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Isolation of Sets of *a*, α, *a*/α, *a*/*a* and α/α Isogenic Strains in *Saccharomyces cerevisiae*

Charlotte Paquin and Julian Adams

Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109, USA

Summary. A simple, quick technique for isolating sets of *a*, α, *a*/α, *a*/*a* and α/α isogenic strains of the yeast, *Saccharomyces cerevisiae* is described. Isogenic *a*/α diploids arise in haploid populations by a rare heterothallic switch of mating type followed by mating of the switched cell with one of the other cells in the population. Sucrose density gradient centrifugation was used to select for large elliptical diploid cells in a population of smaller haploid cells, since diploid cells are larger and more oval than haploid cells. From an *a*/α diploid strain obtained in this manner, *a*/*a* and α/α cells were isolated by selecting for mating ability using a procedure similar to marker recovery. Finally isogenic *a* and α haploids were simply obtained by sporulation and dissection of an *a*/α isogenic diploid strain.

Key words: Haploid strains – Diploid strains – Mating type switch

Introduction

Isogenic strains are commonly used in genetic and biochemical studies to avoid complications and artifacts caused by differences in genetic background. Thus, techniques for the isolation of isogenic strains are useful to many investigators. In *Saccharomyces cerevisiae*, neither of the two methods of obtaining isogenic strains that have been described in the literature is completely satisfactory. One method (Hawthorne 1963), which we refer to as the forced-mating method, consists of the selection of prototrophic diploids from a mixture of two strains with the same mating type but complementing nutritional markers. This method has two disadvantages. The first is the difficulty of selecting appropriate nutritional markers in identical strains without the use of mutagens. The second disadvantage of this technique is that the strains obtained in this way are not truly isogenic because they carry two heterozygous nutritional markers from the parent strains.

The second method, which we call the micromanipulation method (Laskowski 1960), avoids those problems but is tedious and requires an experienced micromanipulator. This method consists of using micromanipulation to isolate large oval cells from a haploid population. Diploid cells are larger and more elliptical than haploids (e. g. Adams 1977), so that some of the large cells isolated in this way are expected to be *a*/α diploids. Such diploids arise spontaneously in haploid populations as a result of a rare heterothallic switch of mating type followed by mating of the switched cell with one of the other cells in the population (Hawthorne 1963; Strathern et al. 1979). The frequency of diploids in a haploid population has been estimated to be only about 1×10^{-6} (Hawthorne 1963), so that many cells have to be screened to isolate an isogenic diploid. In addition neither of these techniques provides a method of isolating isogenic *a*/*a* and α/α strains. Because of the drawbacks of the two techniques described above we have developed a new method of isolating isogenic strains that is simple, quick and allows the isolation of a complete set of isogenic strains, *a* and α haploids and *a*/α, *a*/*a* and α/α diploids.

The procedure consists of three stages as diagrammed in Fig. 1. I) The *a*/α diploids are isolated by enriching a haploid population for the larger, more elliptical diploid cells by means of a series of sucrose density gradient centrifugations. This technique is commonly used to fractionate populations of yeast cells on the basis of cell size (Lieblová et al. 1964; Thuriaux et al. 1978;

Offprint requests to: J. Adams

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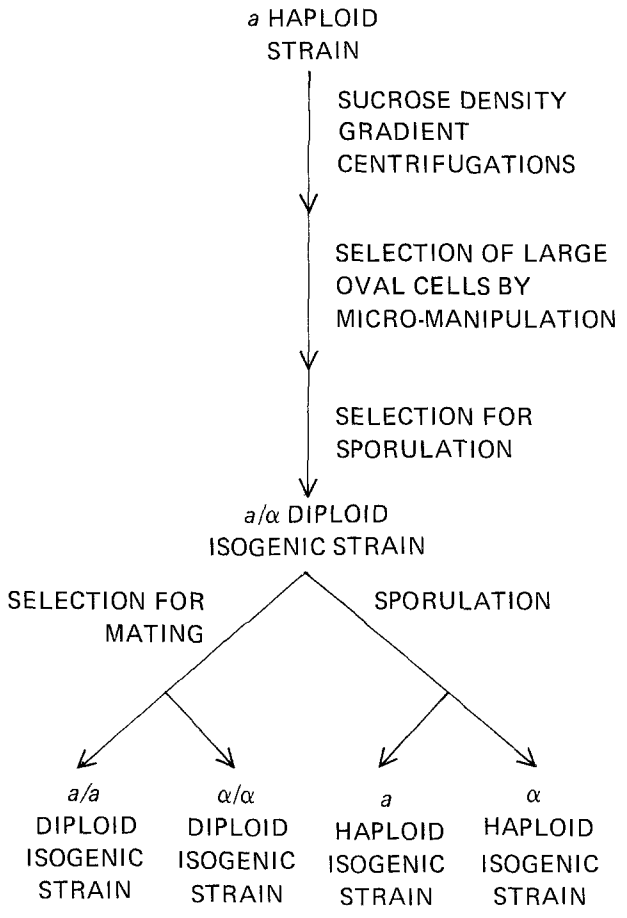

Fig. 1. Protocol for the isolation of a set of *a*, α, *a*/α, *a*/*a* and α/α isogenic strains

Sudbury and Carter 1980). We show here that it can be successfully used to enrich haploid populations for diploid cells by a factor of 10^4. II) Derivative *a*/*a* and α/α isogenic diploids, which arise in isogenic *a*/α populations by mitotic recombination, are isolated using an indirect selection procedure analogous to marker recovery, which assays for cells in the *a*/α population that can mate. III) *a* and α haploids isogenic to the *a*/α, *a*/*a* and α/α diploids are isolated simply by dissecting asci from an *a*/α isogenic diploid.

Materials and Methods

Yeast Strains. The strains used in this study are listed in Table 1.

Media. YEPD and sporulation media are described by Klar (1980). Minimal medium was the defined medium of Hansche and Adams (1974). Supplements to minimal medium were; canavanine: 0.2 mM canavanine sulphate; ethidium bromide: 10 μg/ml ethidium bromide. Glycerol minimal medium contained 2% (v/v) glycerol instead of glucose. Galactose indicator medium contained 2% (w/v) Bacto-peptone, 1% (w/v) Bactoyeast extract, 0.2% Galactose (crystalline Sigma grade) and 8.5 ml/liter of 0.4% (w/v) brom cresol purple in ethanol. 1.4% (w/v) agar was added for solid media.

Sucrose Gradient Centrifugation. The haploid strain from which diploid isogenic cells were to be isolated was inoculated into 10 ml YEPD and grown up overnight at 30 °C in a shaking water bath to a density of about 10^8 cells/ml. The cells were harvested by centrifugation at 1,200 rpm for 10 min at room temperature, the supernatant discarded, and the cells resuspended in 1 ml sterile distilled water. 0.1 ml of this suspension was then layered on top of a discontinuous sucrose density gradient, consisting of 5 ml 20% w/v sucrose, 4 ml 12.5% w/v sucrose and 4 ml 5% w/v sucrose. All solutions were made up in 0.85% NaCl, 0.06% Na_2HPO_4 and 0.3% KH_2PO_4 and filter sterilized. The density gradient was then centrifuged at room temperature at 600 rpm for 5 min, and 0.5 mls from the lowest visible layer of cells (usually just above the interface between the 12.5% sucrose and the 20% sucrose) was drawn off. This aliquot was used to inoculate an overnight culture of 10 ml YEPD to provide the cells for the next round of centrifugation. This centrifugation procedure can be repeated several times to enrich further for large cells.

Canavanine Test for Diploidy. Approximately 1×10^8 cells of the strain to be tested for diploidy were plated on a canavanine minimal plate and incubated at 30 °C for 5 to 7 days. Since canavanine resistance is a recessive marker, no canavanine resistant colonies should appear on the plate if the strain is diploid. Schild et al. (1981) describe a similar procedure, inducing mutations with UV.

Isolation of a/a and α/α Isogenic Strains. A diploid *a*/*a* isogenic strain was plated on YEPD plates at a density of approximately 300 cells per plate. Once colonies were apparent, these were replica plated onto minimal medium plates containing ethidium bromide to produce rho$^-$ cells, thereby marking the cells with the inability to respire, and allowed to grow up at 30 °C for two days. These plates were then replicated onto YEPD plates onto which lawns of either *a* (MH15) or α (110-2B) cells carrying multiple nutritional markers had previously been replicated. The strains were allowed to mate at 30 °C for 3 to 4 h and then refrigerated at about 3 °C overnight to increase zygote formation (Fowell 1969). Finally the mated cells were replicated to minimal glycerol plates to select for zygotes produced by mating between the strains. Colonies that grow on glycerol minimal media are scored, picked from the original master plates and retested for mating.

Sporulation and Micromanipulation. The general procedures are given in Mortimer and Hawthorne (1969). Klar's (1980) method of sporulating *a*/*a* and α/α cells was used. The *a*/*a* and α/α strains are mated to karyogamy defective JC7α and JC7*a* strains (*kar1*, Conde and Fink 1976) respectively and the resultant triploids sporulated. These *kar1* strains, when mated on an *a*/*a* or α/α diploid and sporulated, produce 6-spored asci in which the genetic information of 4 spores is derived from the nucleus of the *a*/*a* or α/α diploid and the genetic information in the other two spores is derived from the nucleus of the *kar1* cell (Klar 1980). Since the nuclei do not fuse there is no genetic exchange between the *a*/*a* or α/α diploid and the *kar1* haploid; therefore, the genetic data from the 4 spores derived from the *a*/*a* or α/α diploid parent may be analyzed as tetrads. These spores may be distinguished from the 2 spores from the *kar1* strain by the *leu1* marker carried by both JC7*a* and JC7α. Asci were dissected using Davidow et al. (1980)

Table 1. Yeast strains

Strain	Genotype	Source or reference
XC500A	*a gal2 mel mal*	YGSC*
XC500B	*α gal2 mel mal*	YGSC*
JC7a	*a kar1 leu1*	A. Klar (1980)
JC7α	*α kar1 leu1*	A. Klar (1980)
MH15	*a met14 ura3 his6 lys9*	J. Pringle
110-2B	*α gal3 trp1 met1 ura1*	YGSC*
110-2A	*a gal3 trp1 his1*	YGSC*
X3163-4C	*a gal2 met1 trp3 arg1 ade5 leu1 ura3*	YGSC*
X3163-2A	*α gal2 met1 trp3 arg1 ade5 lys7*	YGSC*
CP1AB	*a/α gal2/gal2 mel/mel mal/mal*	This paper
CP1AB-1AA	*a/a gal2/gal2 mel/mel mal/mal*	This paper
CP1AB-1BB	*α/α gal2/gal2 mel/mel mal/mal*	This paper
CP1AB-1A	*a gal2 mel mal*	This paper
CP1AB-1B	*α gal2 mel mal*	This paper

* Yeast Genetics Stock Center, Berkeley

Table 2. Sucrose density gradient centrifugation of *a* and α populations

Number of centrifugations	Large, Oval[a] cells in the populations	Diploids[b] obtained by micromanipulation	*a*/α Diploids[c] obtained by micromanipulation
a Populations (strains XC500A and CP1AB-1A)			
0	<0.1%	–	–
1	0.8%	1/25(4%)	0/25(0%)
2	2.7%	4/27(15%)	0/27(0%)
3	5.9%	9/30(30%)	0/30(0%)
4	13.1%	10/23(43%)	2/23(9%)
α Populations (strains XC500B and CP2AB-1B)			
0	<0.1%	6/86(7%)	0/86(0%)
1	7.0%	23/55(42%)	0/55(0%)
2	9.4%	27/54(50%)	0/54(0%)
3	12.4%	40/44(82%)	0/44(0%)
4	22.9%	17/20(85%)	0/20(0%)

[a] The number of large, oval cells in a population was estimated by the number of such cells seen in 1,000 cells counted on a hemocytometer

[b] The number of large oval cells isolated by micromanipulation and subsequently shown to be diploids by the lack of canavanine resistant mutants (see Materials and Methods)

[c] The number of large oval cells isolated by micromanipulation, that were subsequently confirmed to be sporulating diploids

The results shown in columns 3 and 4 are the combined data from four sets of centifugations of the *a* strains and five sets of centrifugations of the α strains

technique, in which each ascus is isolated by micromanipulation before the ascus wall is broken, to ensure that all 6 spores dissected belong to the same ascus.

Verification of Strain Genotypes: The marker *gal2* carried by the parental strain XC500A, was used to verify that the isogenic strains were descended from the parent strains and were not contaminants. The set of *a*/α, *a*/*a*, α/α, *a* and α isogenic strains, CP1AB, CP1AB-1AA, CP1AB-1BB, CP1AB-1A and CP1AB-1B were tested to see if they carried this marker. The *a*/α, *a*/*a* and α/α strains were sporulated and asci dissected, then spores from these strains and the *a* and α strains were allowed to mate with *a* and α *gal2* and *gal3* tester strains (110-2B, 110-2A, X3163-2A and X3163-4C) and tested for growth on galactose indicator plates. None of these strains fermented galactose and all complemented *gal3* but not *gal2* strains of the opposite mating type. Thus, all strains carry *gal2* and we conclude that they are derived from the parent strain XC500A.

Results and Discussion

Isolation of a/α Isogenic Diploids

The results shown in Table 2 demonstrate that repeated sucrose gradient centrifugations can be used to select for diploid cells in haploid populations. In both *a* and α populations the frequency of large, oval cells and the proportion of diploid cells (by the canavanine test) increased with the number of centrifugations. Taking both of these factors into account the frequency of diploids increased from less than 0.1% to approximately 20% in the α populations and from less than 0.1% to approximately 6% in the *a* populations (see Table 2) Using Hawthorne's (1963) estimation of the frequency with which diploids arise spontaneously in haploid populations (1×10^{-6}), this represents a 10^4 fold enrichment for diploids. Diploids selected by this procedure are not necessarily *a*/α diploids. In fact, of 128 diploids isolated and tested from the α haploid population, none sporulated indicating that they were not *a*/α diploids. The frequency of diploids from the *a* population was lower than that from the α population, but 6 out of 37 diploids isolated from the *a* population sporulated, indicating that they were *a*/α diploids. Of these six strains, one was tested further by dissecting asci and this strain showed 2*a*:2α segregation at the MAT locus for all 11 asci, as expected of an *a*/α strain. Furthermore, all spores were shown to be haploid by the canavanine test. This strain was designated CP1AB. These results suggest that different kinds of diploids occur in *a* populations than in α populations. Strathern et al. (1979) have shown that cells with a deletion of the mating type locus are weak *a* maters and so will be able to mate with α cells producing a class of *α/deletion* diploids that will not be present in *a* populations, because there are no α cells for the cells carrying the deletion to mate with. Therefore, a haploid *a* strain should

be used as the parent to isolate a set of isogenic strains. The frequency of a/α diploids after 4 centrifugations is large enough that an a/α diploid may be isolated by picking and testing colonies, without the need to resort to micromanipulation.

Isolation of a/a and α/α isogenic strains

Many of the diploids isolated by the sucrose density gradient centrifugation method are diploid by the canavanine test, mate with *a*'s or α's and do not sporulate, therefore they appear to be a/a or α/α diploids. When these strains were mated to *kar1* strains JC7*a* or JC7α and sporulated, however, they gave aberrant segregations of the mating type locus rather than the $4a:0\alpha$ and $0a:4\alpha$ segregations expected (the 2 *leu* spores from JC7*a* or JC7α in each ascus are ignored). Each of the 3 putative a/a's and 3 putative α/α's tested showed aberrant segregations. Thus isogenic a/a's and α/α's cannot be isolated easily by the sucrose gradient technique that is effective for isolating a/α diploids. On the other hand, a/a and α/α cells will arise by mitotic recombination in a/α populations, and may be selected for by virtue of their ability to mate. Two a/a's and six α/α's were isolated by the indirect selection procedure described in the Materials and Methods section. The frequency of occurrence of a/a and α/α cells amongst a/α cells was approximately 4×10^{-4}. The MAT locus is located 25 cM from the centromere of chromosome III (Mortimer and Schild 1980) and this frequency of occurrence of a/a and α/α diploids is consistent with mitotic recombination of such a locus (Roman 1956) All of the a/a's and α/α's isolated in this manner and tested showed the expected $4a:0\alpha$ and $0a:4\alpha$ segregations respectively. The a/a and α/α isogenic strains isolated by this procedure are designated CP1AB-1AA and CP1AB-1BB. Thus, while isogenic a/a and α/α strains are not easily isolated by the sucrose gradient technique, they can easily be obtained by isolating colonies that mate from an isogenic a/α strain.

Isolation of a and α Isogenic Strains

a and α haploid strains isogenic to the a/α, a/a and α/α isogenic strains isolated as described above may be obtained by sporulating the a/α (CP1AB) diploid, dissecting the resultant asci and testing the spores for mating type. The *a* or α haploid strains so obtained are designated CP1AB-1A and CP1AB-1B.

Conclusion

This paper describes a simple quick method of isolating a set of *a*, α, a/α, a/a and α/α isogenic strains without the drawbacks of the other methods currently available.

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