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# The Dispersed, Repeated Family of *MAL* Loci in *Saccharomyces* spp.

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This report concludes our analysis of the various standard maltose-fermenting strains of *Saccharomyces* spp. We showed that, in addition to either the *MAL2* or *MAL4* functional locus present in the standard *MAL2* and *MAL4* strains, both strains contain two cryptic *MALg* genes mapping to the *MAL1* and *MAL3* locus positions. (Functional *MAL* loci appear to consist of two linked complementing gene functions, *MALp* and *MALg*. Cryptic *MALg* genes lack a linked functional *MALp* gene.) Using a probe containing a DNA fragment derived from the *MAL6* locus, we detected three genomic *Hin*dIII fragments in both the *MAL2* and *MAL4* strains. Each of these *Hin*dIII fragments is shown to segregate in a Mendelian fashion and to be linked to one of the three *MAL* loci in each of the strains. We also detected additional fragments having significant sequence homology to the *MAL6* probe but lacking in *MAL*-related functions.

Early studies of the genes controlling sugar fermentation by *Saccharomyces* spp. (reviewed in reference 1) characterized a polygenic series of loci, called the *MAL* loci, controlling the fermentation of maltose. Strains able to ferment maltose carry at least one of a series of five *MAL* loci: *MAL1*, *MAL2*, *MAL3*, *MAL4*, and *MAL6*. Although the presence of a functional *MAL* locus in a strain is easily identified by standard genetic methods, the status of the genetic information present at the other *MAL* locus positions in these strains, until recently, has remained inaccessible to either genetic or physical techniques.

Two complementing gene functions, *MALp* and *MALg*, required for the fermentation of maltose, have been characterized from studies of naturally occurring maltose-nonfermenting strains (9–11, 14). In 11 strains analyzed, three alleles of the *MAL1* locus were found. The first, *MAL1*, when present in a strain, enabled it to ferment maltose, and thus this locus must be a complex locus containing both *MALp* and *MALg* activity. The other alleles, *MAL1p* and *MAL1g*, complemented each other to allow for fermentation but, when present singly, were cryptic and did not allow for the fermentation of maltose. Mutations in the putative regulatory gene linked to the *MAL6* locus were complemented by the *MAL1p* allele but not the *MAL1g* allele (19). This result implies that *MAL1p* codes for a regulatory protein which is functionally equivalent to the regulatory protein coded for by the *MAL6* locus. The function of *MALg* is as yet not fully defined. A gene coding for maltase has been cloned and shown to be tightly linked to the *MAL6* locus (4, 13). Thus, the *MALg* function may, at least in part, encode maltase.

Genetic analysis of a *MAL6* strain of *Saccharomyces carlesbergensis* (CB11) showed that this strain carries two cryptic *MALg* loci in addition to the functional *MAL6* locus, one linked to *MAL1* and therefore denoted *MAL1g* and the other linked to *MAL3* and therefore denoted *MAL3g* (13). Three genomic fragments were found in strain CB11 which have significant sequence homology to a *MAL6*-derived probe. These fragments segregated as linked to the *MAL6*, *MAL1g*, and *MAL3g* loci. A similar situation of a functional *MAL* locus and an additional cryptic *MALg* locus was seen in two other strains studied, 4059 (a *MAL1* strain) and 1412-4D (a *MAL3* strain) (6). We here report on our analysis of the *MAL2* (1453-3B) and *MAL4* (1403-7A) strains from the Berkeley Yeast Stock Center collection.

**Analysis of the *MAL2* strain 1453-3B.** The *MAL2* strain 1453-3B was crossed to the *mal*$^0$ strain 4-1D, and the diploid formed, W14, was subjected to tetrad analysis. A *mal*$^0$ strain is a nonfermenting strain which complements neither a *MAL1p* nor a *MAL1g* strain. In 20 tetrads from diploid W14, maltose fermentation segregated 2:2. The two nonfermenting segregants from each tetrad were then crossed to tester strains of the genotype *MAL1p* or *MAL1g*, and most of these nonfermenters were found to complement the *MAL1p* strain, indicating the presence of at least one cryptic *MALg* locus. Half-tetrad analysis of the maltose nonfermenters indicated that, in fact, more than one *MALg* gene was segregating in the cross. To determine the number of *MALg* genes involved and to test them for alleles, one tetrad, tetrad 3, from diploid W14 was selected for complete analysis. This analysis is presented in detail in Fig. 1. In summary, the conclusion of these crosses is that the standard *MAL2* strain has the genotype *MAL2 MAL1g MAL3g*.

Five tetrads from diploid W14 were analyzed by gel transfer, using the *MAL6*-derived probe (13). Figure 2 shows tetrads 3 and 7 from diploid W14. Hybridization occurred to *Hin*dIII fragments of 10.7, 8.1, and 7.6 kilobases (kb) in strain 1453-3B. The *mal*$^0$ strain (4-1D) also contained a single 7.0-kb fragment with significant homology to the probe. From tetrad 7 and three other tetrads (data not shown), we can see that two fragments of 8.1 kb are present in strain 1453-3B. All of the fragments segregated in a Mendelian fashion. A comparison of the genotype of each of the segregants of tetrad 3 of W14 with the segregation pattern of the fragments as seen in Fig. 2 shows the 7.6-kb fragment to segregate with the *MAL2* locus, the 10.7-kb fragment to segregate with *MAL1g*, and one of the 8.1-kb fragments to segregate with *MAL3g*. These conclusions are consistent with the results from all five of the tetrads analyzed. In all five tetrads studied, the 7.0-kb fragment present in strain 4-1D segregated in repulsion with the 10.7-kb fragment derived from strain 1453-3B. The 7.0-kb fragment of 4-1D is therefore linked to the *MAL1* locus and is probably the same fragment previously observed in other *mal*$^0$ strains (6, 13). From gel transfer analysis of tetrads

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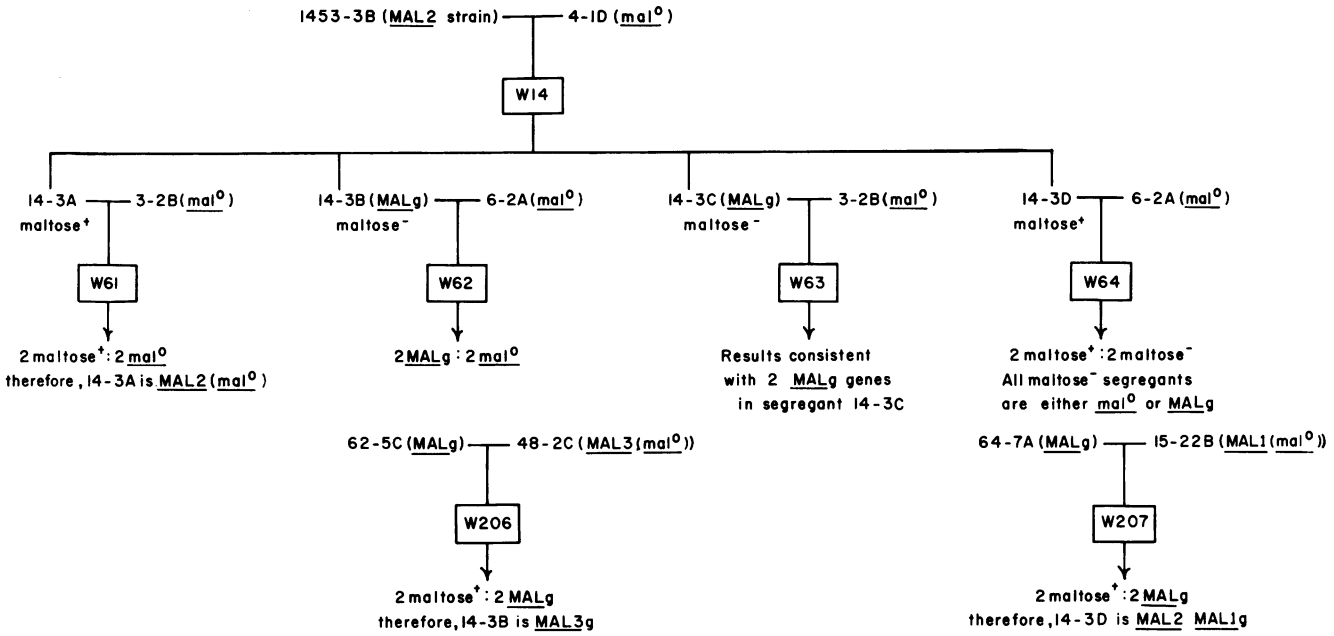


FIG. 1. Genetic analysis of the standard *MAL2* strain 1453-3B. Strain 1453-3B is the standard *MAL2* strain available from the Berkeley Yeast Stock Center collection. The pedigree shown diagrams the crosses used to determine the genotype of strain 1453-3B as it relates to maltose fermentation. This analysis relies on the use of tester strains of the genotypes *MAL1p*, *MAL1g*, and *mal*$^0$ which were derived from natural variants isolated by G. Naumov Institute for the Genetic Study of Industrial Microorganisms, Moscow, as described previously (6, 13). A *mal*$^0$ strain is defined as a strain which does not ferment maltose and does not complement either *MALp* or *MALg* strains to allow fermentation. The presence of a *MALg* locus in a maltose nonfermenting strain is revealed by complementation with a *MAL1p* tester strain. The strain is mated to *MAL1p*, *MAL1g*, and *mal*$^0$ tester strains, and the diploid is tested for its ability to ferment. If a strain carries a *MALg* locus, the diploid formed by mating to a *MAL1p* will ferment. The other diploids will not. Determining the locus position of the *MALg* gene requires maltose-fermenting strains carrying a single functional *MAL* locus and no additional cryptic *MALp* or *MALg* loci. These were constructed from the standard fermenting strains as previously described (13) by crossing the standard *MAL* strain to a *mal*$^0$ strain and selecting fermenting segregants which, when crossed to a *mal*$^0$ strain, yield only nonfermenting segregants of the *mal*$^0$ genotype. These strains are denoted, for example, *MAL1* (*mal*$^0$), indicating the presence of the *MAL1* locus and no other segregating *MALp* or *MALg* loci. To determine the locus position of an unknown *MALg* gene, a strain carrying this gene is crossed to one of these tester strains, and the diploid is dissected and analyzed. If, for example, the unknown *MALg* is linked or allelic to *MAL1*, all of the nonfermenting segregants from the *MALg* to *MAL1* (*mal*$^0$) cross will carry a *MALg* gene, whose presence is detected by its ability to complement a *MAL1p* allele. All procedures of genetic analysis were done by the method of Mortimer and Hawthorne (7). Maltose fermentation is indicated by the production of gas in YP medium–2% maltose in 2 days.

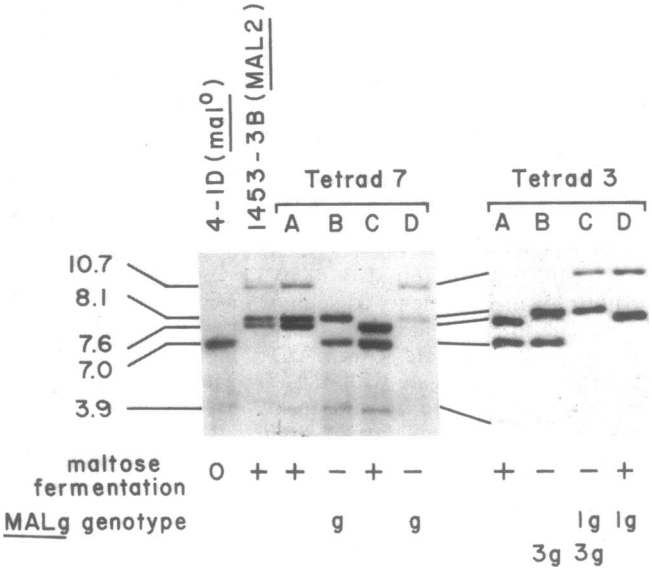


FIG. 2. Gel transfer analysis of tetrads from diploid W14, 1453-3B × 4-1D (*mal*$^0$). The standard *MAL2* strain, 1453-3B was crossed to a *mal*$^0$ strain (4-1D), and the diploid, W14 was dissected. Gel transfer analysis was carried out on the parents and five tetrads resulting from this diploid. The results from two of these tetrads are shown here. The maltose fermentation phenotype as well as the *MALg* genotype of the maltose-nonfermenting segregants are also indicated. Where the locus position of the *MALg* gene was determined, the results are indicated as either 1g or 3g; if not, the genotype was simply designated g. Two procedures were used for preparing total genomic DNA from *Saccharomyces* spp., a modification of the method of Cryer et al. (2), as described by Michels and Needleman (6), and one described by Sherman et al. (16) for preparations from small culture volumes. Restriction digestion was done under conditions recommended by the commercial supplier (Bethesda Research Laboratories, Gaithersburg, Md.). Fragments were separated by using 0.8% horizontal agarose gels. The gels were treated as described by Southern (17). The nitrocellulose filters were hybridized to a $^{32}$P-labeled probe washed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at 65°C and exposed to Kodak XAR film at −70°C by using an intensifying screen. The probe was labeled by nick translation with [$^{32}$P]dCTP (Amersham Corp., Arlington Heights, Ill.) by procedures described by Rigby et al. (15).

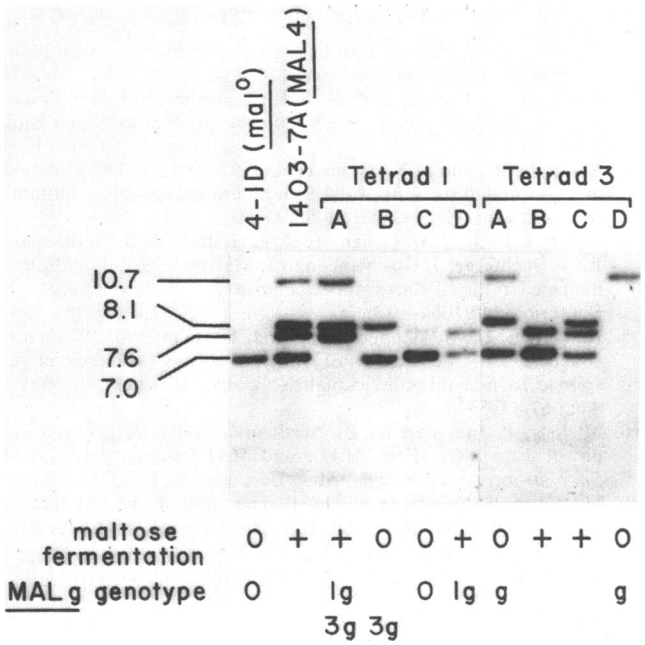


FIG. 3. Gel transfer analysis of tetrads from the diploid W60, 1403-7A × 4-1D (*mal*$^0$). The standard *MAL4* strain 1403-7A was crossed to a *mal*$^0$ strain (4-1D), and the diploid was dissected. The parent strains and two tetrads from this diploid were analyzed by gel transfer as described in the legend to Fig. 2. The maltose fermentation phenotype as well as the *MALg* genotype of the maltose-nonfermenting segregants are also indicated. Where the locus position of the *MALg* gene was determined, the results are indicated.

from diploid W62 (14-3B × 6-2A [*mal*$^0$]), the two 8.1-kb *Hin*dIII fragments originating from strain 1453-3B can be seen to segregate independently of each other, and *MALg* complementing activity is associated with only one of these fragments (data not shown). The other fragment, although having homology to the *MAL6*-derived probe, lacks *MALp* and *MALg* complementing activity and therefore represents another *mal*$^0$ locus not linked to *MAL1*. In two complete tetrads from diploid W64, the 10.7-kb fragment segregated with the *MAL1g* gene, and the 7.6-kb fragment segregated with the ability to ferment maltose, that is, the *MAL2* locus (data not shown).

**Analysis of the *MAL4* strain 1403-7A.** The *MAL4* standard strain 1403-7A was crosed to the *mal*$^0$ strain 4-1D to form the diploid W60. W60 was subjected to a genetic analysis similar to the one described for the *MAL2* standard strain (Fig. 1). The results of this analysis show the genotype of this *MAL4* strain to be *MAL4 MAL1g MAL3g*.

Examples of the results of gel transfer analysis of the tetrads from diploid W60 are shown in Fig. 3. *Hin*dIII fragments of 10.7, 8.1, 7.6, and 7.0 kb, showing homology to the *MAL6*-derived probe, are seen in the *MAL4* parent strain. Two tetrads are shown. Tetrad 1 was genetically analyzed in detail and the four segregants were found to have the following genotype: segregant 1A, *MAL4 MAL1g MAL3g*; segregant 1B, *MAL3g*; segregant 1C, *mal*$^0$; segregant 1D, *MAL4 MAL1g*. Tetrad 3 was not analyzed in the same detail as tetrad 1, but the two nonfermenting segregants, 3A and 3B, were shown by complementation to a *MAL1p* strain to contain *MALg* complementing activity. As can be seen, the 7.6-kb fragment segregates with the ability to ferment maltose and therefore is linked to the *MAL4* locus. The 7.0-kb fragment, again, appears to represent a *mal*$^0$ locus (segregant 60-1C), but clearly this fragment cannot be linked to *MAL1* and therefore must represent a different putative *mal*$^0$ locus. Segregant 60-1B contains the 7.0-kb fragment in addition to an 8.1-kb fragment which appears to segregate with *MAL3g*. Finally, the presence of the 10.7-kb fragment can again be associated with the *MAL1g* locus.

Table 1 summarizes the genotype of each standard *MAL* strain and indicates the size of the *Hin*dIII fragments with

TABLE 1. Summary of the genetic and physical analysis of the standard *MAL* strains

| Strain (functional *MAL* locus) | Genotype | Size (kb) of *Hin*dIII fragments[a] | Linked *MAL* locus |
|---|---|---|---|
| 4059 (*MAL1*) | *MAL1 MAL3g* | 7.3 (3.9)[b] | *MAL1* |
| | | 7.1 | *MAL3g* |
| 1453-3B (*MAL2*) | *MAL2 MAL1g MAL3g* | 7.6 | *MAL2* |
| | | 10.7 | *MAL1g* |
| | | 8.1 | *MAL3g* |
| | | 8.1 | *mal*$^0$, Unknown locus position |
| 1412-4D (*MAL3*) | *MAL3 MAL1g* | 7.3 (4.3, 4.5)[b] | *MAL3* |
| | | 10.7 | *MAL1g* |
| 1403-7A (*MAL4*) | *MAL4 MAL1g MAL3g* | 7.6 | *MAL4* |
| | | 10.7 | *MAL1g* |
| | | 8.1 | *MAL3g* |
| | | 7.0 | *mal*$^0$, Unknown locus position |
| CB11 (*MAL6*) | *MAL6 MAL1g MAL3g* | 7.3 | *MAL6* |
| | | 10.7 | *MAL1g* |
| | | 7.3 | *MAL3g* |

[a] Fragments in each strain showing homology to the *MAL6* probe, D-1 (13).
[b] Fragments showing poor homology to the probe are within parentheses.

homology to the *MAL6*-derived probe that were found in each strain and the *MAL* allele associated with that fragment. The results reported here and in previous reports (6, 13) allow us to make the following conclusions. All of the *MAL* loci are significantly related at the nucleotide level. Each *MAL* strain that we have tested shows multiple genomic fragments with homology to our *MAL6*-derived probe. In our analysis of each strain, we found that one of these fragments segregates with the ability to ferment maltose. Additional fragments in the strain segregate with the ability to complement a *MAL1p* tester strain, and thus these fragments carry *MALg* function. Only two *MALg* genes have been identified in these strains, one which is linked to the *MAL1* locus (called *MAL1g*) and one linked to the *MAL3* locus (called *MAL3g*). Also, there are a limited number of fragments seen in these strains, which have homology to the *MAL6*-derived probe, which neither confer the ability to ferment maltose nor complement *MAL1p* or *MAL1g* tester strains. At least one of these is linked to *MAL1*.

It is clear from this work that the standard *MAL* strains differ from each other depending on the genomic position of this functional *MAL* locus. Although *MAL*-related information was present at or linked to the *MAL1* and *MAL3* locus positions in most strains, *MAL* information is entirely lacking from the *MAL2*, *MAL4*, and *MAL6* locus positions except in *MAL2*, *MAL4*, and *MAL6* strains, respectively. In addition, a number of genomic fragments containing sequences homologous to the *MAL6*-derived probe but lacking any *MALg* or *MALp* activity were characterized. The origin of these nonfunctional copies remains to be determined.

A long history of research on the biochemistry of maltase and its synthesis can be found in the literature (3, 5, 8, 12, 18). It is clear from our work that these studies were carried out with strains which were only partially genetically defined, and thus the conclusions reached must be reevaluated. Of particular interest will be a reassessment of conflicting reports regarding the number of species of maltase enzyme found in the various *MAL* strains. Most reports have indicated that a single maltase of similar physical and enzymatic characteristics is synthesized by the different *MAL* strains (5, 12), but some suggestions to the contrary have been presented (3). Preliminary studies in our laboratory indicate that under certain assay conditions one can distinguish three maltases of differing thermal stability in strain CB11, and each can be associated with one of the three *MAL* loci in the strain.

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