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Isolation of several cDNAs encoding yeast peroxisomal enzymes

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Several candidate clones carrying partial cDNAs for yeast peroxisomal enzymes, such as catalase, carnitine acetyltransferase, isocitrate lyase, malate synthase and acyl-CoA oxidase, were efficiently isolated at a single plating from a phage λgt11 recombinant cDNA library prepared with poly(A)-rich RNA from an *n*-alkane-grown yeast, *Candida tropicalis*, with a mixture of antibodies against the respective purified enzymes. Among them, one candidate clone carrying partial cDNA for catalase was subcloned and subjected to nucleotide sequence analysis. We succeeded in determining that the amino acid sequence deduced from the nucleotide analysis included the sequences derived from the two peptide fragments obtained from the purified enzyme.

cDNA expression library; DNA sequence; Peroxisomal enzyme; Catalase; Alkane-utilizing yeast; *(Candida tropicalis)*

1. INTRODUCTION

We are investigating the biogenesis and development of peroxisomes with an *n*-alkane-assimilating yeast, *Candida tropicalis* [1]. The yeast peroxisomes seem to develop by division, the number of the organelles in the cells increasing during alkane utilization. The yeast system will be very useful to study the development of the organelles in comparing the mammalian system [2], because their development and degradation are easily controlled by changing the growth substrates.

Catalase [3], a marker enzyme of peroxisomes, carnitine acetyl transferase [4,5], isocitrate lyase [6] and malate synthase [7], which we have purified and the properties of which we have examined, are all encoded by nuclear genes, inducibly synthesized and specifically localized in peroxisomes in harmony with the development of the organelles. Synthesis in vitro in the rabbit reticulocyte system with mRNA from alkane-grown *C. tropicalis* cells demonstrated that the size of nascent catalase was indistinguishable from that of mature catalase [8], although we could not exclude the presence of an extra short peptide related to its specific transportation. On the other hand, the size of nascent carnitine acetyltransferase was different from that of mature peroxisomal and mitochondrial enzymes and the precursor was sorted and processed post-translationally [9]. Cytosolic isocitrate lyase and malate synthase detected in the propionate-grown cells of *C. tropicalis* were not different from the peroxisomal enzymes [10].

This paper deals with the isolation of the genes for the peroxisomal enzymes from the yeast cDNA expression library using antibodies and the partial characterization of cDNA for catalase.

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2. MATERIALS AND METHODS

2.1. *Preparation of the cDNA recombinant library of* C. tropicalis

cDNAs were prepared by the method of Norgard et al. [11] by using 20 μg poly(A)-rich

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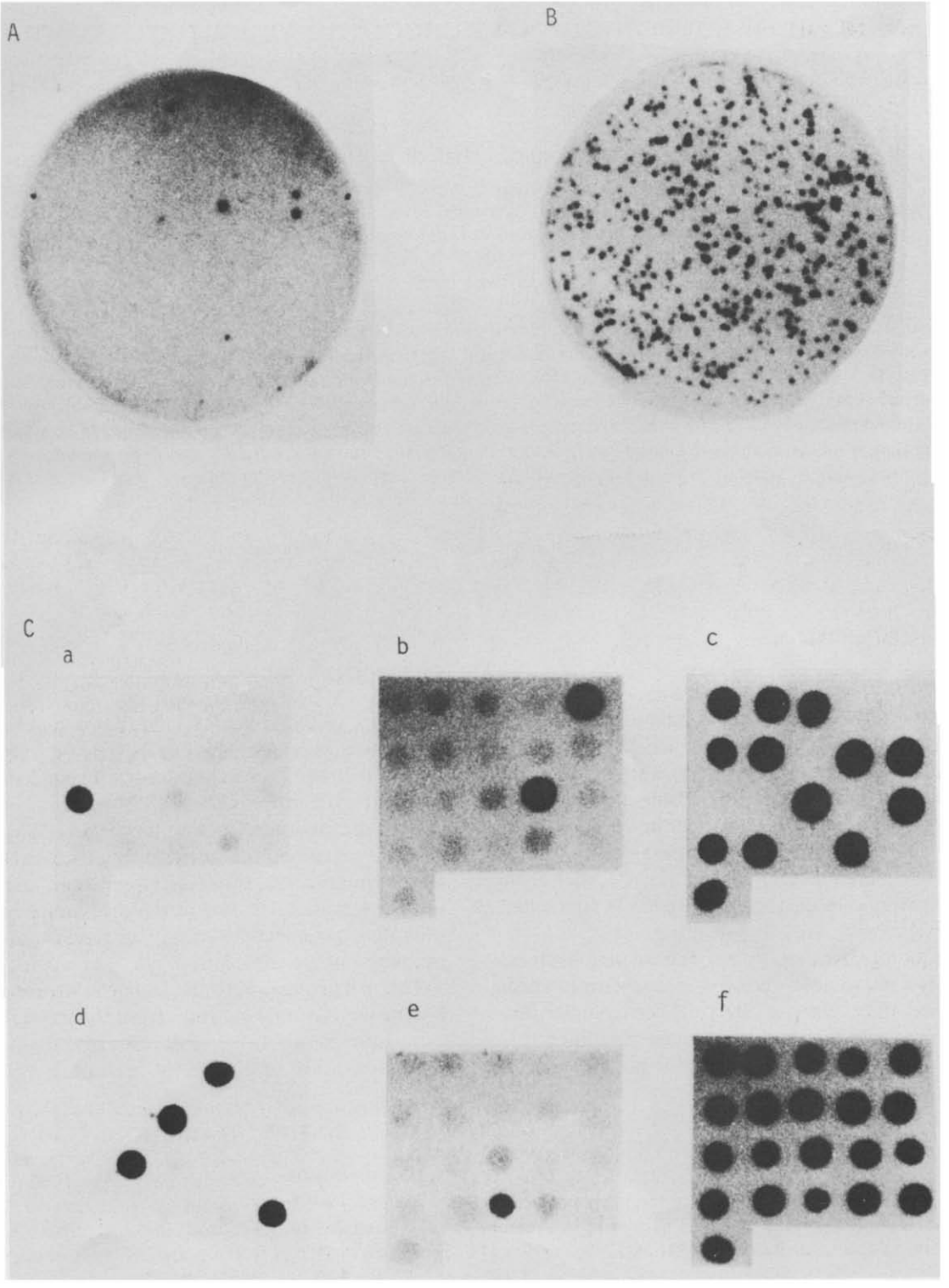
A
B
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RNA from an *n*-alkane-grown yeast, *C. tropicalis* [9]. An expression library with λgt11 [12,13] was prepared as follows: cDNAs ligated with synthetic *Eco*RI linkers were inserted into *Eco*RI-cleaved and phosphatase-treated λgt11 DNA with T_4 ligase. Phage was packaged and amplified in *Escherichia coli* Y1088.

2.2. *Screening of a λgt11 recombinant yeast cDNA library for peroxisomal enzyme candidate clones*

Plaques (1×10^3) on 90 mm plates were screened by using *E. coli* Y1090 according to the method of Young and David [13]. Rabbit antisera against the purified yeast peroxisomal enzymes were used for screening of the clones on nitrocellulose filters transferred from the plates.

2.3. *Sequencing of amino acids and nucleotides*

Amino acid sequences of several peptide fragments obtained after hydrolysis with trypsin and separation by HPLC were analyzed with a gas-phase amino acid sequencer. The isolated and subcloned cDNA was subjected to nucleotide sequence analysis with the dideoxy chain-termination method using M13 vectors [14].

2.4. *RNA blot hybridization analysis*

Total RNA from the yeast cells was denatured with 1 M glyoxal and 50% dimethylsulfoxide, electrophoresed on a 1.5% agarose gel and transferred to a nitrocellulose filter [15]. After the identified cDNA was labelled with a nick-translation system, it was used as the hybridization probe.

3. RESULTS

Rabbit antisera directed respectively against purified catalase, carnitine acetyltransferase, isocitrate lyase, malate synthase and acyl-CoA oxidase were mixed and used to probe a λgt11 library of the yeast cDNA. In a screening of 5×10^3 recombinants, 21 independent clones that produced strong signals were isolated (fig.1A). Respective cDNA clones were further screened until all plaques on the plate produced a signal with the antiserum mixture (fig.1B). Finally, these isolated clones were classified with the respective antisera

(A)

	Amino acid sequence
Native enzyme (N-terminal)	H_2N-Ala-Pro-Thr-Phe-Thr-Asn-Ser-Asn-Gly-Gln-
	Pro-Ile-Pro-Glu- ? -Phe-Ala- ? -Gln- ? -
	? -Gly-Gln-
Fragment	
1	Thr-Leu-Thr-Asn-Glu-Glu-Ala-Gly-Ser-Leu-
	Ala-Gly-Ser-Asn-Pro-
2	Gln-Phe-Ser-Leu-Gln-Glu-Asp-Gln-Glu-Val-
3	Asn-Tyr-Phe-(?)-Ala-Glu-Val-
4	Gln-Ala-Thr-Glu-Leu- ? -Lys-
6	Gly-Phe-Ala-Thr-Lys-

(B)

EcoRI ⟶

------GCGGAATTCAAGCAATTCTCCCTCCAAGAAGACCAAGAAGTCTGGCACGGTGCTGCTACTCCATTCCAC

GlnPheSerLeuGlnGluAspGlnGluValTrpHisGlyAlaAlaThrProPheHis

Fragment 2

TGGAAGGCTACCCCAGCTGATTTCAAGCAAGCCACTGAATTGTGGAAGGTGTTGAAGAAATATCCAAACCAACAA

TrpLysAlaThrProAlaAspPheLysGlnAlaThrGluLeuTrpLysValLeuLysLysTyrProAsnGlnGln

Fragment 4

GAACACTTGGCCCACAATGTTGCTGTCCATGCCTCTGCTGCC------

GluHisLeuAlaHisAsnValAlaValHisAlaSerAlaAla------

Fig.2. Comparison of the amino acid sequences obtained from peptide fragments of purified catalase and deduced from nucleotide sequence analysis of cDNA inserted in λgt11 DNA. (A) Amino acid sequences of several peptide fragments obtained after trypsin digestion of the purified enzyme. The amino acid sequence from the N-terminus was obtained with the intact enzyme. (B) The DNA sequence near the *Eco*RI site in the vector and the deduced amino acid sequence. The underlined regions show the amino acid sequences which coincide with those from the purified enzyme.

Fig.1. Screening of a λgt11 recombinant *C. tropicalis* cDNA library for peroxisomal enzyme clones. (A) Representative plaques (1×10^3) on a 90 mm plate were screened as described in [13] with the use of ^{125}I-labeled protein A and a 1 : 1000 dilution of a mixture of the respective rabbit antisera against catalase, carnitine acetyltransferase, isocitrate lyase, malate synthase and acyl-CoA oxidase. (B) A representative filter after plating and screening repeatedly until all plaques on the plate produced a signal. (C) Probing recombinant cDNA clones for catalase (a), carnitine acetyltransferase (b), isocitrate lyase (c), malate synthase (d), acyl-CoA oxidase (e) and all of the clones (f). 21 λgt11 recombinant cDNA clones were plated in drops of 10^2 PFU on *E. coli* Y1090. The arrays were probed as described in [13] using the respective antisera. Non-recombinant λgt11 phage did not produce the positive signal.

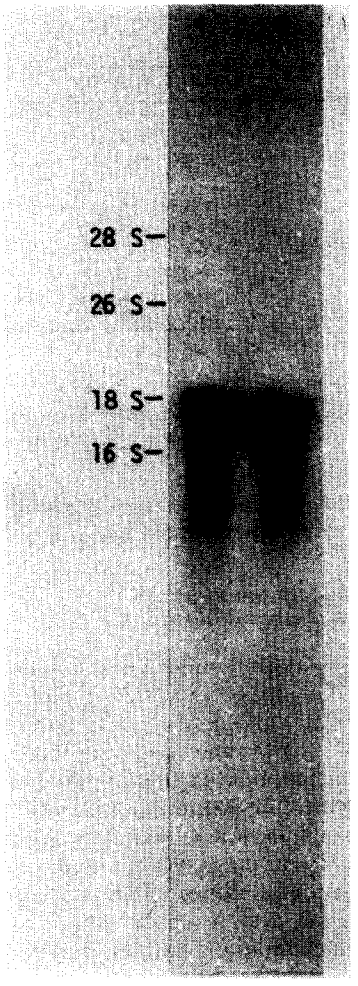

Fig.3. Autoradiogram of blot hybridization analysis of *C. tropicalis* RNA with a cDNA probe for catalase. Total RNA (lane 1, 20 μg; lane 2, 10 μg) from *n*-alkane-grown *C. tropicalis* was analyzed by using calf and *E. coli* rRNA as the size markers.

as shown in fig.1C (one clone for catalase, two clones for carnitine acetyltransferase, thirteen clones for isocitrate lyase, four clones for malate synthase and one clone for acyl-CoA oxidase). The respective groups were found not to be superimposed on each other and candidate clones for all peroxisomal enzymes examined were obtained.

A candidate clone for catalase, for example, carried a cDNA insert of approximately 600 base pairs, which was shorter than the full-length DNA predicted from the size of the protein. The nucleotide sequence of this cDNA insert was partially analyzed with the method of M13 sequencing and compared with the amino acid sequences of peptide fragments obtained from the purified catalase protein (fig.2). Two of them completely coincided with the amino acid sequence deduced from the cDNA. The results indicated that this clone certainly harboured the cDNA sequence for *C. tropicalis* catalase. This nucleotide sequence was detected in the genomic DNA for the enzyme (not shown).

We have also confirmed that several clones for isocitrate lyase carried cDNA corresponding to the partial fragments of the enzyme (not shown).

Blot hybridization analysis of the yeast total RNA with the cDNA probe for catalase revealed only one hybridization positive band with an estimated size of approximately 1.85 kb (fig.3). This RNA has been indicated as representing the mature mRNA encoding the catalase protein of the yeast. The size is enough for the active enzyme to be expressed.

4. DISCUSSION

Candidate clones for catalase, carnitine acetyltransferase, isocitrate lyase, malate synthase and acyl-CoA oxidase were efficiently isolated from only 5×10^3 plaques in the λgt11 recombinant cDNA library of *n*-alkane-grown *C. tropicalis* at a single plating. Comparison of the partial amino acid sequences obtained from the purified enzyme and those deduced from cDNA strongly indicated that, of 21 clones, several clones examined carried cDNAs encoding partial fragments of catalase (this work) and isocitrate lyase (not shown), respectively. Blot hybridization analysis showed that one species of catalase was expressed clearly as a peroxisomal form and that genomic DNA encoding catalase may contain a rather short non-coding region. The latter observation was also confirmed from the analysis of the genomic DNA for catalase (not shown).

This expression library was very convenient for the isolation and characterization of the yeast genes encoding peroxisomal enzymes.

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