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Kluyveromyces lactis electrokaryotyping by Field Inversion Gel Electrophoresis

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Abstract

The chromosome patterns revealed by Pulsed Field Gel Electrophoresis techniques remain one of the most important analyses in establishing species-specific characteristics or detection of intraspecific variation. K. lactis, a very important biotechnological yeast, has been studied in this respect only using Contour Clamped Homogenous Electric Field (CHEF) electrophoresis, due to the relative large size of the chromosome, up to 3 Mpb. In this paper, a simple variant of Field Inversion Gel Electrophoresis (FIGE) is developed. The main advantages of our protocol are the running time (less than 24 h) and the requirements of only few input parameters for a better separation of K. lactis chromosomes.

Introduction

The non-conventional yeast *Kluyveromyces lactis* has become an excellent alternative yeast model organism [1, 2]. Reconsidered to be a distinct species [3, 4], *K. lactis* is an ascomyceteous budding yeast that belongs to the endoascomycetales [1]. There are important reasons for the increased attraction of *K. lactis* of biologists. Genetically, *K. lactis* is closely related to *S. cerevisiae* [1, 5], yet they are physiologically very different [6,7]. This yeast has a distinct property consisting in the ability to use lactose as a sole carbon source [8].

Strictly adapted to aerobiosis, *K. lactis* has been successfully used in biomass industrial production and in large scale expression of relevant gene products. Also, due to its specific DNA-killer system, experiments on *K.lactis* can extend fundamental knowledge about microbial competitions and plasmid biology [9, 10].

According to the different criteria for classification, *Kluyveromyces* species have been subjected to continuous renaming [1,2]. One of the *Kluyveromyces* phylogenetic tree has been proposed by Kock [11], based on phenotypic characters and electrophoretic karyotypes. More recent, chromosomal length polymorphism has proved to be useful either for differentiation of species, verification of synonymies or for the study of the anamorphic/teleomorphic relationships between species.

According to the increased interest for non-conventional yeast [12], especially for *Kluyveromyces* species [13-15], a lot of techniques for chromosome pattern analysis, derived from the conventional electrophoresis, have been developed. For example, Contour Clamped Homogenous Electric Field (CHEF [16]) is still representing one of the most popular electrokaryotyping methods [2, 11].

In this paper is described a short protocol for intact chromosome preparation and the electrokaryotyping techniques applied to *K. lactis*, using a computer controlled Field Inversion Gel Electrophoresis (FIGE [17-19]) apparatus.

Materials and Methods

Yeast Culture

In this experiment we used a *Kluyveromyces lactis* strain received from the Institute of Genetics and Microbiology, University of Paris. A 20 h *K. lactis* culture, grown at 28 ^{o}C in liquid YPG (Yeast extract 1%, peptone 1%, glucose 2%) under aeration conditions was used.

DNA preparations

Solutions and reagents

-EDTA 0.05M, pH = 7.5;
-EDTA 0.5M, pH = 8;
-TE (EDTA 0.05M + Tris-HCl 0.05M, pH = 7.5);
-Lyticase (stock solution 10 mg/ml in 0.05M EDTA);
-Low melting point agarose (LMP) (1% in EDTA 0.05M);
-β-mercaptoethanol;
-Sarcosyl;
-Pronase E or Proteinase K (stock solution 20 mg/ml);
-TBE 5X electrophoresis buffer (stock solution, 0.089 M Tris, 0,089 M Boric Acid, 0.002 M EDTA, pH=8)

Intact chromosomal DNA preparation

Five-ml yeast culture was centrifuged and washed twice in 1.5 ml EDTA 0.05M at 6500 rpm for 6 min. The final sediment was resuspended in 0.9 ml EDTA 0.05M with 0.2 ml of lyticase stock solution. Then, a 0.9 ml LMP solution preheated at 50^{0} C was added. A volume of 1.5 ml mixture was poured in moulds and allowed to harden about 15 min at 4^{0} C. The agarose blocks were sliced from the moulds and placed in 10 ml TE with 750 µl β-mercaptoethanol. After 48 h incubation at 37^{0} C, the solution was poured off and replaced by 7 ml EDTA 0.5M containing 1% Sarcosyl for 10 min at room temperature. This solution was then replaced by 4.5 ml EDTA 0.5M and 0.5 ml Pronase E or Proteinase K (stock solution). The incubation is continued for 48 h at 50^{0} C, then the agarose blocks were washed twice for 15 min at 37^{0} C in 8 ml EDTA 0.5M. Agarose were stocked at 4^{0} C in 10 ml EDTA 0.5 M.

Field Inversion Gel Electrophoresis

FIGE was performed on a 10 cm gel using a pulsed field electrophoresis running gel agarose 1% (SIGMA) in order to allow a wide spread of the chromosomes on the gel. A computer controlled FIGE apparatus (home made) allowed us to carry out the electrophoresis experiment.

The most important parameters are established as follows:

- initial forward time (fwi) = 20 sec;
- final forward time (fwf) = 160 sec;
- reverse time = 1/3 of forward time (for every cycle);
- pause = 1/10 of forward and reverse time (for every cycle);
- running time = 23 h;
- number of cycles = 637;

- linear ramp;
- 10 min premigration at 20 V;
- migration at 40 V;
- electrophoresis buffer TBE 0.5X (prepared from stock solutions by dilution, ethidium bromide added).

Results and Discussions

The chromosomal pattern exhibited by our *K. lactis* strain is shown in **Figure 1**. Individual bands represent intact chromosomes.

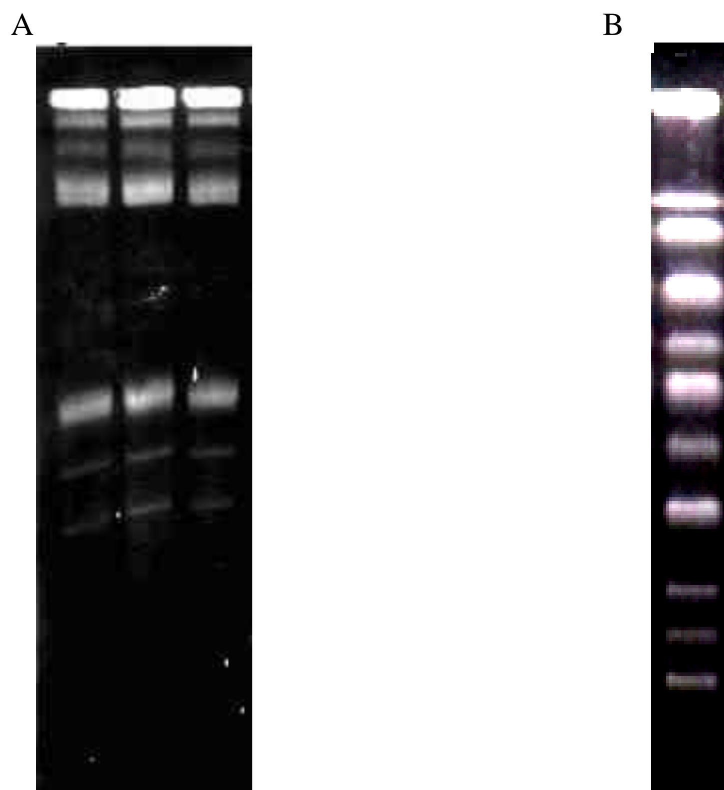

Figure 1. Electrophoretic chromosome pattern of *K..lactis* (A) and *S. cerevisiae S 288C* (B).

Though other electrophoretic techniques were used, several studies have revealed the same number of electrophoretic bands as in our experiment [1,2,11]. Most papers underlined the relative homogeneity of the karyotypes among the *K.lactis* strains, that is 6 chromosomes (from I to VI) with the following average sizes: I – 1 Mbp (Mega base pairs); II – 1,3 Mbp; III – 1,6 Mbp; IV – 1,8 Mbp; V – 2,3 Mbp; VI – 2,7 Mbp.

Usually, the *K. lactis* karyotype is resolved only by CHEF [2, 11], FIGE being considered as having a poor separation capability for DNA larger than 1 Mbp. The FIGE variant we developed proved to have a very good separation up to 2760 kbp, the largest chromosome of *K. lactis*, without using a thermostat or buffer recirculation. Unlike other Pulsed Field Gel Electrophoresis (PFGE) techniques, FIGE is supposed to present the so called “inversion band” [18]. Due to this phenomenon, a large DNA molecule can advance in the electrophoresis gel more than a smaller one. Band inversion is avoided using the switch time ramping, a different forward and reverse migration time for every cycle. The limit of band inversion depends on the switching time and the ramp type.

This type of electrokaryotyping system has a lot of advantages compared to other PFGE devices. First of all, the main part (power supply, gel box, electrophoresis tank) could be provided from a conventional electrophoresis system [19]. Preparation of the agarose blocks is very similar to other PFGE protocols. The running time for a good separation of chromosomes is no more than 24 h; for a CHEF system, *K. lactis* electrokaryotyping lasts about 100 h [2]. Only special FIGE applications, like Asymmetric Voltage FIGE or Zero Integrated Field Electrophoresis requires up to 130 h running time, but increasing separation limit up to 6 Mpb [18].

Conclusions

The most important electric parameters in FIGE electrokaryotyping are the switching time interval and the ramping. Using a computer controlled device, only few parameters must be defined, every cycle consisting of a succession of steps (forward migration, pause, reverse migration, pause) being controlled by computer, according to the chosen ramp and parameters. It is obvious that, without any other precautions, the separation limit could be increased only by choosing the appropriate conditions for DNA migration. In this respect, FIGE could become a very important tool for large size DNA analysis.

In our study we have demonstrated that using appropriate electric conditions, FIGE can be successfully used for a better separation of the *K.lactis* chromosomes.

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