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Genes encoding peroxisomal enzymes are not necessarily assigned on the same chromosome of an *n*-alkane-utilizable yeast *Candida tropicalis*

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We have resolved eight chromosomal bands from an *n*-alkane-assimilating yeast, *Candida tropicalis* pK 233, by using contour-clamped homogeneous electric field gel electrophoresis (CHEF). From the results of hybridization of DNA probes of yeast peroxisomal enzymes — catalase, acyl-CoA oxidase, carnitine acetyltransferase, isocitrate lyase, malate synthase, acetoacetyl-CoA thiolase, and 3-ketoacyl-CoA thiolase — to Southern transfers of CHEF gels, these genes were proven not necessarily to be located on the same chromosome. This fact shows that the genes encoding the enzymes tested were not distributed to be cistronic, although simultaneous and inducible synthesis of peroxisomal enzymes occurred in harmony with the proliferation of peroxisomes, suggesting that their co-ordinated expression might be mainly regulated by certain *trans*-acting factors.

n-Alkane-assimilating yeast; *Candida tropicalis*; Contour-clamped homogeneous electric field gel electrophoresis (CHEF); Peroxisome; Coordinated expression.

1. INTRODUCTION

Peroxisomes, highly proliferated in *n*-alkane-utilizing *C. tropicalis* pK 233 cells, contain enzymes which participate in higher fatty alcohol oxidation, activation of fatty acids, fatty acid β-oxidation, and part of the glyoxylate cycle, carnitine acetyltransferase, hydrogen peroxide-forming oxidases, and catalase, a marker enzyme of peroxisomes [1,2]. These peroxisomal enzymes were encoded by nuclear genes, simultaneously and inducibly synthesized, and specifically localized in peroxisomes in harmony with the development of the organelles [3]. There might be a possibility that these genes are distributed on the same chromosome. Kamiryo and Okazaki [4] supposed the presence of clusters of genes encoding part of the peroxisomal enzymes, including unidentified ones, and of a multi-gene family such as three kinds of acyl-CoA oxidases [5] in *C. tropicalis*. However, it remains unknown whether a kind of the cistronic cluster for the co-ordinated expression of all genes encoding peroxisomal enzymes is really present.

We report here that eight chromosomal bands were detected by the analysis of the *C. tropicalis* genome using a pulsed-field gel electrophoresis and the genes encoding important enzymes for the metabolism of fatty acids derived from alkanes were not necessarily distributed on the same chromosome. The results suggest that the genes encoding peroxisomal enzymes might not be expressed cistronically.

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2. MATERIALS AND METHODS

2.1. *Cultivation of yeast*

C. tropicalis pK 233 (ATCC 20336) was cultivated on a medium containing an *n*-alkane mixture (C_{10}–C_{13}) as described previously [6].

2.2. *Pulsed-field gel electrophoresis*

A contour-clamped homogeneous electric field (CHEF)-DR II Megabase DNA Electrophoresis System (Bio-Rad, Richmond, CA, USA) was used to resolve *C. tropicalis* chromosomes. A sample of the chromosomes was prepared from alkane-grown *C. tropicalis* cells according to the method of Schwartz and Cantor [7], with a slight modification. The cells harvested at the stationary phase were washed twice with 50 mM disodium EDTA (pH 7.5) and resuspended in the same solution to give a concentration of 1.3×10^{11} cells/ml. An aliquot of the suspension (3 ml) was mixed with 5 ml of solution A composed of 0.125 M disodium EDTA (pH 7.5) and 1% (w/v) low melting-temperature agarose (Bio-Rad) kept at 50°C, and then 75 μl of solution B was added for lysis of the cells which was composed of 0.01 M sodium phosphate buffer (pH 7.5), 50% glycerol, and 2 mg/ml Zymolyase 20 T (Seikagaku Kogyo Co., Tokyo, Japan). This mixture was incubated for 15 min at 37°C, and solidified by keeping it for 20 min at 4°C. The solidified sample was chopped into blocks of an appropriate size (each block contains about 3.0×10^9 cells). The blocks were immersed in LET buffer (0.01 M Tris-HCl (pH 7.5) and 0.5 M disodium EDTA) containing 7.5% (w/v) 2-mercaptoethanol and incubated for 16 h at 37°C. The blocks were then transferred to NDS buffer (0.01 M Tris-HCl (pH 9.5) and 0.5 M disodium EDTA) containing 1% *N*-lauroyl sarcosine and 2 mg/ml proteinase K (Merck, Darmstadt, Germany). After incubation for 16 h at 50°C, the blocks were washed once with 50 mM disodium EDTA (pH 7.5) at 25°C and soaked in this solution for 16 h at 50°C. Finally, the blocks were washed again with the same solution and

preserved at 4°C until use. Agarose gels (0.7% (w/v)) prepared with 0.5× TBE solution (5.40 g Tris, 2.75 g boric acid, and 0.372 g disodium EDTA per liter) was used for electrophoresis. The blocks were buried in the starting wells of an electrophoresis gel and the wells were sealed with 0.5× TBE solution containing 0.8% (w/v) low melting-temperature agarose. Electrophoresis was performed at 4°C in 0.5× TBE solution with circulation.

2.3. *Preparation of DNA probe*

All DNA probes were labelled with non-radioactive biotin 7-dATP (BRL Co., Gaithersburg, MD, USA) and used at concentrations of 150–200 ng/ml. A *Dde*I-*Pvu*II fragment of the genomic DNA for catalase [8], a *Nco*I-*Eco*RI fragment of genomic DNA for isocitrate lyase [9], a *Kpn*I-*Hin*cII fragment of genomic DNA for malate synthase (unpublished data), and *Eco*RI-*Eco*RI fragments of the cDNAs for carnitine acetyltransferase, acyl-CoA oxidase [10], acetoacetyl-CoA thiolase, and 3-ketoacyl-CoA thiolase (unpublished data) were used as DNA probes, respectively.

2.4. *Southern blot analysis*

The gel was stained with ethidium bromide after electrophoresis. After irradiation with UV light for 1 min to nick the DNAs, the gel was subsequently soaked in 0.25 M HCl, in de-ionized water, in 0.5 M NaOH solution containing 1.5 M NaCl, and in 1 M Tris-HCl (pH 8.0) containing 1.5 M NaCl. Chromosomal DNAs in the gel were transferred onto a nylon membrane filter (BRL Co.) according to the method of Maniatis et al. [11]. Prehybridization (42°C, 3 h) and hybridization (42°C, 15 h) were carried out as described previously [12]. For detection, a PhotoGene Nucleic Acid Detection System kit (BRL Co.) was used according to the recommendations of the vendor.

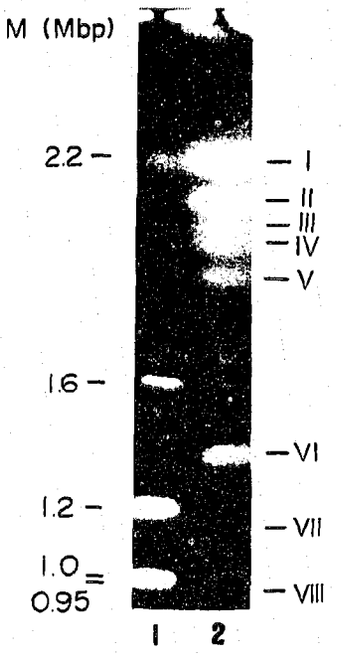

Fig. 1. *C. tropicalis* chromosomes separated by the CHEF system. Lane 1, chromosomes of *S. cerevisiae* as size markers; lane 2, chromosomes of *C. tropicalis*.

3. RESULTS AND DISCUSSION

The CHEF system was used for the resolution of chromosomes of *C. tropicalis* pK 233, because this technique is effective to analyze DNA molecules of a large size, if the operational conditions including the switch intervals for pulsed-field gel electrophoresis are properly adjusted [13,14]. The agarose gel (14.0 × 12.7 cm) was run with a 120-s switch at 150 V (120–130 mA) for 20 h, and then a 600-s switch at 80 V (60–70 mA) for 96 h, followed by a 3600-s switch at 40 V (20–30 mV) for 40 h. Such conditions seemed to provide the maximum resolution. We could detect eight electrophoretically resolved bands from *C. tropicalis* (Fig. 1). Using the size marker from *S. cerevisiae*, the size of the respective bands was determined as follows: 2.2, 2.1, 2.0, 1.9, 1.8, 1.3, 1.1, and 0.9 Mbp, accounting for 13.3 Mbp totally. We numbered them from I to VIII in the order of the size.

Fig. 2 shows the results of a series of Southern blot analyses using the DNA probes of catalase, acyl-CoA oxidase, acetoacetyl-CoA thiolase, 3-ketoacyl-CoA thiolase, carnitine acetyltransferase, isocitrate lyase, and malate synthase. The catalase gene was located on chromosomal bands I and II; the acyl-CoA oxidase gene, III and IV; the acetoacetyl-CoA thiolase gene, I; the 3-ketoacyl-CoA thiolase gene, II; the carnitine acetyltransferase gene, II; the isocitrate lyase gene, I and II; and the malate synthase gene, V. The acyl-CoA oxidase genes which form a multi-gene family [5] were detected on two chromosomal bands. As for the

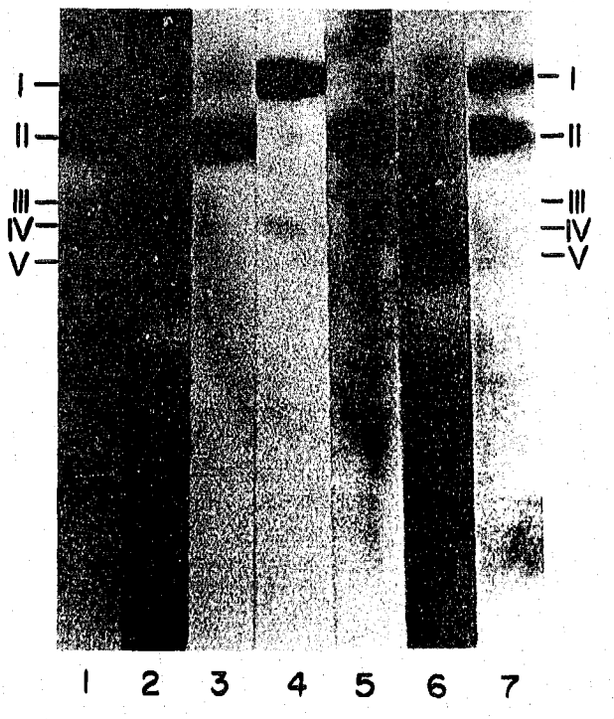

Fig. 2. Assignment of the cloned genes encoding several peroxisomal enzymes on resolved chromosomal bands. Southern blot analysis was carried out with probes of the catalase gene (lane 1), acyl-CoA oxidase cDNA (lane 2), 3-ketoacyl-CoA thiolase cDNA (lane 3), acetoacetyl-CoA thiolase cDNA (lane 4), carnitine acetyltransferase cDNA (lane 5), malate synthase gene (lane 6), and isocitrate lyase gene (lane 7).

catalase and isocitrate lyase genes, we have already isolated one genomic DNA from *C. tropicalis* and proved the presence of only one transcript and one protein in the cells, respectively [8,9]. However, there are possibilities that these genes might form multi-gene families, respectively, and that these transcripts and proteins would be almost the same and indistinguishable even if both genes were expressed. The genes for two thiolases which participate in the fatty acid β-oxidation system were located on different chromosomes. The genes for acyl-CoA oxidase and thiolases, involved in the fatty acid β-oxidation system, were not located on one chromosome. The genes for isocitrate lyase and malate synthase, which are the key enzymes of the glyoxylate cycle, were not on the same chromosome. The gene for carnitine acetyltransferase jointing the fatty acid β-oxidation system in peroxisomes to both the TCA cycle in mitochondria and the glyoxylate cycle between both organelles was detected on chromosomal band II, on which the gene for 3-ketoacyl-CoA thiolase producing acetyl-CoA was. From these facts, it became clear that the tested genes encoding peroxisomal enzymes were not necessarily located on the same chromosome, in spite of the fact that these enzymes were simultaneously and inducibly synthesized and had an intimate metabolic relationship with alkane assimilation. The non-cistronic distribution of the genes suggested that the co-ordinated expression of the genes encoding peroxisomal enzymes would be mainly regulated by some *trans*-acting factors induced in the cells when the cells are assimilating alkanes or fatty acids, although several genes were found on chromosomal band II.

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