THE GENETICS OF ALPHA-METHYL-GLUCOSIDE
FERMENTATION IN SACCHAROMYCES

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Received 3.x.57

1. INTRODUCTION

It has been established that in *Saccharomyces* the fermentation of a given sugar can be controlled by several genes. For example, for the fermentation of maltose and raffinose, four and three polymeric genes, respectively, have been demonstrated by Winge and Roberts (1952). For galactose fermentation, three complementary genes are known (Hawthorne, 1956). Since first reporting a single gene controlling alpha-methyl-glucoside fermentation, Lindegren (1949) has published data on the irregular segregations at this locus, attributing to "gene conversion" the ratios deviating from the 2 : 2 expected in tetrads from a hybrid heterozygous for a single gene (Lindegren, 1953 ; and Lindegren and Lindegren, 1956). The work reported below indicates that a more complex genetic situation exists in our breeding stocks, in that at least five different genes can be demonstrated to be involved in the fermentation of alpha-methyl-glucoside.

2. MATERIALS AND METHODS

The parental haploid strains used in the crosses that follow were either obtained directly from Dr C. C. Lindegren (clones 1426A and 1428) or were derived from cultures originating in his laboratory. With two exceptions, the crosses were made either by pairing haploid cells (Chen, 1950) or by selecting prototrophic diploid clones from a mixture of auxotrophic haploid parents that had been plated on minimal medium (Pomper and Burkholder, 1949). In the two exceptions, crosses 4 and 8, a mass mating of haploid cells was made as described by Lindegren and Lindegren (1943).

The sporulation procedure suggested by Lindegren (1949) was followed. Whenever it was feasible, only linear four-spored asci were dissected (Hawthorne, 1955). Only asci in which all four spores survived were retained for genetic analysis. Since the hybrids were heterozygous for from five to ten genes in addition to the genes controlling alpha-methyl-glucoside fermentation, it was possible to recognise and eliminate from the analysis anomalous asci due to polyploidy (Roman, Hawthorne and Douglas, 1951) or post-meiotic nuclear fusion (Roman and Sands, 1953 ; Winge and Roberts, 1954).

The tests for the fermentation of alpha-methyl-glucoside were made in Kahn tubes containing 1.5 c.c. of the following medium adjusted to pH 6.0 : 1 per cent. peptone, 1 per cent. yeast extract, 1 per cent. alpha-methyl-glucoside, and the indicator bromocresol purple. An alpha-methyl-glucoside fermenter grows vigorously in this medium and causes a change in the colour of the indicator due to acid production. The reliability of this testing procedure has been confirmed by duplicate tests using Durham fermentation tubes.

3. RESULTS

Evidence for the genes MG_1 and MG_2

The first indication that more than one locus was involved in alpha-methyl-glucoside fermentation was obtained from the results of crosses (table 1) in which the haploid parents were non-fermenters. In all four cases, the diploid hybrids turned out to be fermenters and the asci obtained from them exhibited the 2:2, 1:3, and 0:4 segregations expected if two loci were involved. The distributions of these ratios among 35 asci, 9 (2:2), 21 (1:3), and 5 (0:4), suggested that the parental haploids differ in two genes, MG_1 and MG_2 , both of which are needed for alpha-methyl-glucoside fermentation, and that the three ascus types have the following compositions.

Spore <i>a</i>	$MG_1 MG_2$ (+)	$MG_1 MG_2$ (+)	$MG_1 mg_2$ (—)
Spore <i>b</i>	$MG_1 MG_2$ (+)	$MG_1 mg_2$ (—)	$MG_1 mg_2$ (—)
Spore <i>c</i>	$mg_1 mg_2$ (—)	$mg_1 MG_2$ (—)	$mg_1 MG_2$ (—)
Spore <i>d</i>	$mg_1 mg_2$ (—)	$mg_1 mg_2$ (—)	$mg_1 MG_2$ (—)

The genotype of the common parent in the first three crosses, 1011-1*a*, was designated $mg_1 MG_2$. The other parents, 1001-17*a*, 1001-8*d*, and 1001-14*a*, were on this hypothesis $MG_1 mg_2$. The negative segregants could be tested to determine if they were of the genotype $MG_1 mg_2$, $mg_1 MG_2$, or $mg_1 mg_2$ by making crosses to the above haploid parents and then inoculating the mixture containing zygotes into the alpha-methyl-glucoside medium. If the zygotes give rise to fermenters, the haploid being tested is of the complementary genotype. In this way, the genotypes of the parents of the fourth cross were found to be $mg_1 MG_2$ for 133-1*b* and $MG_1 mg_2$ for 1428.

In a series of crosses made to confirm the zygote test described above, non-fermenter segregants of each of the postulated genotypes were crossed with fermenter haploids. The hybrids in every case were fermenters. The crosses involving the genotypes $mg_1 MG_2$ and $MG_1 mg_2$ gave asci with 2:2 segregations as expected. Also as expected, the one cross involving the genotype $mg_1 mg_2$ gave asci with two gene segregations, as shown in table 2.

Evidence for a third alpha-methyl-glucoside gene, MG_3

Two crosses involving non-fermenters of the genotype $MG_1 mg_2$ and the same fermenter parent, 1003-6*c*, had been expected to give asci with only 2:2 segregations, reflecting the segregation of MG_2 . Instead only 19 of the 32 asci of crosses 11 and 12 (table 3), gave 2:2 segregations and the remaining 13 asci showed 1:3 segregations for alpha-methyl-glucoside. These segregations suggested that still another gene was involved in the fermentation of alpha-methyl-glucoside. Additional evidence for the new gene was obtained from the results of a cross in which both haploid parents were non-fermenters. Of the 13 asci from cross 13 (table 3), there were 8 (2:2), 4 (1:3),

TABLE 1

Segregations for alpha-methyl-glucoside fermentation in asci from hybrids of the genotype $MG_1 mg_2/mg_1 MG_2$

Cross	Parents and phenotypes	Hybrid and phenotype	Number of asci segregating as		
			2+ : 2-	1+ : 3-	0+ : 4-*
1	1001-17a (-) × 1011-1a (-) .	133, 135 (+) 136 and 139	3	10	2
2	1001-8d (-) × 1011-1a (-) .	142, 143 (+) 144 and 145	3	8	2
3	1001-14a (-) × 1011-1a (-) .	1015 (+)	2	1	1
4	1428 (-) × 133-1b (-) .	1017 (+)	1	2	0

* Fermenters (+) : non-fermenters (-).

TABLE 2

Segregations for alpha-methyl-glucoside fermentation in asci from hybrids of the following genotypes: $MG_1 MG_2/mg_1 mg_2$ in cross 5, $MG_1 MG_2/mg_1 MG_2$ in crosses 6, 7, 8 and 9, $MG_1 MG_2/MG_1 mg_2$ in cross 10

Cross	Parents and phenotypes	Hybrid and phenotype	Number of asci segregating as		
			2+ : 2-	1+ : 3-	0+ : 4-*
5	1017-2d (+) × 1005-2d (-) .	1018 (+)	0	7	0
6	1017-2b (+) × 1018-4a (-) .	1019 (+)	3	0	0
7	142-4c (+) × 1018-4a (-) .	1036 (+)	13	0	0
8	1019-1d (+) × 136-1b (-) .	1043 (+)	3	0	0
9	1019-1c (+) × 1036-12c (-) .	1047 (+)	4	0	0
10	1018-6a (+) × 1001-16b (-) .	1033 (+)	6	0	0

* Fermenters (+) : non-fermenters (-).

TABLE 3

Segregations for alpha-methyl-glucoside fermentation in asci from hybrids of the following genotypes: $mg_1 MG_2 MG_3/MG_1 mg_2 mg_3$ in crosses 11 and 12, $MG_1 mg_2 MG_3/mg_1 MG_2 mg_3$ in cross 13

Cross	Parents and phenotypes	Hybrid and phenotype	Number of asci segregating as		
			2+ : 2-	1+ : 3-	0+ : 4-*
11	1003-6c (+) × 1001-17b (-) .	1, 2, 5 (+) 6 and 12	11	9	0
12	1003-6c (+) × 1001-14a (-) .	48, 52 (+) and 84	8	4	0
13	129-3c (-) × 143-3a (-) .	1016 (+)	8	4	1

* Fermenters (+) : non-fermenters (-).

and 1 (0 : 4) segregations. One parent, 143-3a, had been tested and found to be of the genotype $mg_1 MG_2$; the other parent, 129-3c, was presumed to be of the genotype $MG_1 mg_2$. However, the preponderance of 2 : 2 segregations over the 1 : 3 and 0 : 4 segregations, as in the results of crosses 11 and 12, could not be accounted for by any simple scheme involving only two genes MG_1 and MG_2 . These results could be explained if it were assumed that there is a third gene, MG_3 , which is equivalent to MG_1 in that a combination of the genes MG_2 and MG_3 will enable the yeast to ferment alpha-methyl-glucoside in the absence of MG_1 . Thus the excess of 2 : 2 asci over 1 : 3 and 0 : 4 asci from cross 13 would be expected if 129-3c were $MG_1 mg_2 MG_3$, and 143-3a were $mg_1 MG_2 mg_3$. Similarly, the 1 : 3 segregations in crosses 11 and 12 can be accounted for if the parents are of genotype

TABLE 4

Segregations for alpha-methyl-glucoside fermentation in asci from hybrids of the following genotypes: $mg_1 MG_2 MG_3/mg_1 MG_2 mg_3$ in crosses 14 and 15, $mg_1 MG_2 MG_3/mg_1 mg_2 mg_3$ in cross 16, $mg_1 MG_2 MG_3/mg_1 mg_2 MG_3$ in cross 17, $MG_1 MG_2 mg_3/mg_1 MG_2 MG_3$ in cross 18

Cross	Parents and phenotypes	Hybrid and phenotype	Number of asci segregating as				
			4+ : 0-	3+ : 1-	2+ : 2-	1+ : 3-	0+ : 4-
14	1003-6c (+) × 145-1c (-)	1035 (+)	0	0	21	0	0
15	1035-6c (+) × 1036-2c (-)	1040 (+)	0	0	11	0	0
16	1003-6c (+) × 133-3a (-)	1034 (+)	0	0	2	9	1
17	1035-2b (+) × 1034-1d (-)	1037 (+)	0	0	9	0	0
18	1036-7a (+) × 1040-7b (+)	1078 (+)	2	9	3	0	0

* Fermenters (+) : non-fermenters (-).

$mg_1 MG_2 MG_3$ (1003-6c) and $MG_1 mg_2 mg_3$ (1001-17b and 1001-14a). If the three genes segregate independently and show no centromere linkage, a hybrid heterozygous for all three would give 2 : 2, 1 : 3, and 0 : 4 segregations in the ratio of 19 : 16 : 1. The actual segregations obtained for the 45 asci of crosses 11, 12, and 13, 27 (2 : 2), 17 (1 : 3), and 1 (0 : 4), do not differ significantly from the expected frequencies : 23.75 (2 : 2), 20 (1 : 3), and 1.25 (0 : 4).

With this indication of a third alpha-methyl-glucoside gene, MG_3 , the crosses reported in the previous section were re-examined. The results are consistent with the interpretation that the parental strains, 1001-8d, 1001-14a, 1001-17a, 1011-1a, 1428, and 1005-2d, and consequently all segregants carried the recessive gene mg_3 .

For further evidence of the correctness of the above explanation, the following crosses were made. The fermenter presumed to be $mg_1 MG_2 MG_3$, 1003-6c, was crossed to a non-fermenter, 145-1c, of genotype $mg_1 MG_2 mg_3$, and as expected from a hybrid homozygous for MG_2 and heterozygous for MG_3 , only 2 : 2 segregations were observed in the 21 asci from this cross (cross 14, table 4). From another

hybrid of this same genotype, $mg_1 MG_2 MG_3/mg_1 MG_2 mg_3$, 11 more asci with only 2 : 2 segregations were obtained (cross 15, table 4).

To obtain haploids of the genotype $mg_1 mg_2 MG_3$, 1003-6c was crossed to a non-fermenter strain of the genotype $mg_1 mg_2 mg_3$, as determined by the zygote tests. The 12 asci from cross 16 (table 4) show a distribution of 2 : 2, 1 : 3, and 0 : 4 segregations close to that expected from two independently segregating complementary genes, *i.e.* 2 : 9 : 1 versus 2 : 8 : 2. A confirmation of the zygote tests of the segregants from cross 16 was obtained when a non-fermenter of the predicted genotype $mg_1 mg_2 MG_3$ was crossed to a fermenter from cross 14, genotype $mg_1 MG_2 MG_3$, to give a hybrid which yielded only the expected 2 : 2 segregations in the nine asci analysed (cross 17, table 4).

A cross of two alpha-methyl-glucoside fermenters was made to substantiate the contention that MG_1 and MG_3 are polymeric genes. One haploid parent, 1036-7a, was from cross 7 where 2 : 2 segregations were obtained for MG_1 and the complementary gene, MG_2 , was homozygous dominant. Similarly, the other parent, 1040-5b, was from cross 15 where 2 : 2 segregations were obtained for MG_3 and again MG_2 was homozygous dominant. The distribution of the segregations, 2 (4 : 0), 9 (3 : 1), and 3 (2 : 2) for the 14 asci (cross 18, table 4) is in accordance with the ratios expected for two independently segregating genes which show little or no centromere linkage.

Evidence for the gene MG_4

When crosses were made to a new fermenter strain, haploid 1426A, received from Dr Lindegren, segregation ratios were observed which indicated that a new gene combination was enabling this haploid to ferment alpha-methyl-glucoside. Haploid 1426A was first crossed to a strain of genotype $mg_1 MG_2 mg_3$, 1018-4a, previously used as the non-fermenter parent in crosses 6 and 7. The segregations in 10 asci (cross 19, table 5) are 1 (4 : 0), 3 (3 : 1), 3 (2 : 2), and 3 (1 : 3). The genotype of the hybrid and therefore 1426A, with reference to the $MG_1 MG_2$, and MG_3 loci, can be adduced from the following considerations. As in cross 18, the 4 : 0 and 3 : 1 ratios indicate that two polymeric genes or combinations of genes are heterozygous in this hybrid. However, the combination $MG_1 MG_2 MG_3/mg_1 MG_2 mg_3$ is precluded since a hybrid homozygous for MG_2 would not give 1 : 3 ratios. Thus the occurrence of 1 : 3 ratios indicates that MG_2 is not present in 1426A and, therefore, that a new gene MG_4 is responsible for the fermentation of alpha-methyl-glucoside in this haploid. The 1 : 3 ratios also eliminate the possibility that MG_4 is alone sufficient for fermentation. Does MG_4 act in concert with yet another new gene? Or, is MG_4 equivalent to MG_2 in requiring either MG_1 or MG_3 for alpha-methyl-glucoside fermentation? For example, if MG_4 and MG_2 are equivalent, all the above ratios can be accounted for by the genotype $MG_1 mg_2 MG_3 MG_4/mg_1 MG_2 mg_3 mg_4$

which would result in the following distribution of ascus types providing there was no case of linkage involved: 6 (4 : 0), 72 (3 : 1), 97 (2 : 2), 28 (1 : 3), and 1 (0 : 4). However, this interpretation was shown to be inadequate when the next cross of 1426A with 1018-2b, a complete recessive $mg_1 mg_2 mg_3 mg_4$, gave 9 asci with these ratios: 3 (2 : 2), 1 (1 : 3), and 5 (0 : 4). If MG_4 is equivalent to MG_2 , the situation would be analogous to that in crosses 11 and 12 and a preponderance of 2 : 2 segregations would be expected. The distribution of the segregation ratios requires either (1) that MG_1 or MG_3 , but not both, is complementary to MG_4 if 1426A is $MG_1 mg_2 MG_3 MG_4$, or (2) that neither MG_1 nor MG_3 are complementary to MG_4 and that a fifth gene must be postulated which in combination with MG_4 will permit fermentation. A further observation favoured the second possibility and provided evidence of the identity of the new gene. The asci from cross 20 (table 5) had 2 : 2 segregations for maltose fermentation (of the parents, 1426A was a fermenter whereas 1018-2b was not), and each alpha-methyl-glucoside fermenter obtained from this cross was also a maltose fermenter. The simplest interpretation is that the maltose gene segregating in cross 20, identified as MA_1 , is involved in the fermentation of alpha-methyl-glucoside. This interpretation was supported by the results of intra-ascus zygote tests with the four haploids from a 0 : 4 ascus of cross 20 :

1028-9a (—) × 1028-9b (—) ... (+)	1028-9d (—) × 1028-9b (—) ... (—)
1028-9a (—) × 1028-9c (—) ... (—)	1028-9d (—) × 1028-9c (—) ... (+)

Under the hypothesis that 2 genes are needed to enable the haploids of 1028 to ferment alpha-methyl-glucoside, these results indicate that 9a and 9c have the same gene and that 9b and 9d have the other gene. The segregants 9b and 9d were maltose fermenters. Since the segregants 9a and 9c were maltose non-fermenters, the gene they carried was designated as MG_4 .

To confirm the participation of the maltose gene MA_1 in the gene combination which enabled haploids of 1028 to ferment alpha-methyl-glucoside, a hybrid predicted to be homozygous for MG_4 and heterozygous for the complementary gene was obtained by crossing 1028-9c with 1028-6c, a fermenter of alpha-methyl-glucoside and maltose. It was expected that this hybrid would yield asci with 2 : 2 segregations for both alpha-methyl-glucoside and maltose fermentation, and if the above interpretation is correct, all the maltose fermenters would be alpha-methyl-glucoside fermenters. In the 11 tetrads that were obtained (cross 21, table 5), maltose segregated 2 : 2, but for alpha-methyl-glucoside there were 9 (2 : 2), and 2 (1 : 3) asci. Again every alpha-methyl-glucoside fermenter was also a maltose fermenter. In the 2 asci with 1 : 3 segregations, the haploids which were maltose fermenters but alpha-methyl-glucoside non-fermenters were found to be "petites" or cytochrome-deficient yeasts as described by Ephrussi, Hottinguer, and Chimenes (1949). Among the alpha-methyl-glucoside

fermenters from this hybrid as well as those from hybrid 1028, there were no petites although about 20 per cent. of the segregants from these hybrids were petites. In an experiment to investigate this relationship, a fermenter haploid, 1042-2*d*, was treated with acriflavine (Ephrussi, Hottinguer, and Tavlitski, 1949) to obtain a petite clone, 1042-2*d'*. This petite strain was then tested on alpha-methyl-glucoside and found to be a non-fermenter. When, however, this petite clone, 1042-2*d'*, and the spontaneous petite segregant, 1042-9*b*, from one of the 1 : 3 asci were checked by means of zygote tests with a normal strain of genotype $mg_1 mg_2 mg_3 mg_4 MA_1$, the presence of MG_4 was indicated since the cultures descended from the normal zygotes were capable of fermenting alpha-methyl-glucoside

1042-2*d'* (—) × 142-1*d* (—)...(+) 1042-9*b* (—) × 142-1*d* (1)...(+)

TABLE 5

Segregations for alpha-methyl-glucoside fermentation in asci from hybrids involving 1426A and its descendants

Cross	Parents and phenotypes	Hybrid and phenotype	Number of asci segregating as				
			4+ : 0—	3+ : 1—	2+ : 2—	1+ : 3—	0+ : 4—*
19	1426A(+) × 1018-4 <i>a</i> (—)	1027(+)	1	3	3	3	0
20	1426A(+) × 1018-2 <i>b</i> (—)	1028(+)	0	0	3	1	5
21	1028-6 <i>b</i> (+) × 1028-9 <i>c</i> (—)	1042(+)	0	0	9	2	0
22	1042-2 <i>d</i> (+) × 142-1 <i>d</i> (—)	1044(+)	0	0	4	1	0
23	1044-1 <i>c</i> (+) × 136-4 <i>c</i> (—)	1057(+)	0	0	7	0	0

* Fermenters (+) : non-fermenters (—).

From these observations, it was concluded that strains of genotype $mg_1 mg_2 mg_3 MG_4 MA_1$ are unable to ferment alpha-methyl-glucoside if they are petites. It should be noted here that petite clones of the other fermenter genotypes are capable of fermenting alpha-methyl-glucoside although there is a delay of usually 3 or 4 days in the expression of the positive test.

To demonstrate the segregation of MG_4 , crosses were made to give hybrids homozygous for MA_1 and heterozygous for MG_4 . Of the 12 tetrads from crosses 22 and 23 (table 5) there were 11 in which there occurred the expected 2 : 2 segregation for the fermentation of alpha-methyl-glucoside. Again, in the 1 : 3 ascus from cross 22, one of the non-fermenters was a petite.

The presence of MG_3 in 1426A was established in the following manner. Zygote tests with the non-fermenters from a 2 : 2 ascus of 1028 indicated that one of the haploids carried a gene complementary to MG_2 . This haploid, 1028-2*d*, was then crossed to a strain of genotype $mg_1 MG_2 mg_3 mg_4$ and from this cross a fermenter segregant with the gene combination $MG_7 MG_2$ was used in the test for allelism. The gene MG_7 was identified as MG_3 when the allelism tests with

1040-7a, genotype $mg_1 MG_2 MG_3 mg_4$ gave only 4 : 0 asci (cross 25, table 6).

Evidence for an irregular segregation of MG_2

An unexpected 1 : 3 segregation was observed in one of the 14 asci from cross 26 (table 6), which had 1028-2d as the non-fermenter parent. The above tests have established the genotype of 1028-2d as $mg_1 mg_2 MG_3 mg_4$. Since the genotype of the fermenter parent, 1037-2c, was $mg_1 MG_2 MG_3 mg_4$ (see cross 17, table 4), only 2 : 2 segregations for the gene MG_2 were expected. The 1 : 3 ratio in the aberrant ascus could be due to a mutation affecting either MG_2 or

TABLE 6

Segregations for alpha-methyl-glucoside fermentation in asci from hybrids of the following genotypes: $mg_1 mg_2 MG_3 mg_4/mg_1 MG_2 mg_3 mg_4$ in cross 24, $mg_1 MG_2 MG_3 mg_4/mg_1 MG_2 MG_3 mg_4$ in cross 25, $mg_1 MG_2 MG_3 mg_4/mg_1 mg_2 MG_3 mg_4$ in cross 26

Cross	Parents and phenotypes	Hybrid and phenotype	Number of asci segregating as				
			4+ : 0-	3+ : 1-	2+ : 2-	1+ : 3-	0+ : 4-*
24	1028-2d (-) × 1093-2c (-)	1124 (+)	0	0	2	0	1
25	1124-3d (+) × 1040-7a (+)	1130 (+)	13	0	0	0	0
26	1037-2c (+) × 1028-2d (-)	1045 (+)	0	0	13	1	0

* Fermenters (+) : non-fermenters (-).

MG_3 . To investigate these possibilities, zygote tests were made with the negative haploids from this ascus :

1045-9a (-) × 1040-6a (-) ... (+) 1045-9a (-) × 1037-9a (-) ... (-)
 1045-9b (-) × 1040-6c (-) ... (+) 1045-9b (-) × 1037-9d (-) ... (-)
 1045-9c (-) × 1040-6a (-) ... (+) 1045-9c (-) × 1037-9a (-) ... (-)

The zygote tests made with the $mg_1 MG_2 mg_3$ test strains, 1040-6a and 1040-6c, were positive for each of the three haploids. Those made with the $mg_1 mg_2 MG_3$ test strains, 1037-9a and 1037-9d, were negative. From these results, it can be concluded that the irregular segregation in this ascus was due to a mutation at the MG_2 locus.

Irregular segregations were also encountered for some of the other genes heterozygous in the foregoing hybrids. The genes for galactose (G_2) and melibiose fermentation, histidine, tryptophane (TR_1), and methionine independence each had a single 3 : 1 segregation. There were two asci with 1 : 3 segregations for uracil independence, and 3 asci with 4 : 0, 3 : 1, and 1 : 3 segregations for sucrose fermentation.

There was no significant evidence of linkage between any of the alpha-methyl-glucoside genes and any of the other genes segregating in these crosses : a/α mating type ; G_1 and G_2 , MEL , SU_1 , and MA_1 , galactose, melibiose, sucrose, and maltose fermentation respectively ; tr_1 and tr_2 , hi_1 , ur_1 , and me_1 , tryptophane, histidine, uracil, and

methionine dependence respectively. The centromere linkage data were not extensive enough to indicate significant centromere linkage for any of the alpha-methyl-glucoside genes.

4. DISCUSSION

It is possible to explain satisfactorily the segregations for alpha-methyl-glucoside fermentation encountered in some *Saccharomyces* hybrids on the basis of 5 interacting genes, designated MG_1 , MG_2 , MG_3 , MG_4 , and MA_1 . The following combinations of dominant genes confer the capacity to ferment alpha-methyl-glucoside: MG_1MG_2 , MG_2MG_3 , and MG_4MA_1 . The segregation ratios expected for a number of the possible heterozygous combinations have been given in the preceding sections. For a cross which gives a hybrid heterozygous for all 5 genes, $MG_1MG_2MG_3MG_4MA_1/mg_1mg_2mg_3mg_4ma_1$, the following distribution of segregation ratios is expected if the genes segregate independently and show no centromere linkage: 19 (4:0), 352 (3:1), 703 (2:2), 216 (1:3), and 6 (0:4).

The need for the presence of two interacting genes to enable a yeast strain to ferment a carbohydrate is not unique to the fermentation of alpha-methyl-glucoside. The fermentation of dextrin or starch by *Saccharomyces diastaticus* is also under the control of 2 genes, one controlling the splitting of the polysaccharide to maltose and the other controlling the fermentation of maltose (Gilliland, 1953). A more analogous situation is found for the fermentation of galactose where the presence of 2 dominant genes, G_1 and G_2 , is essential for rapid fermentation (Leupold and Hottinguer, 1954; and Douglas and Condie, 1954). As a working hypothesis, the role of genes G_1 and G_2 was postulated by the latter investigators to be that G_1 controls the formation of an enzyme involved in the conversion of galactose to glucose-6-phosphate, and G_2 controls a mechanism for transporting galactose into the cell. As yet, the roles of the alpha-methyl-glucoside genes have not been investigated.

The presence of the gene combination MG_4MA_1 also enables a yeast strain to ferment sucrose although at a noticeably lower rate than when one of the specific sucrose genes is present: *i.e.* 48 to 72 hours are required for a full gas trap in a Durham fermentation tube with the MG_4MA_1 combination as compared to 16 to 24 hours with any one of the specific sucrose genes thus far isolated. It was postulated by Gilliland (1949) and demonstrated by Winge and Roberts (1952) that a maltose gene in their stocks, designated M_1 , could enable a yeast strain to ferment sucrose slowly. Winge and Roberts (1955) established that this gene is allelic with the MA_1 of our stocks. However, in our stocks, strains with the gene MA_1 alone do not ferment sucrose at a detectable rate when tested in Durham tubes containing a medium consisting of 1 per cent. peptone, 1 per cent. yeast extract, and 2 per cent. sucrose.

The maltose gene and the 4 alpha-methyl-glucoside genes were isolated from stocks derived from 6 different parental strains. Two of the parental strains were obtained from Dr C. C. Lindegren and 4 were from stocks descended from strains originating in his laboratory. The source of these genes is of importance since Lindegren (1953) also has encountered irregular segregations for alpha-methyl-glucoside fermentation but has interpreted the deviations from a 2 : 2 ratio as cases of "gene conversion". The irregularities in families with only a small proportion of anomalous asci, such as families VII and XI with 1 (4 : 0), 20 (2 : 2), and 2 (3 : 1), 30 (2 : 2) segregations respectively (table 1, p. 627, Lindegren, 1953), might reasonably be attributed to gene conversion. However, some of the cases that are cited may be explained on the basis of multiple genes. For example, the distribution of the segregation ratios in family XV (table 1, p. 627, Lindegren, 1953) with 1 (3 : 1), 20 (2 : 2), 18 (1 : 3), and 1 (0 : 4) asci would be expected, with the exception of the 3 : 1 ascus, from a hybrid heterozygous for the 3 genes MG_1 , MG_2 , and MG_3 . In another case, the irregular segregations may be due to the occurrence of petite clones amongst segregants with the gene combination $MG_4 MA_1$. In family XXVIII (table 1, p. 627, fig. 1, p. 628 Lindegren, 1953), 9 asci out of 13 gave 2 : 2 segregations for both maltose and alpha-methyl-glucoside and in each case, the alpha-methyl-glucoside fermenters were maltose fermenters. In the remaining asci, there were 1 (1 : 3) and 3 (4 : 0) segregations for alpha-methyl-glucoside fermentation. The fermenter parent in this cross, 1426, was shown above to be of genotype $mg_1 mg_2 MG_3 MG_4 MA_1$. If the non-fermenter parent, 12736, is of genotype $mg_1 mg_2 mg_3 MG_4 ma_1$, the observations of Lindegren can be accounted for by the occurrence of petite clones in the asci with the aberrant ratios. It has been noted in crosses with 1426 (crosses 19 and 20, table 5) that about 20 per cent. of the spore cultures are petite and frequently more than one petite spore culture arose from the same ascus.

The 9 asci where maltose and alpha-methyl-glucoside fermentation segregate together are cited by Lindegren as evidence supporting the location of MA and MG on the same chromosome. In the other families that he considers, there is no evidence of linkage for these genes from his summary of the data : 26 parental ditype, 33 non-parental ditype, and 99 tetratype asci. To account for this inconsistency of the data, a crossover suppressor is postulated for family XXVIII. It seems more probable, however, that different alpha-methyl-glucoside genes are being compared. These genes need not necessarily be MG_1 , MG_2 , MG_3 , or MG_4 since it is highly probable that if in a sample of 6 strains there are found 5 different genes involved in alpha-methyl-glucoside fermentation, there exist other genes controlling this ability. Nevertheless, the irregular segregations in some of the families in the pedigree he presents may be explained on the basis of interacting genes, and this possibility must be kept in

mind when considering his data on alpha-methyl-glucoside fermentation as evidence pertaining to gene conversion.

5. SUMMARY

The distribution of the 2:2, 1:3, and 0:4 segregations for alpha-methyl-glucoside fermentation in the four-spored asci of *Saccharomyces* hybrids led to the postulation that 3 genes, MG_1 , MG_2 , and MG_3 , are involved. A yeast with the dominant gene MG_2 and either dominant gene MG_1 or MG_3 is able to ferment alpha-methyl-glucoside. An additional gene combination enabling fermentation, $MG_4 MA_1$, was discovered when a cross which yielded 4:0, 3:1, 2:2, and 1:3 ratios was investigated. Crosses were made which resulted in the segregation of a single gene at a time, and 2:2 ratios were obtained except for 4 anomalous 1:3 asci. Three of the anomalous asci involved the gene combination $MG_4 MA_1$ and can be explained by the occurrence of petite spore clones from these asci since it was demonstrated that petite strains with this gene combination could not ferment alpha-methyl-glucoside. The irregular segregation in the fourth ascus is attributed to the mutation of the gene MG_2 .

Acknowledgments.—The author wishes to thank Drs Howard C. Douglas and Herschel Roman for their advice and interest in this investigation. He is also indebted to Dr Carl C. Lindegren who made available the parental strains from which some of the hybrids used in this study were developed. This investigation received support from the Biological and Medical Research Fund of the State of Washington, and the National Institutes of Health, Public Health Service, Grant No. E-328.

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