SHORT COMMUNICATIONS

Death of Nystatin-resistant Mutants of *Saccharomyces cerevisiae* during Refrigeration

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INTRODUCTION

Nystatin-resistant mutants of yeast are of interest because of their altered cell membranes (Woods, 1971) and their possible significance in medical treatment of yeast infections (Patel & Johnston, 1971). However, some nystatin-resistant mutants of *Saccharomyces cerevisiae* are unstable and are lost after storage in the refrigerator (Coulson, 1970). We have observed a rapid decrease in the percentage of viable resistant mutants present in a mixture of mutant and wild-type cells stored at 4 °C. This investigation was thus undertaken to determine whether the instability of certain mutants is due to death or reversion of these organisms during cold storage, or both.

METHODS

The mutants M9 and N95 were isolated in our laboratory from a haploid strain, x764-S1 (genotype α , *arg4*, *his6*, *hom2*, *ura3*) of *Saccharomyces cerevisiae*. The mutant *nys1* (Ahmed & Woods, 1967) was obtained from Dr R. A. Woods, University of Sheffield.

Growth medium (MYGP) comprised (g/l): malt extract (Oxoid), 3.0; yeast extract (Oxoid), 3.0; glucose, 10.0; bacteriological peptone, 0.5, and for plates 2% of agar (Oxoid No. 1) was added. Nystatin plates (40 units/ml) were made as needed by adding appropriate amounts of a fresh 10000 units/ml stock solution of nystatin (E. R. Squibb and Sons) in dimethyl sulphoxide (BDH Chemicals Ltd.) to MYGP medium.

Cultures were grown overnight in 50 ml flasks containing MYGP medium in an orbital shaker at 30 °C. They were then titered, diluted if necessary, and stored statically at 4 °C. Samples were removed periodically and suitable suspensions of cells spread upon MYGP plates. In some instances, colonies on these latter plates were replica-plated on to nystatin plates.

RESULTS AND DISCUSSION

Upon refrigeration at 4 °C at low yeast concentrations, all three nystatin mutants were found to die, although at different rates (Fig. 1*a*). The mutant *nys1* grew slightly during the first few days of storage and thereafter the cells died at a much slower rate than those of M9 and N95. The control strain, x764-S1, grew at a measurable rate at 4 °C, from 1×10^8 cells/ml to 4×10^7 cells/ml after 34 days, and gave a viable count of 2×10^7 cells/ml after 164 days.

When high cell-concentration cultures of M9, N95 and x764-S1 were stored at 4 °C, all of them showed a fall in viability with time (Fig. 1*b*). There were, however, distinct differences in their death rates. Mutant N95 died faster than did M9 whereas the viability of x764-S1 fell to about 10% of the initial value after 163 days.

When N95, M9 and x764-S1 were stored at 20 °C at a concentration of about 5×10^7

Fig. 1. Percentages of nystatin-resistant mutants and a nystatin-sensitive parent strain viable after storage in MYGP medium at 4 °C for different times. (a) Initial yeast concentration of approximately 10^8 cells/ml; (b) initial yeast concentration of approximately 10^7 cells/ml. ●, x764-s1; ○, N95; ▲, M9; △, *nysl*.

cells/ml, their viability fluctuated slightly with time but nevertheless decreased only marginally during 140 days.

Whether stored at 4 or 20 °C, all viable cells of mutants whose colonies were replica-plated on to nystatin plates gave rise to nystatin-resistant colonies. Therefore no sensitive revertants were detected in these experiments.

Genetical analysis of mutants M9 and N95 has shown that both carry single gene mutations for nystatin-resistance which segregate at a level of 40 units/ml. They also carry modifier genes that increase their resistance to higher concentrations of nystatin. Mutant M9 is resistant to 200 units/ml and N95 to 150 units/ml. The resistance genes in M9 and N95 have been shown to be non-allelic and probably linked to each other. They are both different genes from *nysl*.

Polyene antibiotics act by complexing with membrane sterols, thus causing severe disruption of the cell permeability mechanisms (Lampen, 1966). Polyene resistance has been shown to result from an alteration of sterols in the cell membrane of mutants (Molzahn & Woods, 1972). A possible explanation of the cold-sensitivity of some resistant mutants is therefore an altered permeability and increased leakage of essential ions from cells at lower temperatures.

These results show that some polyene-resistant mutants should be stored at room temperature and not in the refrigerator.

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