YEAST MUTANT REQUIRING ONLY A STEROL AS GROWTH SUPPLEMENT

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SUMMARY

A mutant strain of *Saccharomyces cerevisiae* which requires only a sterol for growth has been isolated. Sterol analysis suggests a block in ergosterol formation at the level of conversion of squalene to lanosterol. The fact that this mutant grows on glycerol but not on hemin shows that it is different from sterol requiring mutants previously isolated.

Ergosterol and 24(28) dehydroergosterol are the major sterols found in *Saccharomyces cerevisiae* and other species of yeast (1). Whilst their precise metabolic role is not clear, sterols are essential for the growth of yeast : during anaerobic growth sterols are not synthesized and must be supplied exogenously (2) ; yeast mutants requiring ergosterol under aerobic growth conditions have been isolated by Resnick and Mortimer (3), Karst and Lacroute (4) and Gollub et al (5). Such mutants have a pleiotropic phenotype : all are petite, several require in addition fatty acid or methionine. It has been shown that these mutants are blocked in porphyrin metabolism (6) leading to the loss of hematin enzymes which have been implicated in sterol biosynthesis (7).

It has been demonstrated that requirement for ergosterol is not stringent in mutants (4) or under anaerobic growth (8), since sitosterol or cholesterol can be used as growth supplement. Furthermore, when ergosterol in the medium is replaced by cholesterol or sitosterol, sterol-requiring mutants are more resistant to nystatin than wild type (4). Therefore we tried to enrich for sterol-requiring mutants by the positive selection of strains which are nystatin-resistant in the presence of cholesterol. Subsequent testing of these strains identified one mutant which requires a sterol for growth.

MATERIALS AND METHODS

Saccharomyces cerevisiae wild type FL 100 was mutagenized with nitrous acid to 1% survival. After growth in liquid minimal medium containing 10 µg/ml of cholesterol, the cells were spread on solid minimal medium containing 20 µg/ml of cholesterol and various concentrations of nystatin (9-20 µg/ml). Those strains which formed colonies after 3 days incubation at 30° were then tested for sterol auxotrophy and for resistance to nystatin on media containing ergosterol.

The genetic analyses were performed by the methods of Mortimer and Hawthorne (9). Difco Yeast Nitrogen Base without amino acids was used as minimal medium. The complete medium contained Yeast Extract (Difco) 1%, Bacto Peptone (Difco) 1% and glucose or glycerol 2%. Tergitol np40 (Sigma) was used to solubilize the sterols.

The non-saponifiable lipids were extracted and separated on Silica gel G plates as described previously (4).

RESULTS

Approximately 2000 strains resistant to nystatin in the presence of cholesterol were analyzed. Only one mutant was found to be nystatin sensitive on ergosterol and unable to grow on minimal or complete medium without a sterol. The efficiency of the enrichment procedure therefore seems very low.

Table 1 shows that hemin or oleic acid do not satisfy the growth requirement of this mutant. Among the precursors of ergosterol tested, only zymosterol and lanosterol relieve the auxotrophy, but lanosterol is a poor supplement compared to zymosterol, ergosterol or cholesterol. In minimal medium supplemented with 2 µg/ml of ergosterol or cholesterol the mutant has a doubling time of 195 min. After a shift to the same medium without sterol the growth rate stays the same for five hours and then slowly decreases (Figure 1). The effect of sterol concentration on the maximal cell yield reached in stationary phase has been studied (figure 2). It appears that 1.5 µg/ml of ergoste-

TABLE 1

Growth of wild type and the *erg*$_1$ mutant on supplemented minimal media

Supplement	FL 100	FK *erg*$_1$
None	+++	0
Oleic acid 100 µg/ml	+++	0
Hemin 30 µg/ml	+++	0
Ergosterol 20 µg/ml	+++	++
Cholesterol 20 µg/ml	+++	++
Zymosterol 20 µg/ml	+++	++
Lanosterol 20 µg/ml	+++	+
Squalene 20 µg/ml	+++	0

The table gives the growth estimated on solid medium. Tergitol np40 (1%) is added to solubilize the lipids.

rol or cholesterol is sufficient to obtain the maximum cell yield. This result is similar to that described by Proudlock et al (8), who showed that about 2 µg/ml sterol gives maximal cell yield in anaerobic cultures of wild type *Saccharomyces cerevisiae*. Figure 2 also shows that for growth of the mutant the concentration of cholesterol must be at least 0.1 µg/ml, whereas with ergosterol at this concentration the cell yield is 25% of the maximum possible. On the other hand, with saturating amounts of sterol, cholesterol gives a higher cell yield than ergosterol. The FK *erg*$_1$ mutant strain is able to grow almost normally on sterol-containing medium with glycerol as sole carbon source ; the strain therefore is not petite.

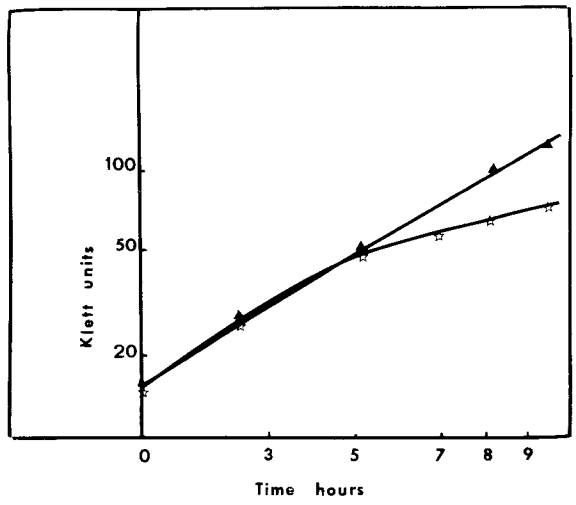

Figure 1. Growth of the FK *erg1* mutant on minimal medium supplemented with ergosterol (2 µg/ml) and after a shift to medium without sterol. The mutant is cultured overnight in minimal medium containing 2 µg/ml ergosterol ; at time zero an aliquot in exponential phase is filtered on a membrane filter (0.8 µ pore size), washed with medium and transfered to fresh medium with (▲-▲) or without (☆-☆) ergosterol.

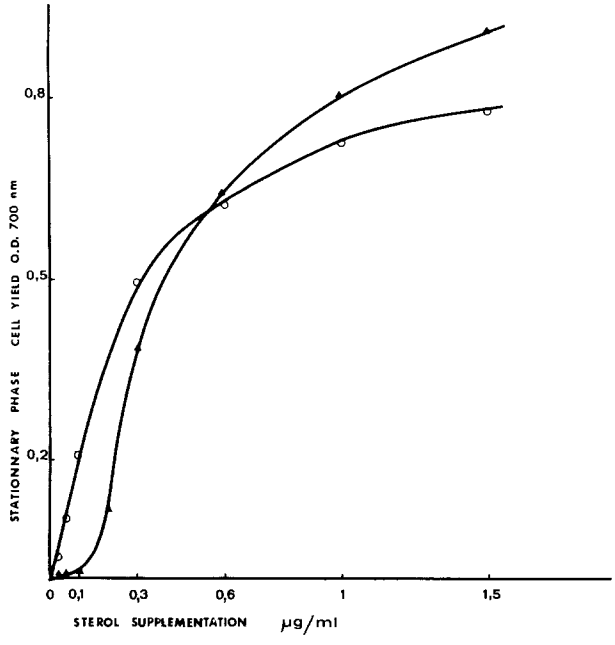

Figure 2. Effect of sterol concentration on the maximal cell yield of strain FK *erg1*. Cultures were inoculated at 2 µg dry weight/ml and sampled in stationary phase (after 48 h) (○-○) ergosterol, (▲-▲) cholesterol.

Heterozygotes produced by crosses between wild type and the mutant grow on minimal medium without sterol. Thus the mutation is recessive. Sporulation of the diploïds gives three types of segregants : in addition to strains with the parental phenotypes, there is a third class which grows, but very poorly, in the absence or presence of sterol. Hence it seems that a second mutation may be necessary for viability of the *erg1* mutant. Further genetic analysis is in progress, but is hindered by low viability of the spores. Complementation tests show that the *erg1* mutation is not allelic to any of the six complementation groups identified amongst the *olerg* mutants (4).

Lanosterol supports growth of the FK *erg1* mutant suggesting that sterol biosynthesis is blocked before lanosterol formation. The non-saponifiable lipids from the mutant give the same Liebeman Burchard colorimetric response (brown) as a sample of squalene. Chromatography on silica gel G plates of the non-saponifiable lipids from the mutant reveals a major band of material migrating with squalene, and a weak sterol band. Colonies of the *erg1* mutant strain have a yellow colour which could be produced by the accumulation of squalene. The amount of radioactive $|^{14}C|$ acetate incorporated into ergosterol and sterol precursors was measured in the FK *erg1* mutant strain. 98% of the radioactivity is recovered in the products migrating with squalene and only 1% in ergosterol and lanosterol (table 2). It appears from these data that the FK *erg1* mutant is blocked at the conversion of squalene to lanosterol.

DISCUSSION

Ergosterol is the only supplement required for growth by the FK *erg1* mutant described in this report. Therefore the FK *erg1* mutant is different from sterol mutants previously described since *ole* mutants (3,10), *olerg* mutants (4) and the *erg* mutants of Gollub et al (5) all require a fatty acid or methionine in addition to sterol. These mutants are petite and are believed to be blocked in porphyrin biosynthesis (6). The *olerg* mutant previously described are defective in sterol synthesis after lanosterol formation and accumula-

TABLE 2

Incorporation of [^{14}C] acetate into ergosterol and its precursors in the strains FL 100, FL 100 *olerg2-1*, FL 100 *olerg6-1* and FK *erg$_1$*.

Strains	Ergosterol	Lanosterol	Squalene
FL 100 (WT)	72	12	16
FL 100 *olerg2-1*	1	33	66
FL 100 *olerg6-1*	1	22	77
FK *erg$_1$*	1	1	98

Cells growing in logarithmic phase are harvested, washed and suspended in 0.1 M phosphate buffer containing 2% glucose and 1 µCi/ml of [^{14}C] acetate. The suspension is vigorously aerated during 2 hours. The non-saponifiable lipids are extracted, chromatographied as described. The results are expressed in percent of the radioactivity incorporated in the 3 fractions. This represents about 70% of the total radioactivity of the non-saponifiable lipids.

te both lanosterol and squalene. On the other hand, the FK *erg$_1$* mutant accumulates only squalene (table 2) and is therefore blocked in the conversion of squalene to lanosterol. This conversion is believed to be a two-step process (11, 12), the first being conversion of squalene to 2,3-oxidosqualene by an oxidase requiring oxygen and NADPH (13) ; the second step is conversion of this intermediate by the 2,3-oxidosqualene-lanosterol cyclase to lanosterol (14). We are trying to discover if either of these activities is defective in the mutant FK *erg$_1$*.

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