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The *Kluyveromyces lactis PDR5* and *RPL28* genes involved in drug resistance originate from chromosome VI

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Kluyveromyces lactis KlPDR5, the drug efflux ABC transporter gene, and *KlRPL28*, a gene encoding the large subunit ribosomal protein, were isolated in our laboratory recently. When overexpressed in *Saccharomyces cerevisiae* host strains, both genes confer an increased resistance to cycloheximide. In this work the chromosomal origin of the cloned genes was determined by Southern hybridization to contour-clamped homogenous electric field (CHEF) separated chromosomal DNA. Both genes were mapped to *K. lactis* chromosome VI.

Key words: drug resistance, yeast, *Kluyveromyces lactis*, chromosomal mapping.

Introduction

Comparative genomics is a new, rapidly growing field, which provides insights into the understanding of molecular evolution. With their relative small genome size, yeasts offer a unique opportunity to explore eukaryotic genome evolution by the comparative analysis of related yeast species. One of the important criteria to judge about the phylogenetic relationship between different yeast species is the chromosome map conservation.

Kluyveromyces lactis, used both in fundamental research and in industrial applications, is closely related to *Saccharomyces cerevisiae.* Interest in this yeast species arose from its distinctive physiological properties compared to *S. cerevisiae. K. lactis* assimilates lactose (CASTILLO, 1989) and belongs to so called petite-negative (BULDER, 1964a,b) and Crabtree-negative yeast species (DE DEKEN, 1966). A major difference in the regulation of glucose metabolism, as compared to *S. cerevisiae*, lies in the absence of glucose repression of respiration. Some *K. lactis* strains contain cytoplasmic linear DNA plasmids conferring the killer phenotype (STARK et al., 1990; WESOLOWSKI-LOUVEL et al., 1996). According to the published

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data concerning the glucose metabolism and its regulation in *K. lactis* it seems that the redundancy of glycolytic genes that exists in *S. cerevisiae* is not found in *K. lactis* (Wesolowski-Louvel et al., 1992; Prior et al., 1993; Bianchi et al., 1996; Billard et al., 1996; Blaissonneau et al., 1997).

Despite the fact that *K. lactis* has only six chromosomes, its genome size (10.6 Mb) and the total gene number are similar to that of *S. cerevisiae* (Bolotin-Fukuhara et al., 2000). The comparison of chromosomal organizations of functionally equivalent genes between *K. lactis* and *S. cerevisiae* reveals many rearrangements, nevertheless, the gene order in some short regions is similar in both organisms (Bolotin-Fukuhara et al., 2000). The conservation of synteny between *S. cerevisiae* and *K. lactis* is about 50% (Ozier-Kalogeropoulos et al., 1998). Most of the *K. lactis* genes characterized so far were isolated on the basis of their structural or functional similarity with *S. cerevisiae.* A genetic map with 75 loci has been published in 1995 (Wesolowski-Louvel & Fukuhara, 1995), an updated survey of databases reveals that about 150 chromosomal genes of *K. lactis* have been cloned and sequenced. The sequence collection of *K. lactis* has been enriched by the addition of nearly 600 random sequence tags (Ozier-Kalogeropoulos et al., 1998) identifying 292 novel genes, but their chromosomal localization is far from being known. The aim of the present work is to map two of the recently isolated and described *K. lactis* genes: *KlPDR5* (Chen, 2001) and *KlRPL28* (Takacova et al., 2002).

Material and methods

Strains and media

The yeast strains used in this study are the *Saccharomyces cerevisiae YKKB-13* (MATα*ura3-52his3-*Δ*200leu-*Δ*1trp1*Δ*63lys2-801*^{amb}*ade2-101*^{ochr}*pdr5::TRP1*) (Bissinger & Kuchler, 1994) and *Kluyveromyces lactis JBD100 (MATa trp1 lac4-1 ura3-100)* (Heus et al., 1990). YPD medium, used for the maintenance of yeast strains and recombinant DNA procedures, contained 10 g/L yeast extract, 20 g/L bactopeptone and 20 g/L glucose. A defined mineral medium supplemented with vitamins was used for physiological characterization of the strains used (Takacova et al., 2002). The nutrients essential for auxotrophic strains were added at 40 μg/mL. The media were solidified with a 2% bactoagar (Difco).

Escherichia coli strains XL-1 blue and JM109 were used for plasmid amplification and preparation. *E. coli* strains were grown in LB broth (Sambrook et al., 1989). Ampicillin was added to a final concentration of 100 μg/mL for plasmid maintenance.

Recombinant DNA techniques

Standard protocols were followed for plasmid isolations, restriction enzyme analyses, ligation, gel electrophoresis (Sambrook et al., 1989). Yeast chromosomal DNA for pulsed-field electrophoresis in a contour-clamped homogenous electric field (CHEF) was prepared according to Teunissen et al. (1993). Electrophoresis was performed in 1% agarose in 1xTAE (Sambrook et al., 1989) for 10 h at 130 V with the pulse times varying linearly from 180 s to 360 s.

DNA blot hybridizations

DNA was blotted onto nylon membranes using the LKB2016 VacuGene vacuum blotting system, with an alkaline blot buffer (0.6 M NaCl, 0.4 M NaOH). Pulsed-field gels were first incubated in 0.25 M HCl for 20 min. DIG-labeled DNA probes were generated by using a commercial DIG DNA Labeling and Detection Kit (Roche Molecular Biochemicals). After hybridization to the target DNA the hybrids were detected by enzyme-linked immunoassay using an antibody conjugate (antidigoxigenin-alkaline phosphatase conjugate) and subsequent enzyme-catalyzed color reaction with 5-bromo-4-chloro-3-indolylphosphate (X-phosphate) and nitro blue tetrazolium salt (NBT).

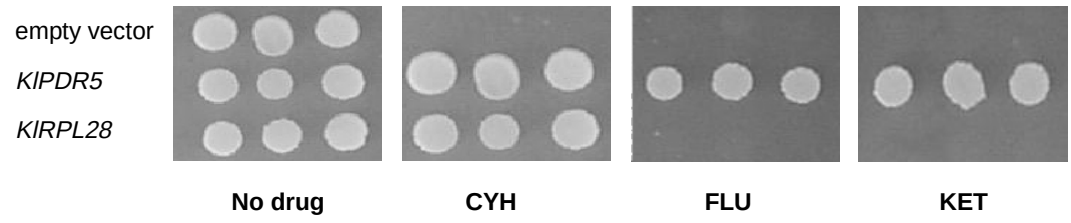

Fig. 1. The drug resistance phenotype of *S. cerevisiae YKKB-13* transformants. Cells were grown on solid mineral medium containing different drugs at indicated concentrations. The growth at 28 ℃ was scored after 4 days. CYH (cycloheximide 0.2 μg/mL), FLU (fluconazole 50 μg/mL), KET (ketoconazole 4 μg/mL).

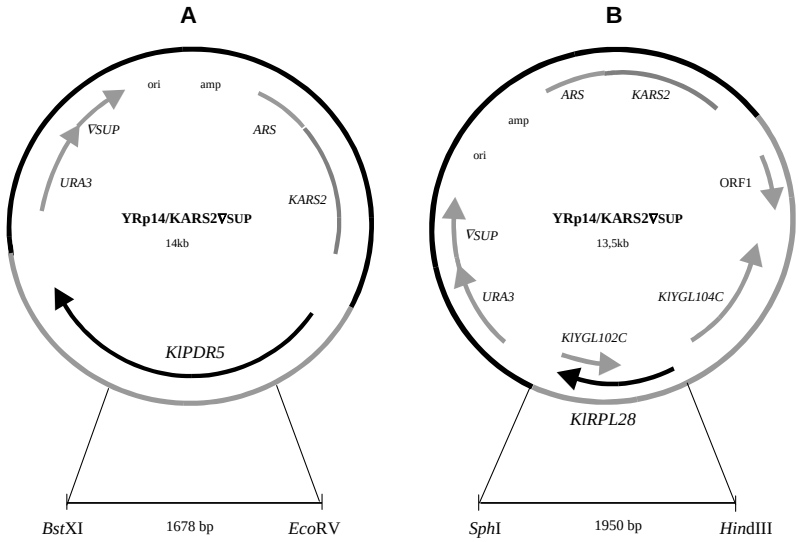

Fig. 2. Construction of the probes used for hybridization. A. DIG-dUTP labelled *PDR5*, B. DIG-dUTP labelled *RPL28*.

Results and discussion

During the search for factors involved in *K. lactis* multidrug resistance we looked for genes that, when overexpressed, confer resistance to cycloheximide in *S. cerevisiae* Δ*pdr5* mutant strain. Functional complementation of *S. cerevisiae* Δ*pdr5* mutant strain hypersensitive to cycloheximide with *K. lactis* genomic library led to the isolation of two different genomic DNA fragments of 5.6 and 4.9 kb, containing the *KlPDR5* and *KlRPL28* genes, respectively, that were responsible for the cycloheximide resistance observed (TAKACOVA et al., 2002). The drug resistance phenotype of heterologous yeast transformants bearing the *KlPDR5* and *KlRPL28* genes, respectively, is shown in Fig. 1.

To determine the chromosomal origin of the cloned genomic inserts, hybridization experiments were performed with chromosomal DNA molecules separated by contour clamped homogenous electric field electrophoresis (CHEF). The DIG-dUTP labelled 1.7 kb *Eco*RV – *Bst*XI internal fragment from the *KlPDR5* gene and 1.45 kb *Hind*III – *Sph*I fragment containing the whole *KlRPL28* gene, respectively (Fig. 2), were hybridized to the CHEF separated chromosomal DNA immobilized on nylon membranes. The results of hybridization experiments are shown in Fig. 3. The chromosomes were numbered in an ascending order with respect to their size in CHEF gels. In both cases we identified a positive signal with the band corresponding

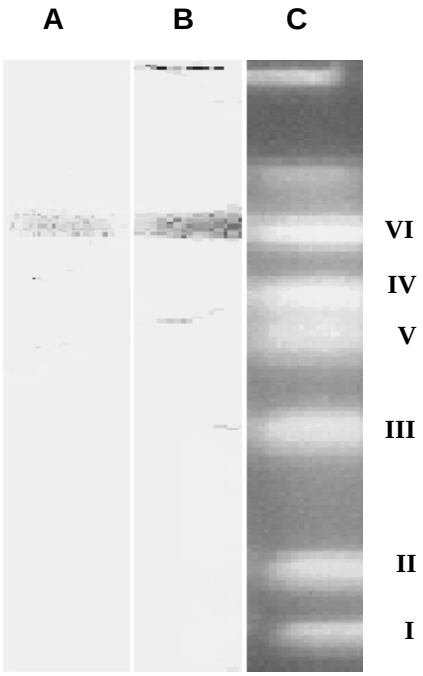

Fig. 3. Mapping of the DIG-dUTP labelled probes by chromosome blotting. The results of enzyme immunoassay after hybridization of the chromosomal blots with the labelled *PDR5* probe (A) and *RPL28* probe (B). On the CHEF separated chromosomal DNA (C) the corresponding chromosome numbers are indicated.

to *K. lactis* chromosome VI. So, the *KlPDR5* and *KlRPL28* genes can be added to the growing list of genes mapped in this biotechnologically important yeast species.

It is supposed that the mapping of chromosomal genes is an important parameter to consider for construction of genetic, physical and molecular maps of respective yeast species as it emphasizes divergence created at the chromosome level rather than at the gene sequence level.

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