SHORT PAPERS

A METHOD FOR OBTAINING ANTIBIOTIC-SENSITIVE MUTANTS IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

We have isolated a temperature-sensitive mutant of *S. cerevisiae* which has proven useful for the isolation of antibiotic-sensitive strains.

OUR understanding of protein synthesis in yeast could be greatly extended if mutants were available which were drug-resistant by virtue of having an altered protein synthesizing system. To date, the only available mutants which are clearly of this type are certain cycloheximide-resistant mutants of *Saccharomyces cerevisiae* which contain an alteration in the 60S ribosomal subunit (GROLLMAN 1966; JIMENEZ, LITTLEWOOD and DAVIS 1972). The difficulty in obtaining a variety of such drug-resistant strains lies in the fact that a number of antibiotics which inhibit *in vitro* protein synthesizing systems from yeast, do not act *in vivo*, presumably because the drugs cannot get into intact cells. For example, emetine, fusidic acid and amicitin are ineffective against whole cells, although they inhibit polyU-directed polyphenylalanine synthesis in the yeast cell-free system (BATTANER and VASQUEZ 1971; JIMENEZ, LITTLEWOOD and DAVIES 1972). Thus, the isolation of yeast strains whose intact cells are sensitive to these antibiotics is the first step in the production of mutants which are drug-resistant because they possess altered protein synthesizing machinery. In the absence of an effective selection technique, the isolation of drug-sensitive mutants involves the screening of large numbers of colonies which consumes substantial amounts of costly and often scarce antibiotics.

We describe in this paper a technique for the isolation of antibiotic-sensitive strains of *S. cerevisiae*, using a novel temperature-sensitive mutant. *S. cerevisiae* BL15, nicknamed "kamikaze," grows normally at 32°C, but loses viability at 42°C. This loss of viability can be prevented by adding cycloheximide to the culture prior to the shift to the nonpermissive temperature. Thus, yeast mutants sensitive to an antibiotic can be selected for by adding the drug to a culture of BL15 and then shifting the culture to the nonpermissive temperature; this high temperature incubation is lethal to cells which are not inhibited by the drug.

This selection procedure allows one to select for mutants sensitive to any drug which inhibits protein synthesis either directly or indirectly. All that is required is that the antibiotic is not itself lethal to the sensitive cells.

MATERIALS AND METHODS

Isolation of BL15: *S. cerevisiae* BL15 α *his4 trp5* was isolated from an ethylmethanesulfonate-treated culture of Y166 α *his4 trp5* (obtained from Dr. H. O. HALVORSON), during the course of an experiment designed to select for temperature-sensitive mutants defective in protein synthesis. The unusual properties of BL15 at high temperature prompted us to investigate the use of this mutant for selection purposes.

Media: Complete (YEPD) and minimal media were prepared as described by FINK (1970).

Microbiological techniques: The techniques used were those described by FINK (1970). To measure the viability of a culture, the culture was serially diluted and 0.1 ml aliquots of several dilutions spread on Petri plates containing solid YEPD medium; the plates were incubated at 30°C and colonies which arose counted after two days.

Chemicals: Cycloheximide was obtained from Dr. G. B. WHITFIELD (Upjohn Co., Kalamazoo, Mich.), emetine hydrochloride from Mann Research Laboratories, New York and trimethoprim-lactate from G. H. HITCHINGS (Burroughs Wellcome and Co., Tuckahoe, N.Y.).

RESULTS AND DISCUSSION

Temperature-sensitivity of BL15: Typical viability curves of BL15 in liquid YEPD are shown in Figure 1. At 32°C, the strain grows normally, with a doubling time of about two hours. When a culture growing at 32°C is shifted to 42°C, the number of viable cells continues to increase for about 40 min and then sharply decreases. The cause of this loss of viability at 42°C is unknown; preliminary studies of ¹⁴C-leucine and ³H-uridine incorporation indicate that protein and RNA synthesis are normal at the nonpermissive temperature.

The addition of cycloheximide (20 μ g/ml) to a 32°C culture stops the cells from growing; the number of colony-forming units remains unchanged for over six hours (Figure 1). When a cycloheximide-inhibited 32°C culture is shifted to 42°C, again, the number of viable cells remains constant for several hours; cycloheximide prevents the loss of viability seen at 42°C in the untreated cultures.

Isolation of an emetine-sensitive mutant: The antibiotic emetine is known to inhibit polyU-directed polyphenylalanine synthesis in the cell-free system from yeast, although it is ineffective against intact wild-type cells of *S. cerevisiae* (JIMENEZ, LITTLEWOOD and DAVIES 1972).

An emetine-sensitive mutant was obtained from BL15 by a simple procedure. A culture of BL15 was mutagenized with ethylmethanesulfonate (FINK 1970), diluted 1:5 in liquid YEPD and grown overnight at 30°C. The culture was then diluted to a concentration of 3.9×10^7 cells/ml in YEPD containing emetine hydrochloride (100 μ g/ml) and grown at 32°C for one hour. At this time, the culture was shifted to 42°C for seven hours, which reduced the concentration of viable cells from 4×10^7 to 7×10^5 cells/ml. Cells from this culture were washed with water to remove the drug and plated on YEPD medium. Eight hundred of the resulting colonies were picked to YEPD plates (100 colonies/plate) and, when grown, replicated to YEPD plates containing 500 μ g/ml emetine-HCl. By this procedure, an emetine-sensitive mutant was obtained whose growth in liquid YEPD is completely inhibited by the addition of emetine-HCl at 80 μ g/ml. Tetrad analysis indicates that the emetine sensitivity of this strain results from two mutations.

FIGURE 1.—Viability curves of BL15 at 32°C and 42°C with and without cycloheximide. At time zero, a log phase culture in liquid YEPD at 32°C was divided in half and 20 $\mu\text{g}/\text{ml}$ cycloheximide added to one portion. After an additional hour at 32°C, the two cultures were again divided in half; half of each culture was shifted to 42°C.

Isolation of a trimethoprim-sensitive strain: Trimethoprim (TMP) is an inhibitor of dihydrofolate reductase in *E. coli* (MIOVIC and PRZER 1971); it inhibits protein synthesis indirectly. It does not affect intact wild-type yeast.

A TMP-sensitive mutant of *S. cerevisiae* was obtained from BL15 using the basic technique described above. The major alteration was that minimal medium, supplemented with histidine and tryptophan, replaced YEPD throughout the procedure. Prior to incubation at 42°C, TMP-lactate was added to the culture at a concentration of 200 $\mu\text{g}/\text{ml}$; colonies were tested by replication to minimal plates containing 300 $\mu\text{g}/\text{ml}$ of the drug. A TMP-sensitive strain was obtained which is inhibited in supplemented minimal medium by TMP-lactate at 100

$\mu\text{g/ml}$. Tetrads analysis indicates that this sensitivity to TMP results from a single mutation.

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LITERATURE CITED

- BATTANER, E. and D. VAZQUEZ, 1971 Inhibitors of protein synthesis by ribosomes of the 80-S type. *Biochim. Biophys. Acta* **254**: 316-330.
- FINK, G. R., 1970 The biochemical genetics of yeast. *Methods in Enzymology* **17A**: 59-78.
- GROLLMAN, A. P., 1966 Structural basis for inhibition of protein synthesis by emetine and cycloheximide based on an analogy between ipecac alkaloids and glutarimide antibiotics. *Proc. Natl. Acad. Sci. U.S.* **56**: 1867-1874.
- JIMENEZ, A., B. LITTLEWOOD and J. DAVIES, 1972 Inhibitors of protein synthesis in yeast. In: Symposium on molecular mechanisms of antibiotic action on protein biosynthesis and membranes. Granada (Spain), 1971, In press.
- MIOVIC, M. and L. PIZER, 1971 Effect of trimethoprim on macromolecular synthesis in *Escherichia coli*. *J. Bacteriol.* **106**: 856-862.