Transformation of Yeast by Agitation With Glass Beads

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ABSTRACT

We have found that agitation of Saccharomyces cerevisiae with glass beads and plasmid DNA using a vortex mixer results in genetic transformation of the yeast cells. This method is less efficient, but considerably more convenient, than other yeast transformation procedures. The fact that the minimal requirements for transformation are simply physical damage and the presence of DNA in an osmotically supportive environment suggests that this process may occur in nature.

EXOGENOUS material can be introduced into many kinds of cells by limited physical disruption of the cell surface. For example, scraping mammalian tissue culture cells from the surface to which they adhere (McNeil et al. 1984) or sonication of Dictyostelium discoideum cells (Fechheimer et al. 1986) allows the entry of dextrans into both cell types. Electroporation (Potter, Weir and Leder 1984; Fromm, Taylor and Walbot 1985; Hashimoto et al. 1985) and bombardment with microprojectiles coated with nucleic acid (Klein et al. 1987; Johnston et al. 1988; Fox, Sanford and McMullin 1988) have allowed introduction of RNA and DNA into mammalian and plant cells and yeast. For sufficiently large cells, microinjection has been widely used to introduce material into individual cells.

The two methods most frequently used to date for genetically transforming yeast rely on treatment with various agents to induce a state of competence in which DNA can be taken up by the cells (Hinnen, Hicks and Fink 1978; Ito et al. 1983). However, the studies cited above raised the possibility that limited physical damage to otherwise untreated yeast cells in the presence of DNA might result in transformation. We show here that a common method of physically disrupting yeast cells, agitating in suspension with glass beads using a vortex mixer, can also be used to introduce plasmid DNA into the cells.

MATERIALS AND METHODS

Yeast strains and media: Saccharomyces cerevisiae strains used were DBY947 (MATa, ade2-101, ura3-52) (Neff et al. 1983; obtained from D. Botstein), GFR18 (MATa, his3-11, his3-15, leu2-3, leu2-112) (Donahue et al. 1983; obtained from G. Fink), DAU2 (MATa, ade2, ura3-Δ; otherwise isogenic to D273-10B, ATCC 25657), and TD28 (MATa, ura3-52, ino1) (Donahue et al. 1983).

YPD medium contained 1% yeast extract, 2% peptone, and 2% dextrose. SD medium contained 0.67% yeast nitrogen base without amino acids and 2% dextrose. Solid media were 2% agar.

Transformation: Cells were grown in YPD medium. At various times, they were collected by centrifugation, washed twice with SD medium made 1 m sorbitol, and resuspended in SD, 1 m sorbitol at 1/10 the original culture volume. Aliquots of 0.2 ml were placed in 15-ml plastic tubes with 5 μg of plasmid DNA in 2.5 μl. Carrier DNA, when added, was 50 μg of sonicated calf thymus DNA (Pharmacia) in SD, 1 m sorbitol. Aliquots of 0.5 g of glass beads (0.45–0.52 mm diameter; Thomas Scientific) that had been weighed into glass tubes and sterilized by baking overnight at 400° were then added to tubes. The tubes containing washed cells, DNA and glass beads were mixed for 30 sec using a Vortex-Genie mixer (Fisher Scientific) at the highest speed. Immediately after mixing was completed, as much of the supernatant as possible was removed from the glass beads and spread on selective medium plates (SD supplemented with nutritional requirements) containing 1 m sorbitol.

RESULTS

Yeast cells mixed with glass beads and DNA can be transformed: Of several strains tested (see below), DBY947 was found to be most efficiently transformed. In an initial experiment, DBY947 was grown to late logarithmic phase (a reading of 500 with a Klett-Summerson photoelectric colorimeter; approximately 2 × 10⁸ cells/ml) in YPD medium and was mixed with plasmid DNA and glass beads using a vortex mixer as described in MATERIALS AND METHODS. Mixing took place in SD medium containing 1 m sorbitol. The plasmid used was pCGE137 (obtained from G. Fink), a pBR322 derivative that carries the URA3 gene and the 2μ origin of replication. After mixing, cells were spread on SD plates containing 1 m sorbitol and supplemented with 20 mg/liter adenine.

Ura⁺ colonies appeared on the plates in 2–3 days. The Ura⁺ phenotype was mitotically unstable and the colonies were Ade⁻, as expected for strain DBY947 carrying pCGE137. DNA from several putative transformants and from DBY947 was analyzed by gel-blot hybridization to radioactively labeled pBR322 DNA (not shown). While DNA from untransformed DBY947 had no homology to pBR322, all of the
putative transformants carried a plasmid hybridizing to pBR322 with the same electrophoretic mobility as pCGE137. The appearance of these Ura<sup>+</sup> colonies was dependent on three factors: added pCGE137 DNA; mixing the cells and DNA with glass beads; and the presence of sorbitol in the plates. Thus we conclude that this phenomenon is genetic transformation. The facts that physical damage must occur and that sorbitol is essential suggest a mechanism in which damaged cells take in some of the medium surrounding them, including plasmid DNA, after which a small fraction of the cells recover when allowed to regenerate on a medium of high osmotic strength. [We have found previously that transformed spheroplasts can regenerate after being spread on the surface of plates containing 0.8 M sorbitol (MUeLLER and FOX (1984)).]

**Factors affecting transformation efficiency:** We investigated the effects on transformation efficiency of the medium in which physical damage occurs by transforming DBY947 in 1 M sorbitol 1 mM EDTA rather than in SD medium, 1 M sorbitol, and by adding carrier DNA. Mixing in SD, 1 M sorbitol gave rise to 50-fold more transformants than mixing in sorbitol-EDTA solution (Table 1). Carrier DNA also increased the frequency of transformation by at least a factor of two (Table 1).

Next, we varied the time of mixing from 0 to 66 sec (Figure 1). Transformation was most efficient between 15 and 45 sec of mixing. At each time point, the extent of survival was determined by diluting and spreading on YPD plates with or without 1 M sorbitol (Figure 1). After 15–45 sec of mixing, about 50–80% of the cells were killed. This figure was in good agreement with a rough estimate, based on microscopy, that 50–75% of the cells were damaged after mixing for about 30 sec. A small, but reproducible, fraction (about 4–5%) of the cells was sufficiently damaged that the cells could not survive on YPD medium alone, but were rescued by plating on YPD containing 1 M sorbitol. This class of cells—those too badly damaged to survive without osmotic support—presumably includes the transformable cells in this population, since none of the cells surviving without osmotic support were transformed.

The efficiency of transformation of yeast by the two most commonly used methods (treatment of spheroplasts with calcium and polyethylene glycol (HINNEN, HICKS and FINK 1978) or treatment of intact cells with metal ions and polyethylene glycol (ITO et al. 1983)) is affected by the growth phase of the cells, with early to mid logarithmic phase cells yielding the most transformants. We tested the transformation efficiency of two strains, DBY947 and GRF18, at various stages of growth (Figure 2). Transformation efficiency was low during the logarithmic phase and increased for both strains in late logarithmic and stationary phases. The maximal transformation efficiencies achieved were about 300 transformants per µg of plasmid DNA for DBY947 and about 70 for GRF18. In contrast, transformation of either strain by treatment with lithium acetate and polyethylene glycol (ITO et al. 1983) at Klett 400 yielded only 10 transformants per µg of plasmid DNA.

Another strain, DAU2, yielded an average of about 6 transformants per µg DNA in late logarithmic and early stationary phases. This strain is poorly transformed by the lithium acetate method under optimal conditions. Strains TD28 (DONAHUE et al. 1983), which is relatively efficiently transformed by the lithium acetate method, gave only about 9 transformants per µg in early stationary phase using the glass beads method. This may be due to the fact that TD28 is sensitive to high osmotic pressure (our unpublished observations). Thus transformability by this method generally corresponded to transformability with the lithium acetate method for the strains tested, with the exception of TD28.

We tested the utility of this method for integrative transformation by transforming DBY947 with the plasmid pMC215, which carries the PET54 gene (Cos-

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**TABLE 1**

<table>
<thead>
<tr>
<th>Medium*</th>
<th>Carrier DNA*</th>
<th>Transformants*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M sorbitol, 1 mM EDTA</td>
<td>+</td>
<td>16, 16</td>
</tr>
<tr>
<td>SD, 1 M sorbitol</td>
<td>−</td>
<td>309, 484</td>
</tr>
<tr>
<td>SD, 1 M sorbitol</td>
<td>+</td>
<td>916, 1207</td>
</tr>
</tbody>
</table>

* Medium in which mixing of cells, DNA, and glass beads took place.

* Fifty micrograms of sonicated calf thymus DNA in 1 M sorbitol 1 mM EDTA or in SD, 1 M sorbitol.

* Number of transformants (per 5 µg of pCGE137 DNA) from each of two experiments.

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**FIGURE 1.** Effects of mixing time on transformation efficiency and cell survival. DBY947 was grown in YPD to a Klett value of 294 (late logarithmic phase) and transformed with 3 µg pCGE137 DNA in SD, 1 M sorbitol in the presence of 50 µg carrier DNA as described in MATERIALS AND METHODS. Number of transformants per µg DNA (filled diamonds) was the average of two experiments. The titer of cells surviving at each time point was determined by diluting in SD, 1 M sorbitol and spreading on YPD plates (open squares) and on YPD, 1 M sorbitol plates (filled squares).
Transformation With Glass Beads

In attempting to optimize transformation efficiency by this method we found several conditions which decreased the yield of transformants. Heat shock (42° for 5 min) after mixing decreased transformation efficiency by about 2-fold. Transforming at 4° was at least 5-fold less efficient than transforming at room temperature. CaCl₂ up to 10 mM in the transformation mixture had no effect; at concentrations greater than 10 mM, efficiency was drastically reduced.

In one variation of this procedure, cells in suspension with glass beads and plasmid DNA were agitated by hand in an empty Petri plate using a glass spreader, and plated on medium containing 1 M sorbitol. While only two transformants were obtained in this manner, this result indicates that relatively mild forces can cause sufficient damage for transformation to occur.

**DISCUSSION**

We have shown that *S. cerevisiae* cells can be transformed by limited physical disruption in the presence of DNA. Although the efficiency of transformation by this method is lower than that achieved with other methods, the convenience of this procedure offers some advantages in cases where optimal efficiency is not important. In contrast to the spheroplasting method (Hinnen, Hicks and Fink 1978) and the lithium acetate treatment method (Ito et al. 1983), for which cells must be grown so that they are in logarithmic phase at the desired time, transformation by agitation with glass beads is possible at any time within a range of at least 36 hr, during late logarithmic and early stationary phases. Also in contrast to those two methods, this procedure requires only one manipulation of the cells after they are grown and washed, and can easily be completed in under 30 min. Finally, unlike the two additional methods available for yeast transformation, electroporation (Hashimoto et al. 1985) and microprojectile bombardment (Johnston et al. 1988; Fox, Sanford and McMullin 1988), agitation with glass beads does not require equipment other than that readily available in a typical laboratory.

Transformation by agitation with glass beads or other methods of disruption may be useful for transformation of other cell types, particularly walled cells such as fungi or plant cells in suspension culture. The fact that cell breakage in the presence of DNA can lead to transformation suggests that exogenous DNA may occasionally enter yeast cells in nature. For example, all three requirements for transformation—DNA, cell breakage, and osmotic support—would probably be met when a hoofed animal steps on a rotting grape.

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LITERATURE CITED


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