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Electrophoretic karyotype analysis of some *Candida Parapsilosis* strains

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Abstract

The goal of our work was to perform the electrokaryotiping by Field Inversion Gel Electrophoresis (FIGE) of two yeast strains with probiotic potential, previously isolated from dairy products. Molecular analysis allowed us to identify those strains as Candida parapsilosis. Usually, the chromosomal pattern and the screening for linear mtDNA are resolved by CHEF (Contour Clamped Homogenous Electric Field), which is a more laborious technique than FIGE. Therefore the method that we have set up presented many practical advantages

Keywords: *Candida parapsilosis*, electrokaryotyping, probiotics, linear mitochondrial genome

Introduction

Studies regarding the benefit of using probiotics have revealed that there are several possible ways in which yeast can stimulate the population of symbiotic bacteria. In mixed microbial populations, probiotic yeast cells synthesize growth factors (malic acid, short chain peptides, lipid compounds, vitamins) that stimulate multiplication of mammal-symbiotic bacteria. On the other hand, yeast cells metabolism lowers the oxygen concentration in mammal intestine, thus facilitating the anaerobic bacteria growth.

Nowadays, most of the studies in this field focus on the enhancement of the probiotic efficacy by selection of new strains, combination of different microbial strains, coupling with synergic agents [1].

In this respect, we have previously isolated two new yeast strains from dairy products. Morphophysiological and genetic studies presumably identified these strains as *Candida parapsilosis* [2]. In the present study in order to more accurately characterize these strains, we aimed to obtain and analyze the karyotype comparatively to a well known strain of *Candida parapsilosis*. Due to their small chromosomes and special mitosis (nuclear envelope remains intact), yeast karyotype can be analyzed only using special electrophoretic techniques [3,4,5].

Electrokaryotyping by FIGE confirmed the preliminary affiliation of yeast strains isolated from dairy product to *Candida parapsilosis*. At the same time Electrophoretic karyotyping with FIGE allowed to reveal the linear mitochondrial DNA [6, 7, 8, 9]. Although the typical mitochondrial DNA (mtDNA) is portrayed as circular molecule, there are yeast species (*Candida parapsilosis, Pichia philodendra* and *Candida salmanticensis)* which have linear mitochondrial genomes terminating with mitochondrial telomeres (Fig.1). Those are characterized by inverted terminal repeats each consisting of several units repeating in tandem and a 5' single-stranded extension. [6, 7].

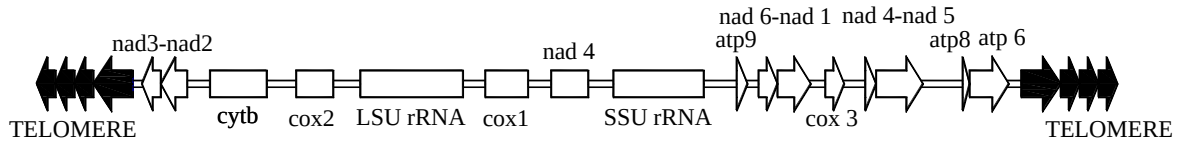

Fig.1 Genetic organization of mtDNA in *Candida parapsilosis* (A.RYCOVSKA & al. [10]). mtDNA of *Candida parapsilosis* displays a linear map terminating with telomeric tandem arrays

Materials and methods

Yeast strains

In this experiment we used yeast strains named *R-BC, R-LC* which was previously isolated from dairy products. As reference strain we used *Candida parapsilosis* strain from the Microbial Collection of the Laboratory of Microbial Genetics and Biotechnology from the Faculty of Biology, University of Bucharest.

Methods

Intact chromosomal DNA preparation

Samples of chromosomal DNA prepared in agarose blocks were obtained as described by [11]. Yeast culture was incubated for 18-20 h at 28°C, in YPG medium (Yeast Peptone Glucose –g/L yeast extract 5; D-glucose 20; peptone 10; pH 6.0). Cells were pelleted and washed twice in 1,5 mL EDTA 0,05M (MERCK- Ethylendinitrilotetraessig–saure Dinatriumsalz-Dihydrat) at 7000 rpm for 7 min and then were re-suspended in 0,9 mL EDTA 0,05 M. Cell suspension was mixed with 0,2 mL of lyticase (SIGMA – stock solution 10 mg/ml in EDTA 0,05M) and 0,9 mL of 1% agarose (SIGMA – Agarose for Pulsed Field Electrophoresis Sample Preparation in EDTA 0,05 M) that was previously melted and kept liquid at 50 to 55°C. 1,5 mL mixture was poured in moulds and incubated for 20 min at 4°C. The agarose blocks were placed in 10 mL TE with750 □l □-mercaptoethanol for 48 h at 37°C.

The solution was replaced by 7 mL EDTA 0,5 M containing Sarcosyl 1% for 10 min at room temperature. Then, the blocks were incubated with 4,5 mL EDTA 0,5 M and 0,5 ml Pronase E (stock solution 20mg/ml) for 48 h at 50°C, and finally washed twice for 15 min at 37°C in 8 ml EDTA 0,5 M . The agarose blocks were stocked at 4°C in 10 mL EDTA 0,5 M [12].

Electrophoretic karyotyping with Field Inversion Gel Electrophoresis

Intact chromosomal DNA prepared as above was separated using a home made computer controlled FIGE system [11, 13]. Electrophoresis was performed for 48 h in 0,9% agarose gel (SIGMA – Agarose for Pulsed Field Electrophoresis Running Gel) in 0,5 X TBE buffer (stock solution, 0.089M Tris, 0.089M Boric Acid, 0.002M EDTA, pH=8) at 2V/cm. The electrophoretic parameters were setup regarding estimated size of chromosomal DNA and were computer controlled as follows:

-Initial forward time (Fwi = 10 sec)
-Final forward time (Fwf = 500 sec)
-Pause of forward (Pfw = 1/10 Fwi)
-Reverse time (Rv = 1/3 Fwi)
-Pause of reverse (Prv = 1/10 Rv)
–Running time = 48 hours

Results and Discussions

Electrokaryotypes variability of the strains was examined by FIGE. Electrophoretic analysis revealed that our strains shared the same karyotypes with *Candida parapsilosis* (Fig.2) that confirming the preliminary identification based on the morpho-physiological and genetic results [2, 14], and led us to an affiliation of *R-BC* and *R-LC* to the specie above.

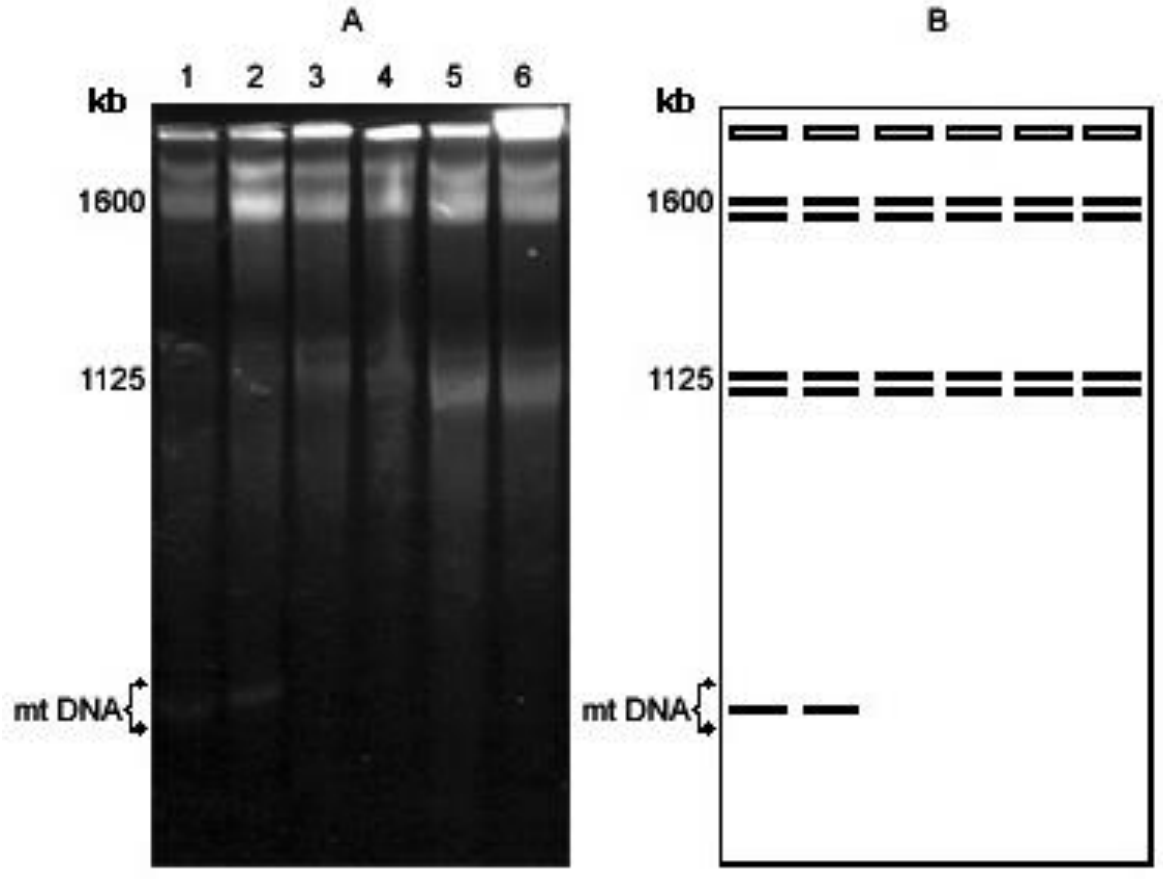

Fig.2 FIGE Electrokaryotypes of yeast strains
A. 1, 2 – C*andida parapsilosis*-reference strain; 3, 4 – *R-BC;* 5, 6 – *R-LC*
B. Schematic representation of electrokaryotypes

Our electrokaryotyping system made possible electrophoretic separation of intact DNA molecules and provided optimal resolution of the chromosome bands with molecular size of 1,125 and 1,600 kb. The high-molecular-weight chromosomal bands (2,200-3,000 kb) could not be separated, suggesting future modifications which need to be made to our protocol. Low-molecular-weight chromosomal bands (less than 900 kb) were absent, this result being similar to those described in literature for Electrophoretic karyotyping with CHEF (Contour Clamped Homogenous Electric Field) for *Candida parapsilosis* [15]

At the same time FIGE analysis revealed a distinct band which presumably corresponding to the linear mitochondrial genome with molecular size of 30 kb [16, 18, 19]. Strains *R-BC* and *R-LC* presumably possess circular-mapping mtDNA which in contrast to linear – mapping mtDNAs, do not exhibit a distinct band in Pulsed Field Gel Electrophoresis (PFGE).These results are in agreement with well known differences in genetic organization of the mtDNA of *Candida parapsilosis* [10].

Usually, the screening for linear mtDNA is resolved by CHEF [10], which suggests that our electrophoretic technique is a competitive one. These results will be followed by

other FIGE variants in order to obtain the complete chromosomal pattern for *Candida parapsilosis* (that is 7 chromosomes - haploid genome) [20, 21].

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