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Short Communication

An Enrichment Method for Temperature-Sensitive and Auxotrophic Mutants of Yeast

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Summary. An enrichment procedure that exploits the difference in heat-sensitivity between exponentially growing and stationary phase cells has been developed for the isolation of yeast mutants. Enrichments of up to 12-fold for temperature-sensitive lethal mutants and of up to 15-fold for auxotrophs have been obtained with single cycles of selection. Still higher enrichments (to frequencies of greater than 90% and 80% for temperature-sensitive lethals and auxotrophs, respectively) have been obtained with multiple cycles of selection. The method requires no special parent strain, and seems adaptable to the selection of a wide variety of types of mutants.

A number of enrichment procedures have been developed for the selective isolation of yeast mutants (Littlewood, 1975; Pringle, 1975; Henry and Horowitz, 1975; Henry et al., 1975; Piedra and Herrera, 1976; Young et al., 1976; Barclay and Little, 1977; Hardie and Dawes, 1977). In addition, many important mutants have been isolated by nonselective screening procedures (Pringle, 1975). However, both selective and nonselective procedures have serious limitations: the former may enrich effectively for only *some* of the mutants of interest, while the latter may fail to isolate some mutants of great interest that happen to occur in low frequency (Hartwell, 1967; Hartwell et al., 1973; Pringle, 1975). Thus, it is desirable to develop and exploit new isolation protocols that may select for mutants that have not previously been obtained. We report here a particularly versatile and powerful new method based on the fact that stationary phase yeast cells are much more resistant than exponentially growing cells to killing by heat (Schenberg-Frascino and Moustacchi, 1972; Parry et al., 1976).

Saccharomyces cerevisiae strain C276-4A*a* (Wilkinson and Pringle, 1974), a prototrophic haploid, was used as the wild type strain for initial experiments, and as the parent strain for all mutagenesis. It was maintained and handled as described elsewhere (Pringle, 1975; Pringle and Mor, 1975). Cells were grown either in the rich liquid medium YM-1 (Pringle and Mor, 1975), or in buffered YNB liquid minimal medium (Wilkinson and Pringle, 1974). Cultures were incubated in Erlenmeyer flasks with vigorous rotary shaking either at room temperature (23° C) or in a New Brunswick G76 water bath at 36° C. YEPD agar plates (Hartwell, 1967) were used to score viability and the per cent temperature-sensitive (*ts*) mutants, while YNB agar plates were used to score the per cent auxotrophs. Constant temperatures for heat-killing were maintained with a Haake Model E52 water bath. Mutagenesis with ethylmethane sulfonate was performed essentially as described by Fink (1970); survival was approximately 5%.

We reasoned that it might be possible to use heat-killing for mutant selection in the following way: if a mutant-containing population of stationary phase cells were diluted into fresh medium under conditions restrictive for the growth of some mutants, the wild type cells present would resume exponential growth and become heat-sensitive, while the mutants that were unable to grow might retain the heat-resistance characteristic of stationary phase cells. A properly timed exposure to heat might then result in a substantial selective mortality of the wild type cells.

The optimal conditions for the heat-killing would presumably be those that led to maximum mortality of growing cells, while permitting most stationary phase cells to survive. To determine these conditions, we measured the survival of strain C276-4A*a* after

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Table 1. Survival of exponentially growing and stationary phase cells of strain C276-4A*a* after exposure to various temperatures

Temperature (°C)	Exponentially growing cells[a]	Stationary phase cells[b]
52	77	83
55	5.5	78
57	0.27	77
59	0.10	38

[a] % survival after exposure for 5 min to the indicated temperature
[b] % survival after exposure for 10 min to the indicated temperature

exposure to various temperatures. From a culture growing exponentially at approximately 10^7 cells/ml in YM-1 medium at 36° C[1], 1 ml aliquots were transferred to prewarmed test tubes and held without agitation at various temperatures. In a parallel experiment, cells from a stationary phase culture that had been grown in YM-1 at 23° C were diluted to approximately 10^7 cells/ml in fresh YM-1 at 36° C; 1 ml aliquots were then immediately transferred to tubes held at various temperatures. In each experiment, samples were removed from each tube immediately after transfer, and at appropriate times thereafter; these samples were diluted, sonicated to break up clumps (Pringle and Mor, 1975), and plated to determine the numbers of viable cells. Representative results from these experiments are shown in Table 1, which illustrates both the temperature-dependence of heat-killing and the dramatic difference in heat-sensitivity between exponentially growing and stationary phase cells. Based on these data, a 7 min exposure to 57° C was chosen as standard conditions for heat-killing.

To determine how long the stationary phase cells should be incubated in fresh medium prior to heat-killing, we used mutagen-treated populations of C276-4A*a*. Cultures were grown to stationary phase in YM-1 at 23° C (conditions permissive for the growth both of *ts* mutants and of auxotrophs). After approximately 24 h in stationary phase, cultures were diluted 100-fold either with fresh YM-1 at 36° C (conditions restrictive for the growth of *ts* lethal mutants) or with fresh YNB at 23° C (conditions restrictive for the growth of auxotrophs). At 2 h intervals after inoculation, 1 ml aliquots were removed from each culture and exposed to 57° C as described above. Survival was determined at intervals during the exposures to 57° C by plating sonicated samples on YEPD plates at 23° C. As expected, the results (Fig. 1) show that the heat resistance characteristic of a stationary phase population is

[1] The growth temperatures used in these experiments were chosen to correspond to those used in our subsequent hunt for *ts* mutants

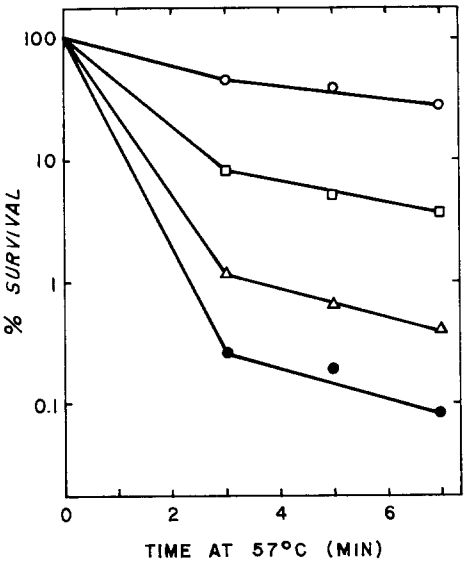

Fig. 1. Heat-killing during treatments at 57° C applied 0 h (○), 2 h (□), 4 h (△), or 6 h (●) after the inoculation of stationary phase cells into fresh YM-1 at 36° C

gradually lost as the population resumes growth in fresh medium. (Similar results were obtained with the population incubated in YNB, except that the loss of heat resistance occurred more slowly, as expected from the lower growth rate under these conditions.)

The colonies resulting from cells that had survived 7 min of heat-treatment in YM-1 or in YNB were replica-plated to YEPD plates at 36° C or to YNB plates at 23° C, respectively, in order to determine the proportions of *ts* lethal mutants or of auxotrophs among the survivors. Figure 2 shows that the mutant frequencies increased steadily with increasing times of incubation in fresh medium prior to the heat-treatment. After 10 h of incubation in YNB, the frequency of auxotrophs among survivors of the heat treatment was 30%. In other experiments (data not shown), we have used repeated cycles of this selection procedure to obtain frequencies of *ts* lethals and of auxotrophs in excess of 90% and 80%, respectively.

In addition to providing high yields of mutants, the enrichment procedure described here seems likely to prove extremely versatile. In contrast to some other powerful enrichment procedures (Henry and Horowitz, 1975; Henry et al., 1975; Barclay and Little, 1977; Hardie and Dawes, 1977), it does not require a special parent strain. Moreover, the method should be adaptable to the selection of various types of mutants (e.g., cold-sensitive mutants, respiratory-deficient mutants, or specific types of auxotrophs) merely by incubating the cells under the appropriate

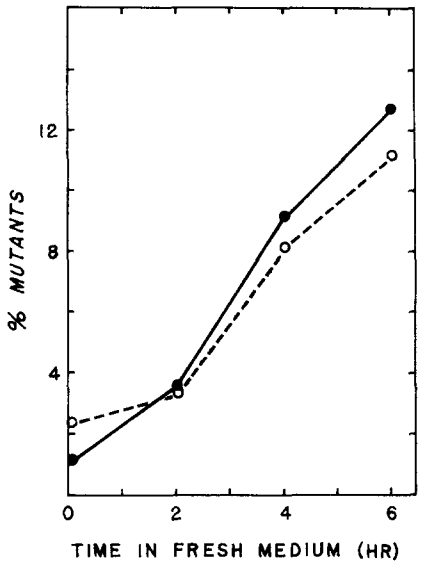

Fig. 2. Proportions of mutants among the survivors of heat treatments (7 min at 57° C) applied at various times after the inoculation of stationary phase cells into fresh medium. ●, per cent *ts* lethal mutants among survivors after incubation in YM-1 at 36° C. ○, per cent auxotrophs among survivors after incubation in YNB at 23° C

restrictive conditions prior to heat-killing. In applying this method, the following considerations should be kept in mind.

1. From the data in Fig. 1 and 2, it seems likely that the use of still longer incubations in fresh medium prior to heat-killing would result in still higher yields of mutants from a single cycle of selection. However, there are presumably mutants that die if incubated too long under conditions restrictive for their growth. Thus, the optimal duration of this incubation probably represents a compromise between the progressive increase in the yield of mutants (Fig. 2) and the presumed progressive decrease in the variety of mutants obtained, and may well vary depending on the goals of the particular mutant hunt.

2. Although the use of multiple cycles of selection results in very high yields of mutants, the variety of mutants obtained may be reduced significantly by the very strong selection operating not only during the incubations under restrictive conditions and subsequent heat-treatments, but also during the prolonged intervening periods of growth under conditions that may not be fully permissive. The greater time involved and the increased risk of contamination are additional drawbacks of a multiple cycle protocol.

3. The conditions selected by us for heat-killing (7 min at 57° C) were adapted to the requirements of a search for *ts* mutants, which involved the use of 36° C as a restrictive temperature. Since yeast cells grown at temperatures less than 36° C are much more heat-sensitive than cells grown at 36° C (Walton and Pringle, in preparation), heat-killing at a lower temperature (e.g., 52° C) should give satisfactory killing of wild type cells, with less risk of killing of interesting mutants, in searches for auxotrophs, cold-sensitive mutants, etc.

4. It is not yet clear what types of *ts* mutants will be obtained using this selection procedure. Mutants blocking energy metabolism or macromolecule synthesis will probably retain the properties of stationary phase cells and thus be selected. Moreover, the observation of a rough temporal coincidence between the onset of budding and the loss of heat-resistance suggests that, among cell cycle mutants, only those that arrest as unbudded, G1 cells (Hartwell et al., 1973) will be selected. Preliminary observations on the mutants obtained to date support these speculations, but more data are needed.

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