Note

Intact Cell Transformation of
Saccharomyces cerevisiae
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A new yeast transformation method using intact cells instead of protoplasts has recently been developed.^{1,2)} This transformation method is based on the treatment of intact cells with alkali cations such as lithium acetate or thiol compounds. It has several advantages compared with the current protoplast method³⁾; for example, since lytic enzyme digestion of yeast cell walls can be avoided, regeneration agar is not necessary and transformant colonies appear significantly faster (2~4 days).

In the course of efforts to develop new yeast host vector systems we have found that intact cells treated with only polyethylene glycol (PEG) can be transformed. In this paper we present evidence for this observation.

The transformation procedure is principally based on the method reported by Ito *et al.*^{1,2)} The *Saccharomyces cerevisiae* strains, AH22 (*a leu2-3 leu2-12 his4-519 can1*), YNN27 (α *trp1 ura3 gal2*) and D13-1A (*a his3-532 trp1 gal2*), were precultured overnight in 1 ml of YEPD (1.0% yeast extract, 2.0% polypepton, 2.0% glucose, pH 5.3). Twenty ml of YEPD containing 0.4 ml of a precultured cell suspension was incubated at 30°C with shaking until cell growth reached the late log phase. Cells were then harvested by centrifugation at 1000 $\times g$ for 5 min and washed once with TE buffer (10 mM Tris-HCl buffer, pH 7.5, containing 1 mM EDTA). Then the cells were suspended at a concentration of 2×10^8 cells/ml in the same buffer. Five hundred μ l of TE buffer containing 0.2 M lithium acetate (lithium acetate method of Ito *et al.*^{1,2)}) or the same volume of TE buffer alone (polyethylene glycol method) was then added to 500 μ l of the cell suspension. After 1 hr incubation at 30°C with shaking, a 100 μ l portion of each cell suspension was added to the plasmid solution containing 10 μ g DNA. As DNA vectors the following plasmids were used: pDB248 (*leu2* for AH22, Ap^r, Tc^r for HB101), YRp7 (*trp1* for YNN27 and D13-1A, Ap^r, Tc^r for HB101) and G1053 (*ura3* for YNN 27. Ap^r,

Tc^r for HB101). After incubation for 30 min at 30°C, either 100 μ l of 70% PEG 4000 in TE buffer or the same volume of TE buffer containing no PEG (as a control) was added and mixed in carefully. These samples were further incubated at 30°C for 1 hr and then kept at 42°C in a water bath for 5 min. Each cell suspension was cooled to room temperature and washed twice with 1 ml of water. A 0.2 ml portion of this cell suspension was spread on a selection agar plate containing 0.7% yeast nitrogen base, 2.0% glucose and 50 μ g/ml of an amino acid or uracil (L-histidine for *Leu*⁺ and *Trp*⁺ transformants of AH22 and D13-1A, respectively, L-tryptophan for *Ura*⁺ transformants of YNN27, and uracil for *Trp*⁺ transformants of YNN27). The culture plates were then incubated at 30°C for 3~5 days and the colonies produced were counted as transformants. For the detection of the plasmid in the cells, each transformed *S. cerevisiae* strain was grown in 4 ml of the selection medium described above, and total DNA was extracted from the cells.⁴⁾ *Escherichia coli* strain HB101 cells were then transformed with these DNA

FIG. 1. Analysis of Plasmids in Transformants Obtained by the Polyethylene Glycol Method.

E. coli HB101 was transformed with DNA extracted from yeast transformants. Amplified plasmids in *E. coli* were analyzed by 0.7% agarose gel electrophoresis. The experimental conditions were given in detail in the text. In lanes 1~7, open circular (upper band) and closed circular (lower band) forms of plasmids can be seen. Lanes 1, 3 and 8 contain marker plasmids of PDB248, YRp7 and G1053, respectively. Lane 2 contains plasmids extracted from *E. coli* strain HB101, which had been transformed with DNA samples from yeast transformants of AH22+PDB248. The original yeast transformants from which DNA was isolated for lanes 4~7, 9 and 10 were YNN27 (ρ^+)+YRp7, YNN27 (ρ^+)+YRp7, D13-1A (ρ^+)+YRp7, D13-1A (ρ^+)+YRp7, YNN27 (ρ^+)+G1053 and YNN27 (ρ^+)+G1053, respectively.

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TABLE I. TRANSFORMATION OF *S. cerevisiae* STRAINS BY DIFFERENT PLASMID VECTORS
WITH THE LITHIUM ACETATE- AND POLYETHYLENE GLYCOL METHODS

A yeast strain containing mitochondrial DNA is designated as ρ^+ and a strain containing no mitochondrial DNA as ρ^- . + shows the treatment of yeast cells with lithium acetate (CH_3COOLi) or PEG. On the other hand, cells treated with buffer instead of CH_3COOLi or PEG are indicated as —.

Strain	CH_3COOLi	Plasmid	PEG	No. of transformants (per 10 μg DNA)	
				ρ^+	ρ^-
AH22	+	PDB248	—		0
	+		+		1420
	—		—		1
	—		+		313
YNN27	+	YRp7	—	2	1
	+		+	1341	506
	—		—	0	1
	—		+	309	15
YNN27	+	GI053	—	0	0
	+		+	139	14
	—		—	0	0
	—		+	235	91
D13-1A	+	YRp7	—	1	3
	+		+	967	93
	—		—	1	2
	—		+	682	12

samples.⁵⁾ Tetracycline and ampicillin resistant colonies were selected as transformants. After amplification in *E. coli*, plasmids were extracted from 5 ml of culture fluid (6) and analyzed by 0.7% agarose gel electrophoresis.⁷⁾

The transformation efficiencies were analyzed as to the following points; a) presence or absence of mitochondrial DNA (ρ^+ or ρ^- cells), b) difference of strains (AH22, YNN27 and D13-1A), c) difference of plasmids (PDB248, YNN27 and GI053), and d) difference of transformation methods (lithium acetate and PEG methods).

Table I and Fig. 1 show that for all *S. cerevisiae* strains used in this experiment, transformation was achieved by only PEG treatment of intact cells, although the efficiency was less than that with the lithium acetate method, with the exception of the transformation of YNN27 with GI053. The transformation efficiency varied depending upon the host strains employed, but it should be stressed that within the same strain, ρ^+ cells were generally more efficient than ρ^- cells.

From the results described above, we propose that treatment of intact cells with PEG is a minimal requirement for successful transformation and the uptake of plasmids is accelerated by alkali cations or thiol compounds.

Since the PEG method is very quick and simple, it has the potential of becoming a convenient and useful pro-

cedure if the transformation efficiency can be raised to a level comparable to that of the current protoplast method. In the course of the preparation of this manuscript, a report was published on transformation by PEG treatment, the efficiency of which is comparable to that of the protoplast method.⁸⁾ In this work the general utility of the PEG method has been shown in a wide variety of organisms including yeast, *E. coli* and cultured human cell lines. Further optimization of the PEG transformation method for yeast cells may be required and the mechanism of the PEG effect in yeast transformation remains to be elucidated.

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