A Modified Procedure for Isolation of Yeast Mitochondrial DNA

Trayana Nedeva[a]*, Ventzislava Petrova[a], Tsonka Hristozova[b] and Anna Kujumdzieva[a]

[a] Department of General and Industrial Microbiology, Faculty of Biology, The Sofia University "St. Kliment Ohridski", 8 Dragan Tzankov St., 1164 Sofia, Bulgaria
[b] Institute of Microbiology, Bulgarian Academy of Sciences, 26 Acad. G. Bonchev St., 1113 Sofia, Bulgaria. Fax: +35 92 66 86 19.
E-mail: nedeva@biofac.uni-sofia.bg

* Author for correspondence and reprint requests

Z. Naturforsch. **57 c**, 960–961 (2002);
received June 6/August 9, 2002

mtDNA, RFLP, Yeast

A modified, rapid and inexpensive method for preparation of mitochondrial DNA (mtDNA), suitable for molecular analysis is proposed. It comprises batch cultivation of *Saccharomyces cerevisiae* strain NBIMCC 583 on a simple nutrient medium at 28 °C; permeabialization of cells from late exponential growth phase with cetyltrimethylamonnium bromide, mechanical disintegration of the cell wall; preparation of a mitochondrial fraction and subsequent isolation and purification of mtDNA. The amount and the purity of the obtained mtDNA have been checked and its application for molecular analysis proven.

The main advantages of the proposed procedure for isolation of mtDNA are introduction of simple nutrient medium, replacement of the enzymatic lysis of the cell wall by the cheaper mechanical one, avoidance of ultracentrifugation steps and use of harmful chemical substances.

Several molecular methods for identification of different *Saccharomyces cerevisiae* strains, based on polymorphism of mitochondrial DNA (mtDNA), have been applied in recent years. These techniques require efficient methods for mtDNA isolation. Most of the simplified procedures proposed include enzymatic digestion of the cell wall for release of the intracellular components (Defontaine *et al.*, 1991) and /or a step of ultracentrifugation (Fox *et al.*, 1991; Querol and Barrio, 1990). The present work proposes a modified, rapid and inexpensive method for preparation of mtDNA, suitable for further molecular analyses, by using mechanical disruption of the cell wall.

A *Saccharomyces cerevisiae* strain NBIMCC 583, provided by Bulgarian National Bank for Industrial Microorganisms and Cell Cultures, was used in this study. A batch cultivation was performed at 28 °C on a rotary shaker in a medium containing 2% glucose, 0.5% yeast extract and tap water, pH 6.0. It has been proven that the simple nutrient medium, composed for this investigation, provokes fast growth of yeasts and facilitates mtDNA isolation due to the formation of large amounts of mitochondria and cell wall composition enhancing its disruption. The cells were harvested in late exponential phase (8 h of cultivation) by centrifugation at 2200 × *g* for 10 min and were washed twice with sterile distilled water. Fresh cell biomass (0.4–0.5 g) was suspended in 1 ml 0.4% cetyltrimethylamonnium bromide (CTAB) in disintegration buffer containing 0.5 M sorbitol, 10 mM EDTA, 50 mM tris[hydroxymethyl]aminomethane-HCl, pH 7.5. The cells were kept in this solution for 10 min at room temperature and then separated by centrifugation at 2200 × *g* for 10 min. The permeabilized pellet was mixed with glass beads (0.15–0.2 mm) and the same buffer in a 1:2:1 ratio and subjected to mechanical disruption in a vibration homogenizer VHG 1 (B. Braun, Biotech International Melsungen, Germany) (three 5-min cycles). A control experiment with the same amount of fresh biomass was performed for isolation of mtDNA following the procedure of Defontaine *et al.* (1991), which comprises enzymatic lysis of the cell wall before mtDNA purification. Afterwards, both crude extracts were centrifuged at 1000 *g* for 10 min to remove cell debris.

The efficiency of the mechanical disruption procedure was evaluated through measurement of the total protein content in the cell free extract, which was estimated as 29 ± 1 mg/ml. At the same time enzyme digestion gave a protein concentration of the cell free extract of 25 ± 1 mg/ml. The comparison between both procedures indicated that the mechanical one is 15% more efficient. Also, about 95.0% disrupted cells were observed microscopically. The cell free extract thus obtained was transferred to microcentrifuge tubes and the mitochondrial fraction was harvested at 16000 × *g* for 20 min. The crude mitochondrial pellet was care-

fully washed three times with disintegration buffer to remove mainly the genomic DNA. The washed mitochondria were sedimented again at 16000 × *g* for 10 min and lysed following the procedure of Defontaine *et al.* (1991). The further purification of mitochondrial DNA obtained through mechanical disintegration proceeded as follows: half volume of 5 M CH_3COOK was added and the mixture was left for 10 min on ice for deproteinization. After centrifugation at 16000 × *g* for 10 min, the supernatant containing mtDNA was mixed with an equal volume of iso-propanol. The mixture was incubated for 10 min at room temperature and the mtDNA pelletted by centrifugation at 11000 × *g* for 10 min, washed with chilled ethanol, dried and resuspended in 10–20 μl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). When not indicated, all procedures were carried out at 4 °C.

The treatment of the enzymatically obtained cell-free extract and the purification of mtDNA were performed according to Defontaine *et al.* (1991).

The amount of mtDNA as well as its purity were quantified spectrophotometrically. Mitochondrial DNA obtained was 50 ± 2 μg/0.5 g fresh biomass and 26 ± 0.9/0.5 g fresh biomass for mechanical and lytic cell wall disruption variants, respectively. Thus, a 2-fold higher concentration of mtDNA, obtained through the proposed procedure, has been achieved. The absorbance ratio $OD_{260/280}$ for both samples was in the range 1.8–1.9. The mtDNA obtained by the mechanical procedure was subjected to RFLP analysis with Eco*RV*, and the results were compared with those for mtDNA isolated according to the control protocol of Defontaine *et al.* (Fig. 1). It can be seen that the mtDNA isolated through the proposed modified procedure yields a digestion profile (Fig. 1, lane 3) comparable with the control (Fig. 1, lane 2).

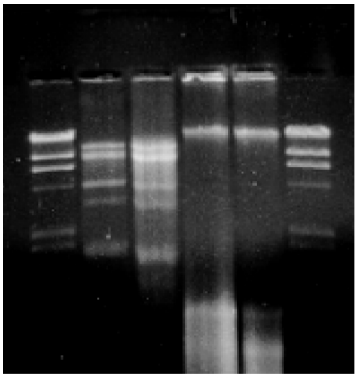

Fig. 1. RFLP analysis of mitochondrial DNA isolated after mechanical disruption of the cell wall and after enzymatic digestion. Lanes 1 and 6: λ phage Hind*III* restriction profile; lanes 2 and 4: digested with Eco*RV* and native mtDNA after enzymatic lysis; lanes 3 and 5: digested with Eco*RV* and native mtDNA after mechanical disruption.

This modified method is easy to perform, does not require special equipment and should take no more than 4 h. Main advantages of this technique are introduction of a cheap nutrient medium, in comparison to YPD (1% yeast extract, 2% bactopeptone, 2% dextrose) routinely used for such kind of analyses, and the following technical changes, making it more applicable: avoiding ultracentrifugation steps and utilization of harmful compounds as phenol and chloroform. In addition, a positive effect of the proposed technique is that the enzymatic digestion is replaced by a cheaper mechanical one.

Defontaine A., Lecocq F. M. and Hallet J.-N. (1991), A rapid miniprep method for the preparation of yeast mitochondrial DNA. Nucl. Acids Res. **19**, 185.

Fox T. D., Folley L. S., Mulero J. J., McMullin T. W., Thorsness P. E., Hedin L. O. and Costanzo M. C. (1991), Analysis and manipulation of yeast mitochondrial genes. In: Guide to Yeast Genetics and Molecular Biology (Guthrie C. and Fink G., eds). Academic Press Inc., San Diego. pp. 158–159.

Querol A. and Barrio E. (1990), A rapid and simple method for preparatuion of yeast mitochondrial DNA. Nucl. Acids Res. **18**, 1657.

Satz und Druck: AZ Druck und Datentechnik GmbH, Kempten