Electrophoretic Karyotyping of Some Yeast Strains Isolated from Oil-Polluted Soils

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Abstract

During the last decades among other molecular biology techniques used for yeast strains characterization, electrokaryotyping has gained a growing importance. Although yeasts have been described as being present in polluted environments, there are still many questions regarding their identification. Our study refers to the analysis of electrokaryotypes of three yeast strains named D3, D4 and D6, isolated from oil-polluted soils, using a FIGE method improved in our laboratory. For D3 we obtained four chromosomes ranging from 225 kbp up to 1050 kbp, and a lane representing, most likely, an extrachromosomal DNA. For D4 we obtained five chromosomes with sizes from 375 kbp up to 845 kbp, and for D6 four chromosomes with sizes from 750 kbp up to 1050 kbp. The comparative analysis of these electrokaryotypes with those obtained in the same conditions for standard strains, along with previous observations, allow us to conclude that D3 and D6 belong both to Candida genus, and D4 to Rhodotorula glutinis.

Keywords: yeasts, oil-polluted soils, electrokaryotyping, FIGE.

Introduction

In order to characterize an yeast strain, taxonomists use a large number of analysis based on classical [3; 13] and molecular biology techniques based on the investigation of chromosomal and extrachromosomal DNA [9; 10; 16]. Electrokaryotyping – obtaining of electrophoretic karyotypes - is one of the most recent techniques used in the

characterization and classification of yeast strains. The idea of a possible electrophoretic separation of intact chromosomal DNA molecules as an alternative for karyotyping by classical methods, was first possible in 1982, when David Schwartz constructed a new kind of electrophoretic system [5].

First electrokaryotypes were obtained for the conventional yeast *Saccharomyces cerevisiae* [6]. Nowadays such experiments are performed on a large number of taxa. Some of them, especially those from *Rhodotorula, Candida* and *Yarrowia* genus, were found in ecosystems polluted with oil compounds [4; 14]. In spite of the fact that enough data has accumulated on n-alkanes and aromatic compounds biodegradation pathways, such yeasts were less studied on a taxonomic approach. Due to the increase of oil - polluted ecosystems and to the high price of technologies used for their rehabilitation, the necessity of bioremediation as alternative tool for conventional remediation occurred [1; 2; 12]. Therefore, there is stringent need for more complex studies including isolation of new yeast strains and molecular analysis of such microorganisms.

Materials and Methods

Yeast strains

Yeast strains named *D3, D4* and *D6* were isolated from oil-polluted soils by the Laboratory of Microbiology from the Institute of Biology, Bucharest and have been previously estimated as belonging to *Candida parapsilosis (D3*), *Rhodotorula glutinis (D4*) and *Candida tropicalis (D6*) [7; O. CSUTAK – unpublished results]. Therefore, in order to increase the taxonomic resolution of what we have determined, we obtained and analyzed the electrokaryotypes of our three strains, comparatively to those of the estimated taxa. The sizes of the chromosomal DNA bands were determined using as yeast electrokaryotype marker the standard strain of *S. cerevisiae* YPH 80 (SIGMA) (**Table 1**).

All the yeasts strains were maintained by cultivation on YPGA medium (g/L yeast extract 10, peptone 10, glucose 20, agar-agar 20).

Preparation of high molecular weight DNA

In order to prepare intact chromosomal DNA molecules we used a new technique, optimized in our laboratory for *S. cerevisiae* strains, based on those described by CARLE & al. [6] and SPENCER & al. [17]. 5 mL yeast culture incubated for 18 – 20 h at 28^{o}C, in YPG medium, were centrifuged for 5 min at 6000 rpm, and the cells were washed twice with 1.5 mL solution EDTA 50 mM, pH 7.5 and then resuspended in 0.9 mL of the same solution. The suspension was mixed with 0.9 mL agarose low melting point 1% (prepared in sol. EDTA 50 mM, pH 7.5, at 45^{o}C). For the lysis of the cellular wall a sol. of lyticase was used to a final concentration of 1 mg/mL for *D3* and *D6* strains, and to a final concentration of 2 mg/mL for *D4*. The mixture was purred in special agarose plugs and incubated for 15 min at 4^{o}C. The agarose plugs were then incubated for 48 h, at 37^{o}C with 10 mL solution EDTA 0.5 M and Tris-HCl 0.05 M, pH 7.5 and 750 ml b-mercaptoethanol. The plugs were incubated for 48 h, at 50^{o}C with sol. EDTA 0.5 M,

pH 8, sarkosyl 1% for strains *D3* and *D6* and 1.5% for *D4*, and proteinase K to a final concentration of 2 mg/ml. After washing them twice with solution EDTA 0.5 M, pH 8, the plugs were incubated at 4°C in the same solution.

Electrophoresis conditions

The specific electrophoresis conditions were established according to the expected dimensions of the DNA molecules to be separated. For electrokaryotyping we used a system designed in our laboratory [D. FOLOGEA – submitted paper].

We used special agarose for pulsed field electrophoresis running gel (SIGMA) concentration 1%, prepared in buffer TBE 0.5X (Tris 0.089 M; boric acid 0.089 M; EDTA 0.002 M, pH 8).

The conditions for electrophoresis were optimized using as starting point the conditions described by SPENCER & al. [17], for the separation of DNA molecules with dimensions up to 2 Mb. Further details of the separation conditions are given in the text.

Results and Discussions

Analysis of D3 and D6 electrokaryotypes

Previous works [8; 11; 15] have determined the approximate number (4 to 7) and average size (1 Mbp) of *Candida*chromosomes.

In our first set of experiments on *D3* we used a total running time of 36 h at 56 V, with an initial forward running time (*Fwdi*) of 10 sec.; final forward running time (*Fwdf*) of 160 sec.; reverse running time (*Rvs*) - 1/3 *Fwdi*; pause for forward and reverse running (*P fwd, P rvs*) – 10 %, and a linear switching ramp.

The electrokaryotype of *D3* strain separated four main bands with approximate sizes of 225 kbp (*I*), 390 kbp (*II*), 750 kbp (*III*) and 1050 kbp (*IV*), from which three are also observed for *C. tropicalis* standard strain (bands *II, III* and *IV*) (**Figure 1**).

To separate also the small DNA molecules, we performed further experiments by reducing the pulse time (*Fwdi* - 40 sec., *Fwdf* – 120 sec., *Rvs* – 1/3 *Fwdi*), the total migration time (20 h) and the voltage (43 V). Under these conditions, we succeeded to separate a new band named *E* (**Figure 2**). Comparative analysis of this *E* band made us presume it represents an extrachromosomal replicon.

For the yeast strain *D6* a number of experiments were performed in order to optimize the electric conditions for electrophoresis. The first set of experiments had a total running time of 24 h at 56 V, with the following steps: initial forward running time (*Fwdi*) – 3 sec.; final forward running time (*Fwdf*) – 140 sec.; reverse running time (*Rvs*) – 1/3 *Fwdi*; pause for forward and reverse running (*P fwd, P rvs*) – 10 %, linear switching ramp (**Figure 3**). For a better separation and resolution of bands, we used for the

second set of experiments a randomic *Fwdi* and *Fwdf*, maintaining the same total running time, voltage, *Rvs* and pause time (**Figure 4**). We succeeded to obtain four bands, identified as: *I* – 750 kbp ; *II* – 850 kbp; *III* – 890 kbp and *IV* – 1050 kbp. Bands *I*, *II* and *IV* are the same as for *C. tropicalis.*

We underline that comparative electrophoretic analysis proved that both our strains *D3* and *D6*, have similar electrokaryotyping profiles to *C. tropicalis*. Even more, the striking FIGE resemblance between *D6* and *C. tropicalis*, confirms our previous results on the taxonomic identification of this strain [O. CSUTAK – unpublished results].

Analysis of D4 electrokaryotype

In **Figure 5** we present the results obtained by FIGE for *D4* and *Rh. glutinis* strains, under the following conditions: total running time of 24 h at 56 V, *Fwdi* – 40 sec.; *Fwdf* – 160 sec.; *Rvs* – 1/3 *Fwdi*; *P fwd, P rvs* – 10 %, linear switching ramp. Thus, for *Rh. glutinis* we obtained 4 bands: I_R - 680 – 690 kbp; II_R – 750 kbp; III_R – 845 kbp and a band named $C_{R,D}$ – representing chromosomal DNA molecules that were not yet separated.

For *D4* we obtained six bands: I_D – 375 kbp; II_D – 700 kbp; III_D – 750 kbp; IV_D – 790 kbp; V_D – 845 kbp; and $C_{R,\,D}$. The comparative analysis of these electrokaryotypes shows a great similarity of the chromosomal patterns, as two of the chromosmomes from *D4* are present also in *Rh. glutinis* strain: bands II_R and III_D; III_R and V_D.

Conclusions

Our analysis on D3 and D6 revealed obvious similarities of their electrokaryotypes. Bands with similar sizes have been observed for D3 as well as for D6: band III from D3 and I from D6, band IV from D3 and IV from D6. These data, along with those already obtained, certify a most possible belonging of D3 and D6 to the same genus - Candida. The analysis of electrophoretic karyotype obtained for D4, allow us to conclude that this strain most probably belong to Rh. glutinis.

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Figure 1. The electrokaryotypes for *D3* and *C. tropicalis* (1, 2 – *D3*; 3, 4 – *C. tropicalis*, 5 – *S. cerevisiae* YPH80)

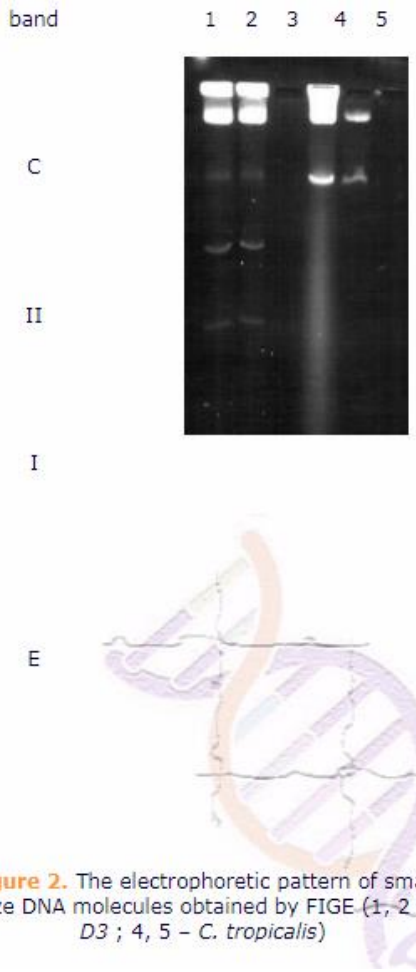

Figure 2. The electrophoretic pattern of small size DNA molecules obtained by FIGE (1, 2 – *D3* ; 4, 5 – *C. tropicalis*)

17.

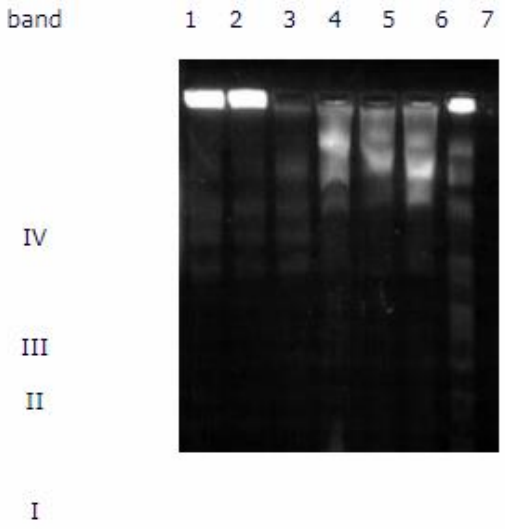

Figure 3. Electrokaryotypes for *D6* and *C. tropicalis* (1, 2, 3 – *D6*; 4, 5, 6 – *C. tropicalis*, 7 – *S. cerevisiae* YPH80)

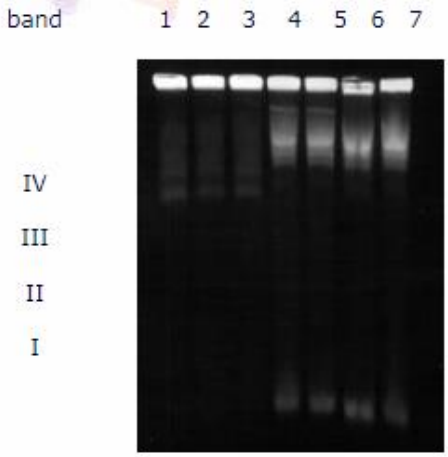

Figure 4. Electrokaryotypes for *D6* and *C. tropicalis* (1, 2, 3 – *D6*; 4, 5, 6, 7 – *C. tropicalis*)

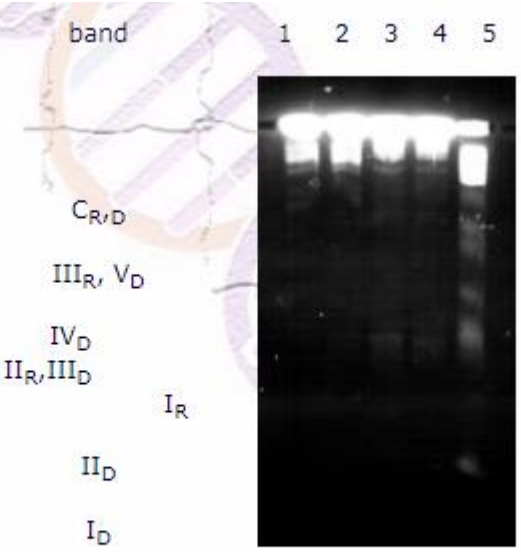

Figure 5. The electrokaryotypes for *Rh. glutinis* and *D4* strains

(1, 2 – *Rh. glutinis*; 3, 4 – *D4*; 5 – *S. cerevisiae* YPH80)

Table 1. Chromosome number and length for *S. cerevisiae* YPH 80 (SIGMA)

Band no.	Chromosome	Size (kbp)
1	XII	2200
2	IV	1640
3	VII	1120
3	XV	1100
4	XVI	945
5	XIII	915
6	II	815
7	XIV	785
8	X	745
9	XI	680
10	V	610
11	VIII	555
12	IX	450
13	III	375
14	VI	295
15	I	225