BIOSYNTHESIS OF YEAST MITOCHONDRIA. DRUG EFFECTS ON THE PETITE NEGATIVE YEAST KLUYVEROMYCES LACTIS.

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Summary

Ethidium bromide, acriflavine, chloramphenicol, erythromycin and nalidixate selectively inhibit growth of Kluyveromyces lactis on a non-fermentable substrate. Cells are able to recover respiratory activity after ethidium bromide treatment. Ethidium bromide, acriflavine and nalidixate considerably lower the level of mitochondrial DNA.

The biosynthesis of mitochondria has been extensively studied in the yeast Saccharomyces cerevisiae. We report here studies carried out on the effect of drugs on the growth ability, respiratory activity, and mitochondrial DNA content of the petite negative (1, 2) yeast, Kluyveromyces (Saccharomyces) lactis.

Materials and Methods

Yeast: The yeast used was a strain derived from K. lactis NRRL Y-1140 which was kindly donated by Dr. A. Herman (3).

Growth conditions: Basic liquid media contained 1% $NH_4H_2PO_4$, 0.2% $MgSO_4.7H_2O$, 0.5% Yeast Extract (Oxoid) and either glucose 0.2M or glycerol 0.4M as carbon source. Solid media contained 1% Yeast Extract (Oxoid), 1% bacteriological peptone (Oxoid), 1.5% Agar (Oxoid No. 3) and either 1% glucose or 1% glycerol. Liquid cultures were grown in 100ml lots in 250ml Erlenmeyer flasks shaking at 250 rpm in a Gallenkamp Orbital Incubator at 30°. Plates were incubated at 30°. Petite mutants were screened for using the tetrazolium overlay technique (4) after 3 or 4 days of colony growth.

Chemicals and drugs: Chemicals used were of the purest grades available throughout. Ethidium bromide was a gift from Boots Pure Drug Co., Nottingham,

England. Acriflavine (euflavine) was a gift from Dr. S. Nagai. Nalidixic acid was used as the sodium salt and was prepared by neutralisation of nalidixic acid (Calbiochem) with sodium hydroxide. Chloramphenicol and erythromycin were purchased from Sigma Chemical Co., London.

Respiration measurements: Oxygen uptake of the cell cultures was determined using a Clark type oxygen electrode (Rank Instruments, Bottisham, Cambridge). Cell density was determined either by direct counting on a haemacytometer or by turbidity measurements and a previously constructed standard curve using a Unicam SP500 spectrophotometer at 550nm. Respiration rates are given as $QO_2 \equiv \mu l\ O_2$ taken up/mg. dry wt./hr.

DNA extractions: These were performed according to the method of Williamson et al. (5).

Analytical ultracentrifugation: This was performed according to Schildkraut et al. (6) using a Beckman Model E analytical ultracentrifuge equipped with a four cell rotor and UV absorption optics. Negatives were scanned with a Joyce Loebl recording microdensitometer. Buoyant densities were computed according to Sueoka (7) using Micrococcus luteus (lysodeikticus) DNA, density 1.731 g/cc as marker.

Results

The effect of drugs on glucose and glycerol growth of K. lactis: The effects on the growth of K. lactis on fermentable and non-fermentable carbon sources, of the petite mutagens acriflavine and ethidium bromide, the mitochondrial protein synthesis inhibitors erythromycin and chloramphenicol and an inhibitor of DNA replication nalidixate were investigated. Fig. 1 shows that both ethidium bromide and acriflavine specifically inhibit glycerol growth at very low concentrations. Slight inhibition of glucose growth occurs under these conditions - a rise in the mean generation time from 1.3 to 1.9 hrs. This result can be explained in terms of a switch from oxidative to fermentative metabolism. Erythromycin exhibits a similar effect, but at much higher concentrations and chloramphenicol is apparently beginning to show the same

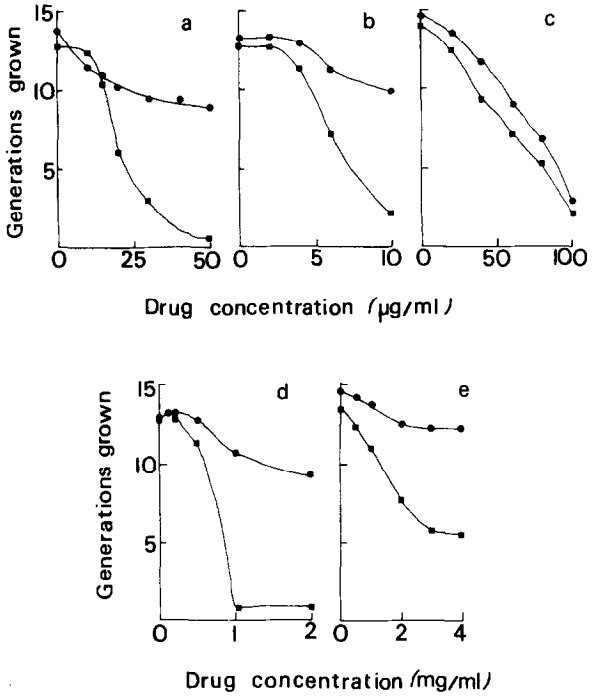

Fig. 1. Effects of drugs on the glucose and glycerol growth of K.lactis. Cells were inoculated at low cell density into flasks containing various concentrations of drugs. Flasks were incubated for 18–24 hrs. The cell densities were determined by haemacytometer counts. Results are expressed as the number of cell-division cycles undergone by the yeast.
a) acriflavine b) ethidium bromide c) nalidixate d) erythromycin
e) chloramphenicol. ●——● glucose ■——■ glycerol.

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effect but is limited by its solubility. In contrast, nalidixate exerts a selective effect on glycerol growth as growth on glucose is considerably inhibited too.

Growth in and recovery from ethidium bromide: K. lactis will grow in glucose medium containing 10μg/ml ethidium bromide apparently indefinitely. In particular we have followed the growth for 40 generations. During this time the mean generation time rapidly rises to 1.9 hrs and the QO_2 of the culture falls from a control value in the region of 200 to about 2. The residual respiratory activity is completely insensitive to cyanide. If such cultures are plated they fail to grow on glycerol medium but colonies develop on glucose medium and when tested by the tetrazolium overlay method or by determination of respiratory activity are found to be respiratory competent. No

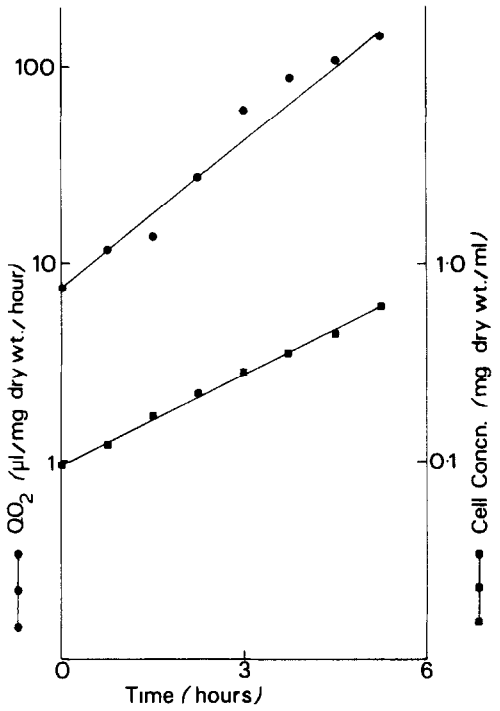

Fig. 2. Recovery of respiratory activity after growth in ethidium bromide. Cells were inoculated into glucose liquid medium containing 100µg/ml ethidium bromide. After nine generations of exponential growth, a sample was taken, washed free of ethidium bromide and used to inoculate fresh, pre-warmed glucose medium containing no drug. The culture was incubated under the usual growth conditions. Samples were taken periodically for determination of dry weight and respiration rate.

petite colonies have been detected and selection of viable respiratory competent cells by plating is considered unlikely since plate counts and haemacytometer counts are in agreement. Further evidence for this view is shown by the experiment detailed in Fig. 2. In this case cells growing exponentially in ethidium bromide have the ability to regenerate mitochondrial activity after transfer to fresh medium in the absence of the drug. The cells continue to grow exponentially after the transfer. Obviously, if a fraction of the cells were petite mutants the rate of adaptation to respiration per cell would be even greater. These experiments do not, however, rule out the possibility that a small fraction of the cells become petite and inviable as suggested by Bulder (1).

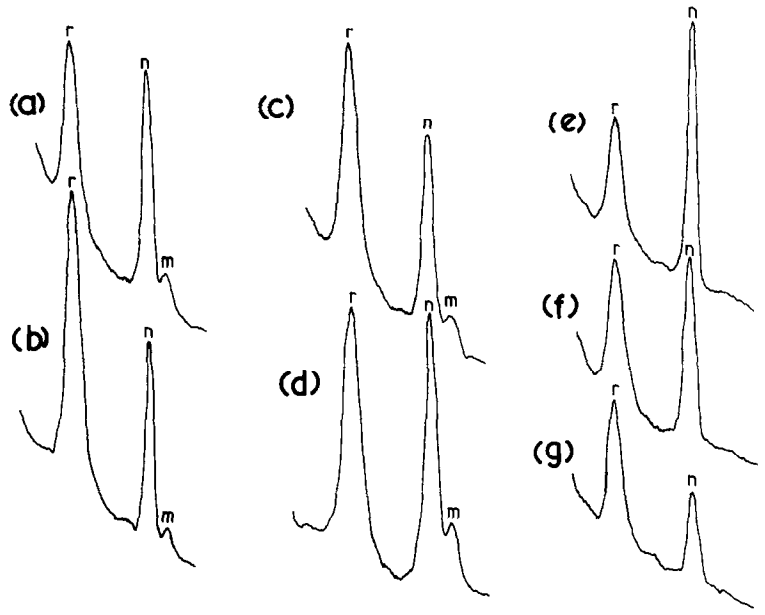

Fig. 3. The effect of drugs on the mitochondrial DNA content of K.lactis. DNA extractions and analytical ultracentrifugation were carried out as described. Growth conditions were as follows:
a) glucose b) glycerol c) glucose + 4mg/ml chloramphenicol
d) glucose + 2mg/ml erythromycin e) glucose + 100μg/ml nalidixate
f) glucose + 40μg/ml acriflavine g) glucose + 10μg/ml ethidium bromide.
m, n and r are mitochondrial, nuclear and reference (M.luteus) DNA respectively.

Mitochondrial DNA content of K. lactis: The effects of drugs on the mitochondrial DNA content of K. lactis is shown in Fig. 3. Cells grown in the absence of drugs in both glucose and glycerol medium show the presence of two DNA components. The major component bands at a buoyant density of 1.699 g/cc and the minor band at 1.691 g/cc. By analogy with other systems and the results of Smith et al. (8) these components are identified as nuclear and mitochondrial DNA respectively. Cells grown in glucose in the presence of chloramphenicol or erythromycin at concentrations which completely prevent glycerol growth both contain mitochondrial DNA in more or less normal amounts. In contrast cells grown in the presence of ethidium bromide, acriflavine or nalidixate contain no mitochondrial DNA which can be detected by this method.

The previous demonstration that K.lactis is able to recover respiratory activity following removal from conditions which eliminate most of the mito-

chondrial DNA suggests that such recovery experiments might prove valuable in the study of mitochondrial DNA biosynthesis.

Discussion

Bulder (1) suggested that petite negative yeasts could not form viable petite mutants, because such species were unable to obtain sufficient energy for growth from fermentation. This interpretation was challenged by DeDeken (2) who found that a number of petite negative species could grow exponentially in a concentration of acriflavine sufficient completely to inhibit respiratory enzyme synthesis. This view is confirmed and extended by these observations. Not only can K.lactis grow for 40 generations in the presence of a repressing concentration of ethidium bromide, but it can regenerate mitochondrial activity when removed from the presence of the drug.

The maintenance of mitochondrial DNA under chloramphenicol and erythromycin repression is consistent with their known effects as protein synthesis inhibitors of bacterial and plastid systems. In the light of recent ideas concerning the existence of mitochondrial control systems (9), it is clear that mitochondrial protein synthesis is not required for initiation of mitochondrial DNA synthesis. The loss of mitochondrial DNA during treatment with ethidium bromide, acriflavine and nalidixic acid is of considerable interest. There are a number of possible explanations. It is possible that ethidium bromide, acriflavine and nalidixate, all DNA synthesis inhibitors, set up a new and very low steady state concentration of mitochondrial DNA. We know of no specific mechanism whereby, under these circumstances, the passage of a mitochondrial genome to the daughter cells could be ensured. One would expect to find that a significant fraction of daughter cells would give rise to mitochondrial DNA-less petite clones. Two versions of the master copy hypothesis, originally suggested by Wilkie (10) may also be considered. The first possibility would be that a master copy of mitochondrial DNA remains in the cytoplasm. In the case of K.lactis this would require the existence of a mechanism by which all daughter cells received a copy of mitochondrial DNA.

A second possibility is that a master copy of mitochondrial DNA remains in the nucleus, which would explain how the genetic information could be precisely maintained. Cohen et al. (11) and Fukuhara (12) have presented evidence that in S.cerevisiae there is some base sequence complementarity between nuclear and mitochondrial DNA. The loss of the nuclear complement following cytoplasmic petite mutation would support the idea of a nuclear master copy. The authors do point out, however, that the level of complementarity is close to the limit of detection.

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