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Chromosomal polymorphism of the yeast *Yarrowia lipolytica* and related species: electrophoretic karyotyping and hybridization with cloned genes

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Abstract. Significant differences in electrophoretic karyotyping patterns were found among 27 strains of *Y. lipolytica*. Twenty-one of these strains were classified into four groups of similar karyotypes while six strains showed unique karyotypes. Chromosomal DNAs of different strains were hybridized with cloned genes of *Y. lipolytica* (*URA3, LEU2, ARS18* and *ARS68*), which revealed four different bands in most strains. We conclude that the haploid chromosome number of *Y. lipolytica* is at least four, and possibly five or six. Electrophoretic karyotyping and hybridization with cloned genes of *Y. lipolytica* provided evidence of a large divergence between *Y. lipolytica* and related species of *Saccharomycopsis*, *Endomycopsella* and *Endomyces*.

Key words: Karyotype – *Yarrowia lipolytica* – Chromosome polymorphism – Hybridization

Introduction

The dimorphic yeast *Yarrowia lipolytica* (syn. *Candida lipolytica*) has been used for the industrial production of citric acid, of single-cell protein (Phaff 1985), and more recently of heterologous proteins (Buckholz and Gleeson 1991). Although *Y. lipolytica* has been studied by genetic and molecular methods for more than 20 years it is still a difficult model object for biological investigations. A combination of low mating frequencies, low fertility of hybrids, irregular meiotic segregation, and mitotic haploidization, make it difficult to study natural isolates of this yeast or the hybrids obtained when different inbred lines are crossed (Gaillardin et al. 1973; Esser and Stahl 1976; Ogrydziak et al. 1978, 1982; Kurischko 1986). A possible explanation for these genetic anomalies in *Y. lipolytica* could be the genetic heterogeneity of the taxon. We decided, therefore, to explore this possibility by studying natural isolates of *Y. lipolytica* from different sources originating from various geographic regions.

Pulse-field gel electrophoresis of chromosomal DNAs, followed by hybridization with cloned genes, has been successfully used to study species and strain peculiarities (Johnston et al. 1988; Magee et al. 1988; Steensma et al. 1988; Sor and Fukuhara 1989; Iwaguchi et al. 1990; Rikkerink et al. 1990; Asakura et al. 1991; Naumov et al. 1992). We have used this technique for comparing the karyotypes of 27 *Y. lipolytica* strains.

A second aim of this study was to explore the genetic relatedness between *Y. lipolytica* and three other genera which until recently were grouped in the same genus, *Saccharomycopsis*. The genus *Yarrowia* has been created (van der Walt and von Arx 1980) because the former species *Saccharomycopsis lipolytica* was the only one in the genus *Saccharomycopsis* that did not have plasmodesmata (Kreger van Rij and Veenhuis 1973). However, it is not obvious that this morphological difference should correspond to a large taxonomic distance. It was thus interesting to investigate with molecular tools the relatedness between these different yeast species which all display mycelium formation. Based on the 18 *s* rRNA sequence it had already been shown that *Y. lipolytica* was very distant from most other yeast genera including several *Candida* species (Barns et al. 1991). Evaluating the molecular relatedness between the genetically well-known *Y. lipolytica* and potentially close genera could provide the necessary background for developing new tools for gene manipulation among these species.

Materials and methods

Strains. The strains of *Y. lipolytica*, and the *Saccharomycopsis*, *Endomycopsella* and *Endomyces* species which were used are listed in Tables 1 and 2.

Preparation of chromosomal DNA. Yeast cells were grown in 25 ml of YPD medium at 28 °C overnight, then harvested and washed with 50 mM EDTA, pH 7.5. They were resuspended at a concentration of 1×10^9 cells/ml in an aqueous solution containing 0.9 M

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Table 1. List of the *Yarrowia lipolytica* strains

Strain received as	CLIB number	Mating type	Geographic origin	Source of isolation
CBS 6124.1[a]	CLIB 77	A	USA, Illinois	Ex maize-processing plant
CBS 6124.2[a]	CLIB 78	B	USA, Illinois	Ex maize-processing plant
CBS 599	CLIB 200	A	Netherlands	Ex rancid margarine
CBS 2074	CLIB 201	A	Italy	Ex olives
CBS 2078	CLIB 202	A	Netherlands	Ex soil
CBS 5570	CLIB 203	B	Argentina	Ex lung
CBS 5589	CLIB 204	A	Argentina	Ex lung
CBS 6660	CLIB 205	A	Russia	Ex soil
CBS 7033	CLIB 206	B	Unknown	Ex soil
H222	CLIB 80	A	Germany	Ex soil
A-15	CLIB 83	Unknown	Poland	Ex soil (near oil-well)

CBS, Centraalbureau voor Schimmelcultures Delft, Holland
CLIB, Collection de Levures d'Intérêt Biotechnologique, Thiverval-Grignon, France
CBS 6124.1 and CBS 6124.2 are monosporic segregant of the type culture CBS 6124
[a] We received these strains from the NRRL collection as NRRL 423-3 and NRRL 423-12

sorbitol, 0.1 M Tris-HCL pH 8.0, 0.1 M EDTA, 0.3 mg/ml zymolyase 100T and 0.28 M beta-mercaptoethanol. The mixture was incubated for 15–20 min at 37 °C. To this suspension was added an equal volume of 1% low-melting-point agarose prepared in 0.25 M EDTA solution (pH 8.0) and cooled to 42 °C. The mixture was allowed to gel at 4 °C and plugs were incubated in lysis buffer (0.5 M EDTA pH 8.0; 1% N-lauroylsarcosine; 1 mg/ml proteinase K) at 50 °C for 48 h. The lysis buffer was then replaced by 0.5 M EDTA pH 9.0 and the plugs stored at 4 °C.

Chromosome separation. A CHEF-DRIITM apparatus (Bio-Rad, Richmond, Calif., USA.) was used for separating chromosomal DNA of the *Saccharomycopsis*, *Endomycopsella* and *Endomyces* species. The electrophoresis buffer was 0.5 × TAE cooled to 12 °C. Electrophoresis was carried out at 50 V for 48 h with a switching time of 2400 s and then for 70 h with a switching time of 3000 s. Standard sets of chromosomes of *Saccharomyces cerevisiae* YNN295 and *Schizosaccharomyces pombe* 972 h^- were obtained from commercial sources (Bio-Rad). Chromosomes of *Y. lipolytica* were separated on a Geneline™ apparatus (Beckman Instruments, Palo Alto, Calif., USA), run at 10 °C in 1 × TAFE buffer (10 mM Tris, 4 mM acetate, 0.5 mM EDTA). Electrophoresis was carried out at a constant current of 18 mA for 162 h with a switching time of 70 min.

Southern-blot analysis. Southern-blot analysis of chromosomal DNA was carried out with a LKB 2016 VacuGene Blotting System (LKB Biotechnology AB, Bromma, Sweden). Chromosomal DNAs, separated by CHEF or TAFE, were depurinated, denaturated, neutralized and transferred to nitrocellulose filters. The *URA3* gene probe was a 1.7-kb *Sal*I fragment isolated from pLD55 (Davidow and Zeeuw 1985). The *LEU2* gene probe was a 5-kb *Sal*I fragment isolated from pINA62 (Gaillardin and Ribet 1987). The centromeric *ARS18* probe was a 2.1-kb *Bam*HI fragment isolated from pINA119 (Fournier et al. 1991). The centromeric *ARS68* probe was a 2.1-kb *Bgl*II-*Sty*I fragment isolated from pINA397 (P. Fournier, unpublished data). The probes used in hybridization were prepared according to Maniatis et al. (1982) using the GeneClean kit (Bio 101 Inc., LaJolla, Calif., USA) and labelled with digoxigenin-11-dUTP using a nonradiactive DNA Labeling Kit (Boehringer Mannheim, Mannheim, Germany). Hybridization was performed overnight at 68 °C in 5 × SSC (0.15 M NaCl, 0.015 M Sodium citrate) containing 0.1% N-lauroylsarcosine, 0.02% sodium dodecyl sulphate (SDS) and 1% blocking reagent. Detection of hybridization was done as recommended by the supplier. The filters were stained in the dark for 4–12 h.

Results

Electrophoretic karyotypes of Yarrowia lipolytica strains

Chromosomal DNAs of 27 strains of *Y. lipolytica* isolated from different geographic regions and various sources (see Table 1) were compared by CHEF electrophoresis. The strains showed large variations in their karyotyping patterns (data not shown). Summarizing the electrophoretic data from several gels we could classify most strains of *Y. lipolytica* into four groups including individuals of similar karyotype: (1) H222, CBS 2072, CBS 2073, CBS 2074, CBS 2075, CBS 6317, CBS 6614, CBS 7034; (2) A-15, A-101; (3) CBS 5589, CBS 2787, CBS 5699, CBS 5919, CBS 6125; (4) CBS 7033, CBS 2070, CBS 6012, CBS 6114, CBS 6303, CBS 6659. However, six strains (CBS 599, CBS 2078, CBS 5570, CBS 6124.1, CBS 6124.2 and CBS 6660) differed both from the four previous groups and from one another.

Representatives of each group and the six unclassified strains were further studied on TAFE gels (Fig. 1 A). Chromosomal sizes were estimated using chromosomal standards of *S. cerevisiae* (YNN 295), *S. pombe* (972 h^-) and *Kluyveromyces lactis* (CBS 2630). Interpretation of the patterns was complicated by the fact that, in several *Y. lipolytica* strains, bands with stronger relative intensity were observed: we assumed that these bands corresponded to doublets (or triplets in the case, for example, of CBS 599; see below). In several strains the upper bands had a lower mobility than chromosome I of *Schizosaccharomyces pombe* (5.7 Mb) which rendered the evaluation of chromosome size only approximate. Taking all these caveats into account, we estimated that the total genome size of natural *Y. lipolytica* isolates varied between 12.7 and 22.1 Mb. Chromosomes were resolved into 3–6 bands ranging in size from 1.4 Mb to 6.2 Mb. All strains carried two small chromosomes, rather similar in size, in the range of 2.3 to 2.8 Mb. However, in five strains these two bands migrated as doublets (Fig. 1 A, lanes 4, 6, 13, 14, 15). In two strains (CBS 6124.1 and CBS 6660) one of the short chromosomes was apparently smaller than in the other strains (1.4 and 1.8 Mb respectively; see Fig. 1 A, lanes 2 and 7).

To compare karyotypes of different *Y. lipolytica* strains in more detail, TAFE gels were transferred to nitrocellulose filters and then hybridized with four cloned genomic probes of *Y. lipolytica*: *URA3* (Fig. 1 B) and *ARS68*, *LEU2* and *ARS18*. Data from several gels were pooled and are summarized in Fig. 2. *ARS18* and *ARS68* are known from genetic data to be centromeric DNA belonging to two different chromosomes (Fournier et al. unpublished). *ARS18* and *ARS68* probes hybridized to the two smallest bands. When these were separated, the *ARS68* probe hybridized to the smaller one and *ARS18* to the larger of the two, except in two strains, CBS 6660