of *FLO* to *TRP*1, less than 95% significant (7).
[e]Linkage of *FLO* to *ADE*1, 96.9% significant (7).

TABLE VII
Genetic Analysis of Crosses Between Strains with *FLO*? and *FLO*1[a]

Hybrids[b]	Segregation of Flocculence 0:4	1:3	2:2	3:1	4:0
9c × *FLO*1	0	0	0	0	23
9d × *FLO*1	0	0	0	0	38

[a]Ascospores 9c and 9d from hybrid F were mated with tester strains carrying a known *FLO*1 gene.
[b]All other genetic markers segregated 2:2.

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LITERATURE CITED

1. Gunge, N., and Tamaru, A. *Jpn. J. Genet.* 53:41, 1978.
2. Maraz, A., Kiss, M., and Ferenczy, L. *FEMS Microbiology Lett.* 3:319, 1978.
3. Morgan, A. J., Heritage, J., and Whittaker, P. A. *Microbios. Lett.* 4:103, 1977.
4. Mortimer, R. K., and Hawthorne, D. C. Page 355 in: The Yeasts. Rose, A. H., and Harrison, D. C., eds. Academic Press, New York, 1969.
5. Mowshowitz, D. B. *J. Bacteriol.* 137:1200, 1979.
6. Perkins, D. D. *Genetics* 38:187, 1953.
7. Reader, H. P., Ph.D. thesis. Genetic Analysis of Flocculation in *Saccharomyces cerevisiae*. University of Strathclyde, Glasgow, Scotland, 1979.
8. Rudert, F., and Halvorson, H. O. Bull. Res. Council Israel 11A4:337, 1963.

9. Russell, I., Stewart, G. G., Reader, H. P., Johnston, J. R., and Martin, P. A. *J. Inst. Brew.* 86:120, 1980.
10. Russell, I., and Stewart, G. G. *J. Inst. Brew.* 85:95, 1979.
11. Skatrud, P. L., Jaeck, D. M., Kot, E. J., and Helbert, J. R. *J. Am. Soc. Brew. Chem.* 38(2):49, 1980.
12. Skatrud, P. L., Kot, E. J., and Helbert, J. R. Fusion of Brewer's Yeast. Presented at The Molecular Biology of Yeast Meeting. Cold Spring Harbor, NY, 1979.
13. Stahl, U. *Mol. Gen. Genet.* 160:111, 1978.
14. ten Berge, A. M. A. *Mol. Gen. Genet.* 115:80, 1972.

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