New cell fusion method using polymer membrane

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A porous polymer membrane of nitrocellulose or tetrafluoroethylene (TFE) was employed for fusion of *Saccharomyces cerevisiae* (AH22 and D13-1A) protoplasts. Protoplasts were adsorbed on the membrane with slight suction. Some part of the protoplasts was trapped in pores of the membrane as observed by electron microscopy. The membrane retaining protoplasts was placed on a selective medium. Several colonies appeared on the medium after 5–7 days incubation at 30°C. The fusion of the two strains was ascertained by DNA content and genetic markers. Fusion frequency was 1.2×10^{-6} in the case of the TFE membrane.

Cell fusion *Porous polymer membrane* Saccharomyces cerevisiae

1. INTRODUCTION

Cell fusion is well understood as a useful technique to develop a new strain of microorganism [1–4]. The cell fusion was induced by chemical reagents such as polyethyleneglycol (PEG) and polyvinylalcohol, or by viruses such as HVJ and SV5. Viruses are used for the cell fusion of mammalian cells having specific receeptors for the viruses. In the case of microorganisms, however, viruses are not applicable, because no receptors for these viruses exist on the cell surface of microorganisms. On the other hand, PEG can be used for various cells including microorganisms. This reagent, however, was often lethal for cells. Therefore an efficient and useful method was required for the cell fusion of microorganisms.

Here, polymer membranes were used for the cell fusion of different strains of *Saccharomyces cerevisiae*. Advantages of this physical fusion method using membrane were also discussed.

2. MATERIALS AND METHODS

2.1. *Strains*

Heterothallic haploid strains of *S. cerevisiae* AH22 (a Leu2-3, 2-112, His4-519 [5] and D-13-1A (a His3-532 Trp1) [6] were employed for experiments. These yeasts were kindly provided by Dr Gunge (Mitsubishi-Kasei, Institute of Life Science).

2.2. *Preparation of protoplasts*

Each strain was cultivated in YPG medium (1% yeast extract, 2% polypepton, 2% glucose) and harvested at log phase. The cells were suspended in TS buffer (Tris-HCl 0.05 M, sorbitol 0.8 M, pH 7.5) containing 2-mercaptoethanol (final concentration 20 mM), and then converted to protoplasts with the lytic enzyme, Zymolyase 60000 (Kirin Brewery, final conc. $50\,\mu g \cdot ml^{-1}$). Protoplasts were collected by centrifugation at $1000 \times g$ for 15 min, washed twice with TS buffer and resuspended in the same buffer.

2.3. *Protoplast fusion with use of porous membrane*

The protoplast suspensions of both strains were mixed and 0.5 ml of mixed suspension containing 2×10^7 cells were dropped on a porous membrane with controlled suction (fig.1). Then the porous membranes were put onto plates containing selective medium (2% glucose, 0.7% Difco yeast nitrogen base, 0.8 M sorbitol, 3% agar), and plates were incubated at 30°C for 5–7 days.

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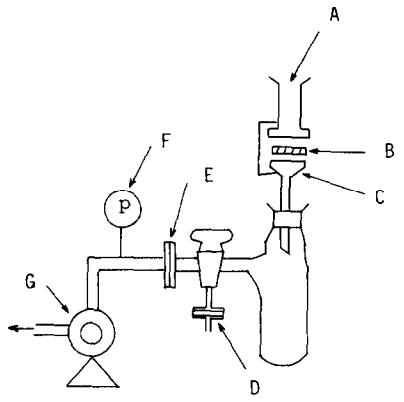

Fig. 1. Apparatus for cell fusion: A, protoplast suspension inlet; B, porous membrane; C, glass filter; D, valve; E, air filter; F, pressure gauge; G, vacuum pump.

Properties of porous membranes used are listed in table 1. Tetrafluoroethylene (Junkosha) nitrocellulose (Toyoroshi) and polycarbonate (Nuclepore) membranes were employed in these experiments.

Fusion frequency *(F)* was defined as follows:

$$F = \frac{\text{number of colonies on the selective medium}}{\text{number of protoplasts trapped in the membrane}}$$

Table 1

Fusion frequency by various membranes

Membrane	Physical properties			Fusion frequency ($\times 10^{-6}$)
	Pore size (μm)	Thickness (mm)	Porosity ratio (%)	
Tetrafluoroethylene	1.5	0.28	80.9	0
	2.6	0.10	80.9	0.5
	3.6	0.11	87.4	1.2
	5.3	0.09	89.3	1.0
	6.7	0.09	89.1	0.3
Nitrocellulose	1.0	0.15	80	0.3
	3.0	0.15	81	0.4
	5.0	0.15	81	0.3
	8.0	0.15	/	0
Polycarbonate	2.0	0.01	<30	0
	3.0	0.01	<30	0
	5.0	0.01	<30	0

2.4. *Determination of DNA content and mating type*

DNA was extracted by the method of [7] and determination of DNA content was carried out as in [8].

Mating types were determined by observing the zygote formation after mixed inoculation of cells with strains A5-1d(a) and R2N1C (α) as the standards.

2.5. *Electron microscopy*

Samples were fixed in 2.5% (w/v) glutaraldehyde solution containing TS buffer for 2 h at 4°C, and post-fixed with 2% OsO_4 in the same buffer for 1 h at 4°C. Then fixed samples were dehydrated in a graded ethanol series and dried by a critical point dryer (Hitachi, HCP-2). They were sputter-coated with gold by an Eika Ion Coater and examined with a scanning electron microscope (Hitachi, S-415).

3. RESULTS AND DISCUSSION

S. cerevisiae D13-1A and AH22 cannot grow on the selective medium, since these strains require histidine, leucine and tryptophan for their growth. When protoplasts of D13-1A were genetically fused with protoplasts of AH 22, the fusants can grow on the selective medium.

Two protoplasts from yeast strains were adsorbed on the porous membrane with suction as illustrated in fig. 1. The suction process was performed at low pressure (30–270 mmHg) for 30–90 sec. Various membranes were used for the cell fusion experiments. The results obtained are summarized in table 1. The fusion frequencies were $0.3–1.2 \times 10^{-6}$, when either TFE or NC membrane was used for the experiments. On the other hand, no fusant appeared on the selective medium when the PC membrane was used for fusion.

In order to investigate the adsorbed protoplasts, the membranes were observed by electron microscopy. As shown in fig. 2, protoplasts were aggregated on or in a porous TFE membrane. In the case of the PC membrane, protoplasts remained only on the surface of the membrane. These results suggested that the trapping of the protoplasts in the membrane pores should be necessary for cell fusion.

As the control, parent strains were treated by the

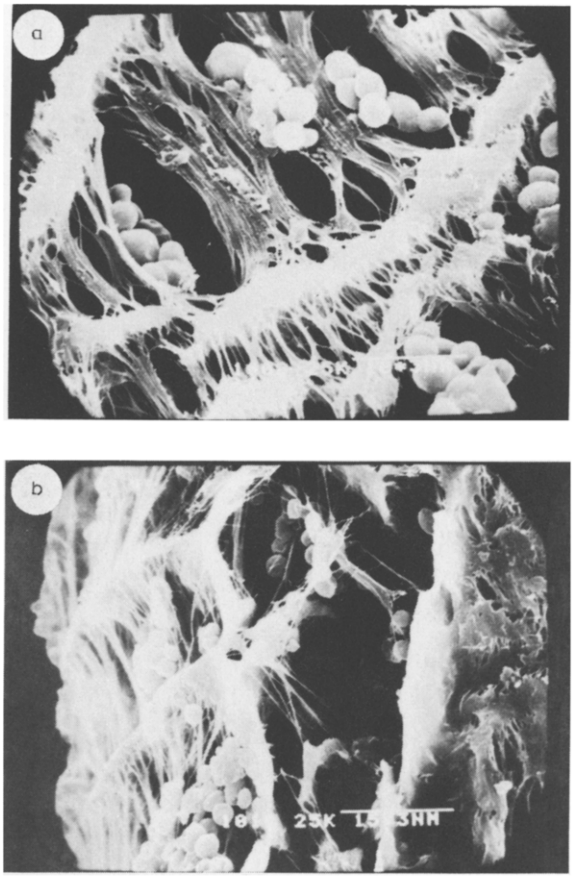

Fig. 2. Scanning electron microscopy of (a) the surface and (b) the cross section of the TFE membrane-maintaining protoplasts. Scale bars: (a) 10 μm; (b) 15 μm.

Table 2

Characteristics of fusion products

	Mating type	Auxotrophic markers				Relative DNA content
		His3	Trpl	His4	Leu2	
D13-1A	a	+	+	−	−	1.0
AH22	a	−	−	+	+	0.7
F-1	a	+	+	+	+	1.5
F-2	a	+	+	+	+	1.7
F-3	a	+	+	+	+	2.1
F-4	a	+	+	+	+	2.7

same procedure without mixing the two strains. No back mutation occurred on the selective plates.

12 colonies were obtained from the TFE membrane with pore size of 3.6 μm. 4 of the colonies were selected and genetically characterized as shown in table 2. DNA content and genetic markers indicated that these colonies were fused products of AH22 and D13-1A protoplasts.

As reported previously, *S. cerevisiae* protoplasts could revert to whole cells only when they were entrapped in polymer matrix such as agar and gelatine [9]. This fact suggested that physical protector around protoplasts might be necessary for the regeneration of cell wall. In the present study, polymer membranes played a similar role of agar and gelatine matrix. Another role of polymer membranes might be initiation of aggregation. The physical treatment described above accelerated efficiently contact of both the protoplasts. Thus, viruses or chemical reagents were not required to induce cell fusion.

In conclusion, synthetic polymer membranes could be used for a novel method of cell fusion. Further developmental studies in this laboratory are being directed toward improving the fusion frequency. Electric stimulation and chemical modification of the membrane surface can be used for improvement of this method.

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