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A simple method for extraction of DNA from fungi and yeasts with anhydrous hydrogen fluoride

Satoru Oshiro, Nobuhiko Katsura, Kunio Kitada* and Norio Gunge*

Department of Oral Biochemistry, School of Dentistry, Nagasaki University, 7-1, Sakamoto-machi, Nagasaki 852 and **Department of Applied Microbial Technology, Kumamoto Institute of Technology, 4-22-1, Ikeda, Kumamoto 860, Japan*

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A novel method is described for the extraction of DNAs from fungi and yeasts. Anhydrous hydrogen fluoride (HF) selectively cleaves their cell walls under mild conditions (for 5 min at 0°C), enabling the effective extraction of DNAs from organisms with a cell wall. A possible mechanism for this method concerning the selective cleavage of O-glycosidic linkages in cell walls has been described previously [(1977) Anal. Biochem. 82, 289–309]. The extracted DNA is intact: in fact, the yeast DNA is directly applicable for restriction analysis and transformation of *Escherichia coli*.

Hydrogen fluoride; DNA; Cell wall; (*Saccharomyces cerevisiae*, Fungi, Yeast)

1. INTRODUCTION

Until now, the most popular method for the extraction of DNA from fungi or yeasts has been the protoplast lysate procedure using cell wall lytic enzymes [1,2]. This procedure is simple and efficient but involvees limitations in that a range of species are susceptible to lytic enzymes. It has been reported that HF cleaves the *O*-glycosidic linkages of polysaccharides [3]. We therefore applied this chemical method to the disruption of fungal and yeast cell walls for DNA extraction. Our procedure is a technically simple method for isolating fungal DNA and also yeast plasmid and chromosomal DNA. Here, we describe a novel method for the extraction of DNA from microbes and compare it with the established technique.

Correspondence (present) address: S. Oshiro, Department of Biochemical Genetics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo 113, Japan

Abbreviations: YPG, 0.5% yeast extract, 1% polypeptone and 2% glucose, pH 6.0; TE, 10 mM Tris, 1 mM EDTA, pH 8.0; HF, anhydrous hydrogen fluoride.

2. MATERIALS AND METHODS

2.1. *Strains, plasmids and media*

Chaetomium gracile SANK 18667 (a gift from Sankyo Co., Ltd, Tokyo) was cultured as in [4]. *Candida albicans* serotype A, strain K [5] (provided by Dr Aoki), was cultured aerobically in YPG medium. *Saccharomyces cerevisiae* F102 is a cell fusant of *S. cerevisiae* AH22 *(a his4 leu2)* with *Kluyveromyces lactis* carring the linear DNA killer plasmids, pGKL1 and pGKL2 [6]. Cells were grown in YPG medium and the DNA extracted by HF treatment for digestion with restriction enzymes.

S. cerevisiae AH22 carring plasmid YEp13 (selective markers: *LEU2* Ap^r Tc^r [7] and *Schizosaccharomyces pombe* JY226 (h^+ *ural his2 leu1*) carring plasmid pYGU1 (selective markers: *LEU2 URA3*) [8] were grown in selective medium and subjected to DNA extraction for *E. coli* transformation. *E. coli* strain C600 (*leuB thr thi*)

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was used for transformation. Plasmids YEp13 and pYGU1 are shuttle vectors which replicate in *S. cerevisiae, Sch. pombe* and *E. coli*. Logarithmically growing fungal and yeast cells were harvested by centrifugation, resuspended in water, and then the centrifuged and pelleted cells (wet wt 0.6–1.0 g) were subjected to with treatment HF after lyophilization. After 17 h lyophilization, the pressure and temperature of the bottles used for freeze-drying reached about 3 Pa and room temperature (16°C), respectively.

2.2 *HF treatment of fungal and yeast cell walls*

Dried cells were treated with HF according to [3] in an HF reaction apparatus (type II, Protein Research Foundation, Osaka), consising of an HF cylinder, reservoir vessel, reaction vessel and trap filled with calcium oxide. The dried samples were added to the reaction vessel which was then evacuated. The pressure in the entire line was maintained at 20 mmHg. After introducing HF from the cylinder into the reservoir vessel, samples were exposed to HF in the reaction vessel at the appropriate temperature. When performing the reaction at 0°C, the reaction vessel was chilled using an ice bath. At completion of the reaction, the reaction vessel was evacuated via the calcium oxide trap to remove HF.

2.3. *Isolation of DNA from HF-treated fungal and yeast cells*

HF-treated cells were suspended in 0.1 M Tris-HCl, 0.1 M NaCl, 1% SDS (pH 8.0) and centrifuged at 5000 rpm for 10 min. The resulting supernatant was used as the sample for DNA isolation. The DNA fraction was prepared as in [9] except that guanidine-HCl was substituted for SDS.

2.4. *Isolation of DNA from yeast cells by an established method*

The procedure for yeast isolation essentially follows that of [9], developed for the rapid isolation of yeast plasmid and chromosomal DNA.

2.5. *Restriction enzyme digestion*

S. cerevisiae F102 DNA extracted by treatment with HF was digested with *Eco*RI, *Hin*dIII and *Bam*HI using 14–28 U enzyme/μg yeast DNA, samples being incubated for 2 h at 37°C. Restriction fragments were subjected to 0.3% agarose gel electrophoresis. The gel was stained with ethidium bromide.

2.6. *Transformation of* E. coli

E. coli transformation was carried out according to the $CaCl_2$-mediated transformation technique of Mandel and Higa [10].

3. RESULTS AND DISCUSSION

The size of fungal DNAs extracted after various HF treatments was determined by agarose gel electrophoresis. Severe conditions with HF (24°C, 6 and 1 h) destroyed not only the fungal cell wall but also the DNA. A milder treatment with HF (0°C, 1 h) caused deavage of the DNA to a lesser extent. The mildest treatment with HF (0°C, 5 min) gave high-M_r DNA. We obtained a reproducible yield of about 0.3–0.4 mg DNA/g wet wt by HF treatment of *C. gracile*. This value is comparable to that obtained using French press treatment [11]. The recovery of *S. cerevisiae* DNA after treatment with HF was 4 μg DNA/3 $\times$ 10^8 cells or approx. 45% of total DNA. This recovery was based on an estimate of the DNA content of *S. cerevisiae* made by Holm et al. [9].

As shown in fig.1 DNAs prepared by the HF method from fungi (*C. gracile*, lane 2; *Ca. albicans*, lane 3) were of high molecular size. To investigate the effectiveness of the HF extraction procedure in the isolation of fungal and yeast DNA, we attempted to extract yeast DNA from three different yeast strains. The strains were chosen as model yeasts, since they have well-characterized plasmid DNAs. *S. cerevisiae* F102 carries two linear plasmids, viz. pGKL1 and PGKL2. The former contains one recognition site for each of *Eco*RI, and *Bam*HI and two for *Hin*dIII. The latter strain contains one *Bam*HI site and six *Eco*RI sites [8]. Therefore, the purity and integrity of DNA after HF extraction were checked by means of digestion with these endonucleases (*Eco*RI, *Hin*dIII, *Bam*HI) and agarose gel electrophoresis. As shown in fig.2, digestion of the chromosomal and plasmid DNAs resulted in several DNA fragments, indicating that the restriction patterns of the plasmids pGKL1 and PGKL2 were identical to those of preparations following the previously published protoplast lysate procedure (not shown). These results were compatible

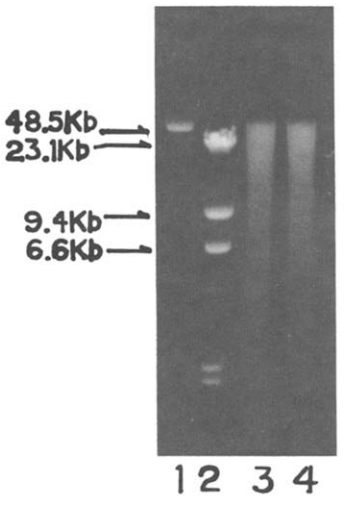

Fig.1. Electrophoresis of isolated fungal DNA. Fungal DNA was isolated after treatment with HF at 0°C for 5 min and subjected to electrophoresis in 0.3% agarose gel at 1 V/cm for 16 h in a cold room. Lanes: 1, λ DNA; 2, λ DNA digested with *Hin*dII; 3, DNA isolated from *C. gracile*; 4, DNA isolated from *Ca. albicans*.

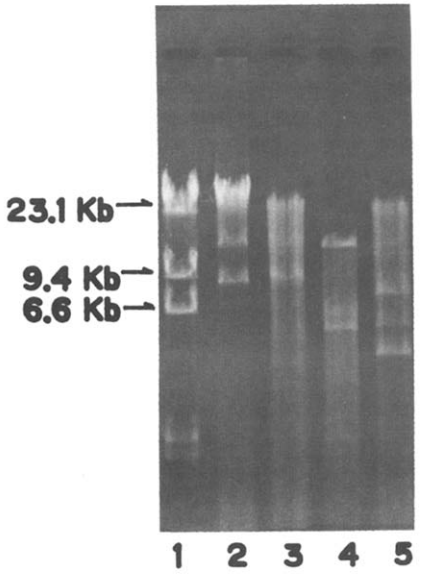

Fig.2. Restriction endonuclease fragment patterns of DNA extracted from *S. cerevisiae* F102 after HF treatment. Lanes: 1, λ DNA digested with *Hin*dII; 2, DNA from *S. cerevisiae* F102 carrying the linear plasmids pGKL1 (8.9 kb) and pGKL2 (13.4 kb); 3, yeast DNA partially digested with *Eco*RI; 4, yeast DNA digested with *Hin*dIII; 5, yeast DNA digested with *Bam*HI.

Table 1

Transformation of *E. coli* with DNA from yeast extracted by HF treatment

DNA source[a]	Selection	No. of transformants
AH22(YEp13)	Ap^r	317
AH22(YEp13), protoplast lysate	Ap^r	290
JY226(pYGU1)	LEU	43
JY226(pYGU1), protoplast lysate	LEU	30

[a]Details of plasmid and yeast strains are given in section 2.1

with the restriction nuclease maps of pGKL1 and pGKL2. Thus, HF treatment caused no chemical degradation of the extracted DNAs.

We also attempted to transform *E. coli* with DNA extracted from yeasts carring plasmids (table 1). *E. coli* C600 cells were transformed with 20 μl TE containing DNA from *S. cerevisiae* AH22 carrying plasmid YEp13, selecting Ap^r on complete medium (LB broth agar) containing ampicillin (50 μg/ml). As shown in table 1, 317 Ap^r transformants were obtained. Furthermore, *E. coli* C600 cells were transformed with 20 μl TE containing DNA from *Sch. pombe* JY226 bearing plasmid pYGU1, selecting LEU^+ on minimal medium (M9 agar) without leucine. As shown in table 1, 43 LEU^+ transformants were obtained. The transformation frequency was almost the same as that of DNA extracted using the conventional protoplast lysate procedure.

From these results, we conclude that yeast DNAs extracted by employing HF treatment are biologically intact and are able to transform *E. coli* to Ap^r and LEU^r according to the plasmids employed. This newly developed HF method should be useful for the extraction of chromosomal and plasmid DNAs from fungi and yeasts, and may be applicable on an industrial scale.

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