MUTANTS OF SACCHAROMYCES CEREVISIAE RESISTANT TO THE 
α MATING-TYPE FACTOR1

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Manuscript received September 10, 1975
Revised copy received November 20, 1975

ABSTRACT

Mutants that are resistant to α-factor have been isolated from α mating-type 
haploid strains of yeast by direct selection on agar medium containing partially 
purified α-factor. All resistant mutants isolated were found to be sterile. They 
were characterized and compared with mutants previously isolated as non-
mating. Among 93 able to mate at low frequency and to sporulate, none 
showed linkage to the mating-type locus. The results support the hypothesis 
that the response to α-factor by cells of mating-type α is essential for mating.

IN the yeast Saccharomyces cerevisiae, mutations at a number of loci, including 
the mating-type locus, impair the ability of haploid cells to participate in 
sexual conjugation. Nonmating mutants, derived from α and α mating-type 
haploids have been described previously (MACKAY and MANNEY 1974a,b). These 
sterile mutants represent at least five different genetic loci and probably con-
siderably more. They were all deficient in one or more additional functions 
related to conjugation, including production and response to hormonelike diff-
usible mating-type factors.

The existence of diffusible mating-type factors in this yeast was first reported 
by LEVI (1956) and has been characterized in some detail by others (DUNTZE, 
MacKay and Manney 1970; Throm and Duntze 1970; Bucking-Throm, 
et al. 1973; Duntze, et al. 1973; MacKay and Manney 1974a; Wilkinson and 
Pringle 1974). Mating-type α cells secrete into their growth medium an oli-
gopeptide, α-factor, which acts specifically on cells of mating type α, arresting 
them, temporarily in the GI stage of the cell division cycle. Arrested cells un-
dergo a characteristic morphological change; they assume an asymmetrical elong-
gated form which has been termed “schmoo-shaped,” for whimsical reasons, and 
for convenience (see MacKay and Manney 1974a).

An analogous α-factor is apparently produced by α mating-type cells and has 
a similar effect on α cells. This reaction is more transitory and less is known 
about the diffusible molecule and the details of the response.

There is strong circumstantial evidence that these factors play an important 
role in conjugation; but direct evidence that they are essential for mating has not 
been produced. It has been speculated that these reactions serve to synchronize

1 This investigation was supported in part by USPHS Research Grant No. 19175.

the two cell cycles of mating cells as a prelude to conjugation (Bucking-Throm, et al. 1973; Hartwell 1973).

Many of the sterile mutants isolated from each mating type retained the ability to produce their respective diffusible mating-type factor. However, all of the \(a\) mutants had lost the ability to respond to \(\alpha\)-factor, and only one of 107 \(a\) mutants tested retained the ability to respond to \(\alpha\)-factor. These observations led to the idea that resistance to \(\alpha\)-factor as a growth inhibitor might be useful as another method for selecting mutations affecting conjugation and related process. If such resistant mutants were also sterile, it would provide additional support of the hypothesis that the processes mediated by these factors are essential for mating. Isolation of mutants by this method and their characterization is the subject of this paper.

**MATERIALS AND METHODS**

*Yeast strains:* The genotypes of the heterothallic strains of *Saccharomyces cerevisiae* are given in Table 1. They were derived from strains obtained from R. K. Mortimer at the University of California at Berkeley.

*Media:* The standard culture media used in these experiments have been described previously. Partially purified \(\alpha\)-factor on the surface of YEPD agar (Difco Yeast Extract, 10 grams/liter; Difco Bacto-Peptone, 20 g/l; dextrose, 20 g/l; agar, 20 g/l), the day it was to be used. In Experiments 1 and 2, plates used for selection were prepared by mixing the \(\alpha\)-factor with the agar before it was poured into the plates. However, the above method proved to be superior and was thereafter adopted. The \(\alpha\)-factor was prepared by the method of Bucking-Throm, et al. (1973) and used without further sterilization.

*Isolation of \(\alpha\)-factor resistant mutants:* Parent haploid strains were grown to stationary phase in liquid YEPD on a rotary shaker at 30°. Cells were diluted in distilled water. Samples of \(10^5\) cells were plated on 2 ml medium containing 16 units of \(\alpha\)-factor in \(35 \times 10 \text{ mm petri dishes.}

UV irradiated samples were exposed without lids to a General Electric germicidal lamp for a dose yielding approximately 50% survivors. In the preliminary experiments, (1 and 2) the number of cells/plate was varied from \(10^4\) to \(10^6\) and the \(\alpha\)-factor was increased up to 8 times the above amount. SC medium was also tried in place of YEPD. The conditions described above gave the greatest recovery of mutants and the most economical use of \(\alpha\)-factor.

**TABLE 1**

*Genotypes of yeast strains*

<table>
<thead>
<tr>
<th>Parents of mutants</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>XT1177-S24</td>
<td>(a) leu1 trp5-18 can1 gal2</td>
</tr>
<tr>
<td>XT1177-S47</td>
<td>(a) ade2-1 his2 lys1-1 trp5-18 gal2</td>
</tr>
<tr>
<td>Complementing (a) strains</td>
<td></td>
</tr>
<tr>
<td>XT1172-S153</td>
<td>(a) lys1-1 ura1 gal2</td>
</tr>
<tr>
<td>XT1172-S245c</td>
<td>(a) ade6 his6 leu1 trp5-1 met1 can1 gal2</td>
</tr>
<tr>
<td>Mating-type testers</td>
<td></td>
</tr>
<tr>
<td>XT1219-1A</td>
<td>(a) ade1 trp1 his2 gal1</td>
</tr>
<tr>
<td>XT1219-18A</td>
<td>(a) ade1 trp1 his2 gal1</td>
</tr>
<tr>
<td>XT1172-S110</td>
<td>(a) leu1 ade6 lys1 his6 ura1 gal2</td>
</tr>
<tr>
<td>Mating type factor testers</td>
<td></td>
</tr>
<tr>
<td>X2180-1A</td>
<td>(a) gal2</td>
</tr>
<tr>
<td>X2180-1B</td>
<td>(a) gal2</td>
</tr>
<tr>
<td>PA1</td>
<td><em>stel</em> mutant of XT1172-S153</td>
</tr>
</tbody>
</table>
After 2 to 3 days of incubation at 30°, colonies were picked and purified by cloning on non-selective YEPD agar. In Experiments 1 and 2, mutants were isolated from XT1177-S24, in experiment 3 from XT1177-S47.

Isolates were replica-plated to YEPD containing 3 units of α factor per ml and examined microscopically for morphology and growth after 3 to 5 hours at 30°.

Detection of mating and isolation of diploids: High-frequency and low-frequency mating was detected by the methods described by MacKay and Manney (1974b). Diploids were obtained from mating-defective mutants by prototroph selection as described previously (MacKay and Manney 1974a). Mutants derived from XT1177-S24 were crossed with XT1172-S153, and those derived from XT1177-S47 with XT1172-S245c.

Random spore analysis: Random spore samples were prepared and tested by a modified version of the simple method described previously (MacKay and Manney 1974b). Sporulation mixtures treated with Glusulase (Endo Laboratories, Inc.) were streaked on YEPD to obtain single colonies. After 3 days' incubation at 30°, colonies were subcultured onto master plates of YEPD, incubated for one day and replica-plated to determine mating response and nutritional phenotype. Mating type was scored by cross-streaking replicas with the mating-type testers and replica-plating them after 24 hours to suitable media for detecting complementation.

RESULTS

Isolation of α-factor resistant mutants: Mutants which have lost the ability to respond to α mating-type factor were isolated by direct selection on agar media containing partially purified α-factor, in three experiments. In the first two the selective plating conditions were varied as described in METHODS and MATERIALS to optimize the procedure. In the third experiment, the optimal conditions were used to isolate a larger sample of mutants, and to search for temperature-sensitive mutants. Colonies that grew up on α-factor-containing plates in each experiment were picked, purified and tested for inhibition of budding and morphological transformation (schmooing) in the presence of α-factor. After subsequent screening, nonsporulating α-factor resistant isolates having the nutritional phenotype of the original α mating-type haploid were further characterized. All mutants that were resistant to inhibition by α factor also failed to schmoo. Properties of these mutants are summarized in Table 2.

The frequency of UV-induced mutants can be estimated from the results of Experiment 3. The 163 mutants isolated arose from approximately 10⁶ irradiated cells at the survival of approximately 50%. Accordingly, there were approximately 3.2×10⁻⁴ mutants/survivor. In Experiment 2 the frequency of mutants selected under the same condition without irradiation was only about 5×10⁻⁴, or about 1% of the induced frequency. Five of the mutants isolated in Experiment 3 were temperature-sensitive; they have the mutant phenotype at 36° but behave as wild type at 22°. In all three experiments only one case of mutation of α to α was detected.

Mating behavior: All of the mutants isolated were tested for their ability to mate with a normal mating-type α strain at or near wild-type frequency, as judged by formation of prototrophs in cell mixtures. Ambiguous cases were verified by microscopic examination for visible zygotes. These and subsequent semi-quantitative tests proved that none of the 283 mutants isolated mated at greater than about 1% of wild-type frequency. In every case loss of the ability to respond to α-factor was accompanied by loss of the ability to mate.
TABLE 2

Characteristics of α-factor resistant mutants

<table>
<thead>
<tr>
<th>Source of mutants</th>
<th>Number of mutants</th>
<th>α-factor production</th>
<th>Low-frequency mating</th>
<th>Recombination with mating type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number tested</td>
<td>Percent positive</td>
<td>Number tested</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unirradiated</td>
<td>17*</td>
<td>17</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>Irradiated</td>
<td>19</td>
<td>19</td>
<td>53</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unirradiated</td>
<td>20*</td>
<td>20</td>
<td>55</td>
<td>3</td>
</tr>
<tr>
<td>Irradiated</td>
<td>64</td>
<td>64</td>
<td>28</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irradiated</td>
<td>163</td>
<td>0</td>
<td></td>
<td>158</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>51</td>
</tr>
<tr>
<td>Total</td>
<td>283</td>
<td>123</td>
<td>33</td>
<td>259</td>
</tr>
</tbody>
</table>

* Mutants in Experiments 1 and 2 which arose spontaneously may not be of independent origin, however they do vary in their phenotypes.

Production of α factor: To further characterize these mutants for comparison with previously studied sterile α mutants, we tested the mutants isolated in Experiments 1 and 2 for their ability to produce α factor. The reaction between α cells and α-factor is much weaker than that between α cells and α-factor. Consequently, the α-factor production test, described previously (Mackay and Manney 1974a) is only marginal in its ability to detect normal levels of α-factor, and very likely does not detect a-factor production in some mutants that are only slightly impaired. To increase the sensitivity of the test, the α sterile mutant PA1, which responds to α factor but does not produce α factor, was mixed directly with the mutant being tested and the mixture was examined for schmooes. In several cases, schmoo-shaped cells were isolated and tested; all had the phenotype of the α strain PA1. Approximately one-third of the α-factor resistant mutants tested produced detectable a-factor (Table 2).

Low-frequency matings: In order to analyze these mutations for linkage to the mating-type locus, we isolated diploids by prototroph selection using a suitable normal mating-type (αI strain. Seventy percent of those tested formed diploids at low frequency (Table 2).

One isolate of each diploid was picked for genetic analysis. Of these 188 diploids, all but 17 sporulated; 2 of these were subsequently found to be unmated haploids and 6 were petite, leaving 9 non-sporulating diploids.

Random spore analysis: Random samples of up to 32 spores from each of 116 sporulating diploids were analyzed for mating-type and nutritional marker segregation. In this analysis, recovery of sterile spores confirms the presence of the mutation in the diploid. If the mutation is at, or closely linked to the mating-type locus, approximately equal numbers of sterile and α spores, but no α spores, are expected. However, if the mutation is free to recombine with mating type, α-sporoses, as well as α and sterile spores are expected, their relative frequency
depending on whether the mutation is specific or nonspecific with respect to $a$ and $\alpha$, and on its distance from the mating-type locus.

In 23 cases no sterile spores and approximately equal numbers of $a$ and $\alpha$ spores were recovered. While we cannot rule out the possibility that some of these are nonchromosomal mutations, previous studies (MacKay 1972) indicate they most likely resulted from reversions of the sterile mutation which were selected by the low-frequency mating procedure. In the remaining 93, sterile, as well as both mating-type $a$ and $\alpha$, spores were recovered (Table 2). Recovery of $a$ mating-type spores in these small samples proves that none of these 93 mutations is at, or closely linked to, the mating-type locus, and therefore, none are mutations of the $a$ allele. The number of spores analyzed was insufficient to determine mating-type specificity of the mutations.

**DISCUSSION**

This new criterion for selecting mutations affecting conjugation provides further insights into the relationships among mating-type associated functions in this yeast. The finding that all mutants selected as resistant to $\alpha$-factor are also sterile, strongly supports the hypothesis that the response to this hormone-like peptide is essential for mating. It also provides a rapid method for isolating sterile mutants from $a$ mating-type strains. Failure to find any sterile mutants that did not recombine with the mating-type locus confirms previous observations.

The combination of phenotypic characteristics of the mutants isolated in this study bear a striking similarity to those of mutants isolated as sterile (MacKay and Manney 1974a,b). They are compared in Table 3. The method used to select the nonmating mutants most likely discriminated against any mutants that were not also resistant to $\alpha$-factor. Consequently, the coincidence of sterility and resistance in those mutants may only reflect this bias in the selection method. In the direct selection of $\alpha$-factor resistant mutants, however, there is no apparent artificial bias selecting for sterility. Therefore, the coincidence of these defects must reflect a functional relationship.

In the previous study of nonmating mutants, all of the 28 that could be analyzed recombined freely with the mating-type locus. In the present study we analyzed an additional 93 mutants in a search for mutations of the $a$ mating-type allele. Our continued failure to find any is taken as strong circumstantial evidence

**TABLE 3**

*Comparison of sterile and $\alpha$-factor resistant mutants*

<table>
<thead>
<tr>
<th>Percent of mutants tested</th>
<th>Sterile</th>
<th>$\alpha$-factor resistant</th>
<th>$\alpha$-factor producing</th>
<th>Low-frequency mating</th>
<th>Sporulating with mating type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated as nonmating*</td>
<td>100</td>
<td>100</td>
<td>17</td>
<td>58</td>
<td>80</td>
</tr>
<tr>
<td>Isolated as resistant to $\alpha$-factor</td>
<td>100</td>
<td>100</td>
<td>33</td>
<td>70</td>
<td>95</td>
</tr>
</tbody>
</table>

* MacKay and Manney 1947b.
that they do not occur. This is in marked contrast to the case for $\alpha$ mutants, where 20% of the diploids analyzed were closely linked to the mating-type locus (MacKay and Manney 1974b).

We infer from these results that if sterile mutations occur at the $a$ locus they must also abolish the ability to mate even at low frequencies or to sporulate. This provides strong support for the previous suggestions that there is a significant asymmetry between the mutations affecting the $a$ and $\alpha$ forms of the mating-type locus (MacKay 1972; MacKay and Manney 1974b).

We wish to thank Lela Riley for isolating the mutants in Experiment 3.

LITERATURE CITED


Corresponding editor: F. Sherman