Expression Cloning of Catalase Genomic Gene: Genomic DNA Expression Library of *Candida boidinii* in *Saccharomyces cerevisiae*

By

Jian ZHENG*, Haruyuki ATOMI*, Mitsuyoshi UEDA* and Atsuo TANAKA*

(Received June 18, 1992)

Abstract

The genomic DNA expression library of a methylotrophic yeast, *Candida boidinii* (*Kloeckera* sp.) 2201, was prepared in *Saccharomyces cerevisiae* by the electroporation method. Five transformants harbouring a catalase gene were independently isolated with an anti-*C. boidinii* catalase antibody from this library. Furthermore, exhibition of catalase activity in these transformants demonstrated that *C. boidinii* genes could sufficiently function even in *S. cerevisiae*.

1. INTRODUCTION

Catalase is one of the essential enzymes involved in the metabolism of methanol in a methanol-assimilating yeast, *Candida boidinii* (*Kloeckera* sp.) 2201.[1] This enzyme exclusively exhibits peroxidatic activity (not catalatic activity) to utilize the molecular oxygen efficiently for methanol oxidation by coupling with alcohol oxidase in the peroxisomes. Peroxisomal enzymes including catalase are inducibly synthesized and specifically localized in peroxisomes when the yeast is grown on methanol.[2,3] The analysis of these mechanisms on the molecular level is very attractive.

Expression cloning of the genomic gene derived from *Saccharomyces cerevisiae* in the same yeast has been carried out.[4] Recently, there have been several reports demonstrating that some of *Candida* genes are functional in *Saccharomyces* yeasts.[5,6] In this paper, we constructed a genomic DNA expression library of the methylotrophic yeast, *C. boidinii*, with a convenient transformation method, "eletroporation", in *S. cerevisiae* and expression cloning of the genomic gene encoding catalase was examined by using catalase antibody.

* Department of Industrial Chemistry, Kyoto University, Kyoto, Japan.

2. MATERIALS AND METHODS

Strains and medium.

Saccharomyces cerevisiae MT8–1 (*his, leu, ade, trp, ura*) was used as the host cell. The methylotrophic *Candida boidinii* (*Kloeckera* sp.) 2201 was cultivated in the medium reported previously.[7]

Isolation of genomic DNA.

The isolation of genomic DNA from *C. boidinii* cells was carried out by the method described previously.[8] The *C. boidinii* cells cultivated for 12 h on the medium containing glucose were used for this purpose.

Enzyme assay and protein estimation.

Catalase activity was measured spectrophotometrically according to the previous paper.[9] Protein was determined by the method of Lowry *et al.*.[10]

3. RESULTS AND DISCUSSION

Preparation of recombinant plasmids

The genomic DNA from *C. boidinii* 2201 was partially digested with the restriction enzyme *Sau* 3AI (Toyobo Co., Osaka, Japan). DNA fragments with the size of 2–12 kbp were fractionated with agarose gel electrophoresis followed by electroelution. The plasmid pMT34–(+ 3) is a shuttle vector containing Amp (β-lactamase gene), 2 μm DNA origin of replication, URA3 and *GAL7* promoter gene.[11] After digestion with *Bgl* II, the vector DNA was treated with the bacterial alkaline phosphatase (BAP). This vector DNA was ligated with the genomic DNA fragments prepared and transformed to the host cell, *S. cerevisiae* MT8–1 (Fig. 1).

Transformation and construction of genomic DNA library

Unlike transformation of *E. coli* cells, that of yeast cells is generally difficult. Most of yeast genomic DNA libraries have been constructed in *E. coli* cells with help of phages or in yeasts using the protoplast-transformation method. In the latter case, the yeast cells have been usually treated with a lytic enzyme, followed by their subsequent cell-wall regeneration in solid media. However these treatments are tedious and time-consuming. To construct the *C. boidinii* genomic DNA expression library, two methods of transformation were examined. The lithium acetate method[12] was first applied, but the transformation efficiency was too low to construct the library in *S. cerevisiae*. An electroporation method,

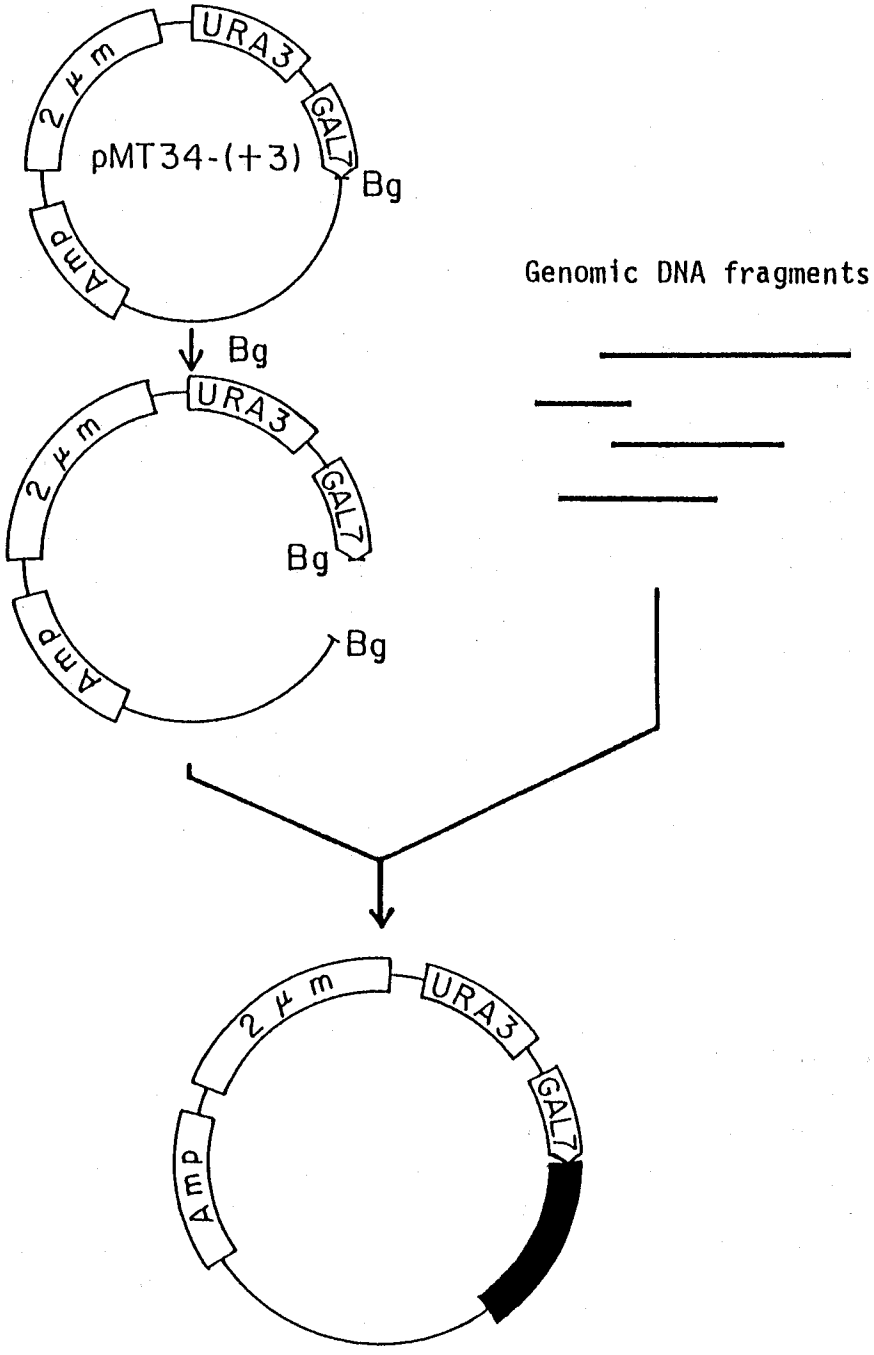

Fig. 1 Construction of the recombinant DNA. Bg, the site of the restriction enzyme *Bgl* II.

a convenient transformation method, was then carried out. Host *S. cerevisiae* cells (100 ml broth, $A_{600} = 1.2$) cultivated for 12 h in the YPD medium (1 % (w/w) yeast extract, 1 % peptone and 2 % glucose) were washed twice with cold water and once with 1 M cold sorbitol solution, then suspended in 0.5 ml of 1 M cold sorbitol solution. An aliquot (60 μl) of the suspension was mixed with 1 μl of plasmid DNA solution (0.15 – 1.0 μg DNA). After standing for 5 min on ice, the mixture was applied in a 0.2 cm cuvette of the electroporation apparatus (Gene Pulser, BIO-RAD Laboratories, Richmond, CA, USA), and treated with an electric pulse under the conditions to be mentioned below. The cells treated were immediately mixed with 1 ml of 1 M cold sorbitol solution, spread on SD (0.67 % Bacto-yeast nitrogen base w/o amino acids, 2 % glucose) plates containing 1 M sorbitol solution and incubated at 30°C for 2–3 days.

The transformation efficiency was significantly affected by the voltage supplied in the electric pulsed field (Table 1). In our case using pMT 34–(+ 3) (8.6 kbp), the maximum transformation efficiency was obtained at 1.0 kV, 25 μF of capacitance, 200 ohm of current resistance and 4.3 ms of pulse time. The sorbitol solution contained in the selective plates as well as the cell concentration was very essential to provide osmotic support for the cells. The number of colonies obtained was satisfactory to screen the clones harbouring the *C. boidinii* catalase gene.

Table 1. The effect of voltage on transformation efficiency of pMT 34–(+ 3) with the electroporation method.

Voltage (kV)	Cell survival (%)	Transformation efficiency (colonies μg DNA^{-1})
1.3	19	1857
1.0	38	4214
0.9	43	1500
0.7	37	214

Screening and identification of clones harbouring the *C. boidinii* catalase gene

The *C. boidinii* genomic DNA expression library (20,000 colonies) was screened directly with the anti-*C. boidinii* catalase antibody, with the similar method reported in *E. coli*,[13] except for using Zymolyase 100 T for lysis and the ProBlot immunoscreening system kit (Promega Co., Madison, WI, USA) for detection. Some of the colonies which produced positive signals were picked up and purified. The DNAs of five transformants finally obtained were isolated and digested with *Eco* RI. Southern blot analysis using the probe DNA of *C. tropicalis* catalase[1,14] indicated that these transformants harboured *C. boidinii* catalase gene (data not shown). To examine whether or not these transformants express catalase proteins, catalase activity was measured using their cell-free extracts prepared with

Table 2. Catalase activities in transformants obtained.

Yeast cells	Activity before induction	Activity after induction with		
		none	galactose	methanol
		(μmol · min^{-1} · mg protein^{-1})		
Host	nil	nil	13.5	nil
Transformant				
No. 1	90.5	98.0	104	124
No. 2	103	94.5	82.3	114
No. 3	121	121	101	128
No. 4	90.4	126	98.8	138
No. 5	90.5	90.8	100	98.8

the method described previously.[15] After the transformants cultivated for 15 h on YPD medium were washed and suspended in deionized water, they were inoculated into SGlyLac medium (0.67% Bacto-yeast nitrogen base w/o amino acids, 2% glycerol, 2% sodium DL-lactate and 0.04% casamino acid).[11] After 40 h cultivation, 2% galactose or 0.5% (v/v) methanol was added as the inducer into the broth. The cells, after 10 h, were harvested and suspended in 50 mM potassium phosphate buffer (pH 7.2). The cells were disintegrated by sonication.[15] The supernatant obtained after centrifugation at 10,000 x*g* for 15 min was used as the cell-free extract. As shown in Table 2, these transformants clearly exhibited far higher catalase activities than the host cell, *S. cerevisiae* MT8–1. Induction of catalase with galactose was not observed, but the addition of 1% methanol resulted in a slight increase in activity. *GAL7* promoter originally harboured in plasmid pMT 34–(+ 3) was not functional, but some upstream activating regions incorporated together with the coding regions from the *C. boidinii* genomic DNAs might be functional in *S. cerevisiae*. It will be necessary to analyze the plasmids from the transformants and the coding, 5′-flanking and 3′-flanking regions of the genes.

In conclusion, some of *C. boidinii* genes including coding and flanking regions can be expressed in *S. cerevisiae* and the expression cloning reported here was demonstrated to be possible.

Acknowledgements

We wish to thank Prof. T. Fukasawa, Keio University, for his generous gift of *S. cerevisiae* MT8–1 and plasmid pMT 34–(+ 3).

References

1) S. Mozaffar, M. Ueda, K. Kitatsuji, S. Shimizu, M. Osumi and A. Tanaka; Eur. J. Biochem., **155**, 527 (1986)
2) A. Tanaka, S. Yasuhara, M. Osumi and S. Fukui; Eur. J. Biochem., **80**, 193 (1977)

3) S. Fukui and A. Tanaka; J. Appl. Biochem., **1**, 171 (1979)
4) N. Shiomi, H. Fukuda, H. Morikawa, Y. Fukuda and A. Kimura; Appl. Microbiol. Biotechnol., **29**, 302 (1988)
5) C. Chen, T. Turi, D. Sanglard and J. C. Loper; Biochem, Biophys. Res. Commun., **146**, 1311 (1987)
6) D. Sanglard, I. Beretta, M. Wagner, O. Käppeli and A. Fiechter; Biocatalysis, **4**, 19 (1990)
7) S. Fukui, A. Tanaka, S. Kawamoto, S. Yasuhara, Y. Teranishi and M. Osumi; J. Bacteriol., **123**, 317 (1975)
8) M. Ueda, M. Hikida, H. Atomi and A. Tanaka; Mem. Fac. Eng., Kyoto Univ., **52**, 15 (1990)
9) T. Yamada, A. Tanaka and S. Fukui; Eur. J. Biochem., **125**, 517 (1982)
10) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall; J. Biol. Chem., **193**, 265 (1951)
11) H. Shimada, T. Fukasawa and Y. Ishimura; J. Biochem., **105**, 417 (1989)
12) H. Ito, Y. Fukuda, K. Murata and A. Kimura; J. Bacteriol., **153**, 163 (1983)
13) D. M. Helfman, J. R. Feramisco, J. C. Fiddes, G. P. Thomas and S. H. Hughes; Proc. Natl. Acad. Sci. USA, **80**, 31 (1983)
14) H. Okada, M. Ueda, T. Sugaya, H. Atomi, S. Mozaffar, T. Hishida, Y. Teranishi, K., Okazaki, T. Takechi, T. Kamiryo and A. Tanaka; Eur. J. Biochem., **170**, 105 (1987)
15) M. Ueda, H. Okada, A. Tanaka, M. Osumi and S. Fukui; Arch. Microbiol., **136**, 169 (1983)