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Isolation and Characterization of Mitochondrial DNA from the Alkane Yeast *Saccharomycopsis lipolytica*

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Summary. Mitochondrial (mt) DNA of the alkane yeast, *Saccharomycopsis lipolytica,* was isolated. Its buoyant density in CsCl was found to be of 1.687 g/cm^3, indicating a GC content of 27.5% and its melting point T_m = 79.5 °C, indicating a GC content of 24.9%. The corresponding values for nuclear (n) DNA, are 1.709 g/cm^3 (GC: 49.5%) and T_m = 90.5 (GC: 51.7%) respectively. Electron microscopy revealed that mtDNA has a circular structure with a contour length of about 14.5 μm corresponding to 45.5 kb per molecule. The size estimated from restriction analyses performed with 7 endonucleases was 48.35 kb/molecule. A restriction map was constructed, using the cleavage data of 4 endonucleases.

Key words: *Saccharomycopsis lipolytica* – mtDNA

Introduction

Saccharomycopsis lipolytica (formerly *Candida lipolytica*) is a petite negative yeast which is able to grow on alkanes as a sole carbon source. Therefore, it is used in biotechnology for the production of single cell protein (Knecht et al. 1977). In contrast to many other organisms used for this purpose, *Saccharomycopsis lipolytica* can be manipulated genetically via a sexual cycle (Wickerham et al. 1969, 1970; Gaillardin et al. 1973; Esser and Stahl 1976). This facilitates strain improvement. To widen the genetic basis for breeding it seems desirable to investigate extrachromosomal genetic information, such as mitochondrial (mt) DNA, especially because this petite negative yeast depends on the chondriome for energy production. A clarification of the structure of mtDNA provides the possibility of using isolated DNA for transformation experiments which, to our knowledge have been performed up to now only with intact mitochondria (Ferenczy and Maraz 1977; Gunge and Sakaguchi 1979). In addition, a comparison with other yeasts would be of interest because both the length and the GC content of mtDNA are quite different in the two well studied species *Saccharomyces cerevisiae* (25μm, 24.5%; Hollenberg et al. 1970) and *Schizosaccharomyces pombe* (8.5 μm, 29.6%; Tabak and Weijers 1976).

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Material and Methods

Strains: Wild-type strain H 5027 *Saccharomycopsis lipolytica* was kindly given to us by Dr. P. Präve (Hoechst A.G., Frankfurt, FRG). According to Esser and Stahl (1976) this strain is haploid and carries the minus mating type. Two other wild-type strains were kindly furnished by Prof. Fiechter (Zürich): KW2 (minus) and KW4 (minus). Bacteriophage lambda DNA was isolated from *E. coli* K12 (λ, cI857, Sam7) obtained from W. Rüger (Bochum).

Media and Culture Conditions

Cells of *S. lipolytica* were grown aerobically at 28 °C in yeast extract (YE) medium (2% yeast extract, 0.5% glucose, pH 7.3) and were harvested in log. phase (growth time 24 h).

Spheroplast Preparation

Yeast cells were harvested by centrifugation (8,000 x g, 10 min, 4 °C) and spheroplasts were prepared by the method of Cryer and coworkers (1975). Spheroplasts were used for isolation of mitochondria and DNA.

Isolation of Mitochondria and mtDNA

Spheroplasts were suspended in SET-1 buffer (0.25 M sucrose, 50 mM EDTA, 100 mM tris, pH 7.5) and homogenized with a

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Teflon homogenizer. Homogenates were centrifuged (2,500 × g, 5 min, 4 °C) to remove cell debris. Mitochondria in the supernatant were centrifugend again (12,000 × g, 20 min, 4 °C) and the resulting pellet of mitochondria was resuspended in SMT buffer (0.5 M sucrose, 5 mM $mgCl_2$, 20 mM tris, pH 7.5) plus 200 µg DNAse per ml. After incubation (30 min, 0 °C) diethylpyrocarbonate was added (final concentration 0.05%) for DNAse inhibition. Mitochondria were pelleted by centrifugation (12,000 × g, 20 min, 4 °C) and resuspended in SET-2 buffer (0.44 M sucrose, 50 mM EDTA, 0.1 M tris, pH 7.5), followed by a sucrose density gradient according to Tudzynski and Esser (1979) for purification. The mitchondrial band was reisolated and diluted with three volumes of TE buffer (10 mM tris, 1 mM EDTA, pH 8.5). The mitochondrial pellet obtained by centrifugation (12,000 × g, 20 min, 4 °C) was suspended in 4% SDS dissolved in TES buffer (50 mM tris, 50 mM NaCl, 5 mM EDTA, pH 8) and lysed for 15 min at 60 °C. After incubation with proteinase K (0.25 mg/ml, 90 min, 4 °C) mtDNA was dialysed against TES buffer and its buoyant density determined in the analytical ultracentrifuge.

Analytical Ultracentrifugation

All mtDNA, isolated by preparative ultracentrifugation or by lysing of isolated mitochondria, was identified by analytical ultracentrifugation in a Beckman model E. Buoyant densities were calculated according to Szybalski (1968) in using *M. lysodeikticus* DNA as marker (1.731 g/cm^3).

Melting Analysis

Both nDNA and mtDNA were isolated, and thermal denaturation midpoints were determined according to Stahl and Esser (1979). DNA base compositions were calculated using the formula of Marmur and Doty (1962).

Large Scale Preparation of mtDNA

Method I. Mitochondrial DNA was isolated from spheroplasts by the method of Stahl and coworkers (1980).

MtDNA fractions were monitored by analytical CsCl density centrifugation and dialysed against TE buffer. Purified mtDNA was used for restriction enzyme analysis.

Method II. Yeasts spheroplasts were lysed as by Stahl and coworkers (1980). From the cleared lysate DNA was precipitated by polyethyleneglycol 6,000 (Humphreys et al. 1975). The resulting pellet was dialysed for 36–48 h against TES buffer. DNA was centrifuged to equilibrium in a CsCl ethidium bromide (EB) gradient in a VTi 65 vertical rotor (45,000 rpm, 21 h, 15 °C). The nDNA and mtDNA bands were visualized under UV light and isolated from the top of the tube with a pasteur pipette to avoid shearing of DNA molecules. Bound EB was extracted with isopropanol and mtDNA dialysed for 12–18 h against TES buffer was used for electron microscopic preparations.

Electron Microscopy

For critical electron microscopy studies of mtDNA the droplet method of Land and Mitani (1970) was adopted. In routine experiments the ammonium acetate technique of Davis et al. (1971) was used. Shadowing, measuring and calibrating was done according to Stahl and coworkers (1980). PM2 DNA (3.22 µm according to Stüber and Bujard 1977) was used as internal length standard.

Restriction Endonuclease Analysis

Restriction endonucleases *EcoR*I, *Hpa*II, *Pst*I, *Sal*I, *Bgl*II, *Hind*III (Boehringer, Mannheim, FRG) and *Hae*III (New England Bioblabs, Boston MA, USA) were used in these studies; buffer systems used were as specified by the manufacturers. For double digestion of mtDNA with restriction endonucleases, conditions of the enzyme requiring the lower salt concentration were used. Incubation time was 4–6 h. Reactions were stopped by addition of 0.25 volumes of 60% sucrose solution, containing 20 mM EDTA and 0.025% bromphenol blue. Electrophoretic separations were done in horizontal agarose (Seakem, Marine Colloids) slab gels (McDonell et al. 1977) with 1.0% or 0.4% agarose, respectively, in tris acetate buffer (Palmer et al. 1979), and were run at 100 mA for 15 h at room temperature or in tris borate buffer (Meyers et al. 1976), at 50 mA for 15 h. Gels were stained with ethidium bromide (1 µg/l). Molecular weights of fragments were calculated from relative mobilities in agarose of *Hind*III and *Sal*I generated fragments (0.4% gels) of lambda DNA as internal length standard (Murray and Murray 1975; Arrand et al. 1978). The physical map was constructed from single and double digestions of mtDNA with endonucleases *EcoR*I, *Hpa*II, *Pst*I and *Sal*I. Different fragments isolated from preparative agarose gels by the freeze and squeeze method of Thuring and coworkers (1975) were analysed by a second digestion.

Results and Discussion

To obtain highly purified mtDNA of *S. lipoytica* for further characterization the following sequence was used: cells were harvested after aerobic growth, treated with zymolyase to release spheroplasts, and homogenized. Mitochondria were isolated and purified and mtDNA was obtained after mitochondrial lysis.

Buoyant Density

Highly purified mtDNA from strain H 5027 was assayed for buoyant denstiy in a CsCl gradient. For comparison mtDNA not treated with DNAse prior to mitochondrial lysis and also bulk DNA were submitted to the same procedure (Fig. 1). The bulk DNA profile exhibits a major peak at 1.709 g/cm^3 and a minor peak at 1.687 g/cm^3. The inverse picture is obtained with mtDNA not treated with DNAse. The highly purified mtDNA shows only the peak at 1.687 g/cm^3 which, therefore represents mtDNA. The other peak predominant in the bulk DNA represents nuclear DNA.

From repeated experiments standard deviations of buoyant densities were calculated to be as follows: mtDNA 1.6874 ± 0.0017 g/cm^3 (n = 14) and nDNA 1.7091 ± 0.0013 g/cm^3 (n = 10) giving to GC contents of 27.5% and 49.5%, respectively. this confirms the find-