Transformation of Escherichia coli with DNA from Saccharomyces cerevisiae Cell Lysates

ANA CRISTINA ADAM, GRACIA GONZALEZ-BLASCO, MARTA RUBIO-TEXEIRA,† AND JULIO POLAINA*
Instituto de Agroquimica y Tecnologia de Alimentos, Consejo Superior de Investigaciones Cientificas, Apartado de Correos 73, E-46100 Burjassot, Valencia, Spain

Received 18 March 1999/Accepted 16 September 1999

We developed a system to monitor the transfer of heterologous DNA from a genetically manipulated strain of Saccharomyces cerevisiae to Escherichia coli. This system is based on a yeast strain that carries multiple integrated copies of a pUC-derived plasmid. The bacterial sequences are maintained in the yeast genome by selectable markers for lactose utilization. Lysates of the yeast strain were used to transform E. coli. Transfer of DNA was measured by determining the number of ampicillin-resistant E. coli clones. Our results show that transmission of the Amp gene to E. coli by genetic transformation, caused by DNA released from the yeast, occurs at a very low frequency (about 50 transformants per ug DNA) under optimal conditions (a highly competent host strain and a highly efficient transformation procedure). These results suggest that under natural conditions, spontaneous transmission of chromosomal genes from genetically modified organisms is likely to be rare.

The increasing use of genetically modified organisms (GMOs) in biotechnology has generated controversy concerning the safety of these organisms. Specifically, whether recombinant sequences are transferred from hosts to other organisms is of interest. Also, if transfers occur, it is necessary to determine by what mechanism and at what frequency, since safety regulations require estimates of potential risk (10).

The flow of genes between species, which is termed horizontal transfer, is well-documented (19). Therefore, transfer of genetic material from GMOs to other organisms, which may lead to undesirable effects, is possible. For example, in vivo transfer of an antibiotic resistance gene from lactic acid bacteria to mouse intestinal bacteria has been reported (9).

The mechanisms by which horizontal transfer can occur are conjugation, transformation, and virus-mediated transduction (2, 11). Conjugation, which can mediate gene exchange in virtually all eubacteria, also can transfer genetic material from bacteria to yeasts and plants (8, 17, 22). Transformation and transduction are known to occur spontaneously only in bacteria. Although yeasts and other eukaryotic organisms can be transformed, the conditions required for this to occur are quite different from conditions found in the natural environment.

The aim of this study was to determine the conditions under which and the frequency at which heterologous DNA present in a recombinant Saccharomyces cerevisiae strain could transform Escherichia coli. The yeast S. cerevisiae is a convenient experimental organism since it is widely used in the food industry and has been well-charactered genetically. Transformation is the most likely mechanism by which DNA can be transmitted from a eukaryotic organism to bacteria. While transformation of E. coli by plasmids extracted from yeast cells is a routine laboratory procedure, transfer of genes integrated into a chromosome has not been extensively studied. We studied this process by using a S. cerevisiae strain that carries multiple copies of a recombinant plasmid integrated into its genome.

MATERIALS AND METHODS

Strains, plasmids, and culture conditions. The E. coli strain used in this work was strain DH5α (7). Cultures of this strain were grown in Luria-Bertani (LB) medium at 37°C.

The S. cerevisiae strain used was strain MRY247. This strain carries the ribosomal DNA (rDNA) locus (RDNI in chromosome XII) multiple copies of two plasmids, pAA11 and pMR4 (1, 13) (Fig. 1). Plasmid pAA11 (length, 9.4 kb) contains a complete copy of pUC18 (21), the defective leu2 gene of S. cerevisiae, the bgf4 gene encoding a β-glucosidase from Paenibacillus (Bacillus) polymyxa under control of the S. cerevisiae CYC-GAL promoter, and a fragment of DNA from S. cerevisiae that targets the plasmid in the yeast genome (1). The β-glucosidase of P. polymyxa has some β-galactosidase activity, which allows it to cleave lactose (15). S. cerevisiae strains expressing the bgf4 gene can assimilate lactose if they have permease activity for sugar uptake. Plasmid pMR4 (length, 9.6 kb) contains the leu2 gene, pUC18, and the Saccharomyces rDNA fragment (as in pAA11). It contains the LAC12 gene encoding the lactose permease from Kluyveromyces lactis under control of the CYC-GAL promoter (13). Growth in a medium containing lactose as the carbon source selects for lactose and lactose permease functions supplied by the integrated plasmids. The lactose activity of the bgf4 β-glucosidase is quite low. The gene must be overexpressed for the host yeast to grow on lactose. The permease activity provided by pMR4, however, is not required at such high level. Thus, many copies of pAA11 are present (ca. 200 copies per genome), whereas there are fewer copies of pMR4 (ca. 20 copies per genome).

Yeast cultures were grown at 30°C in standard media prepared as described by Sherman et al. (16) with either glucose or lactose as the carbon source. Minimal media were supplemented with the necessary auxotrophic compounds.

Plasmid pUC18 DNA (21) was used as a control for E. coli transformation.

Preparation of transforming DNA. We used cell lysates of S. cerevisiae MRY247 as the source of transforming DNA. Cultures of this strain were grown overnight in liquid YPD medium. The cells were collected by centrifugation (4,000 × g, 15 min), washed with TE buffer (10 mM Tris, 1 mM EDTA; pH 7.5), and resuspended in 4% of the original volume of the same buffer. The cells were broken either by mechanical disruption or by chemical lysis. Mechanical disruption was accomplished by vortexing a cell suspension in the presence of 0.25 g of glass beads (diameter, 425 to 600 μm; Sigma Chemical Co., St. Louis, Mo.). The cells were vortexed for 1 min and then placed on ice for another 1 min to avoid excessive heating. This process was repeated several times. Cell disruption was monitored by visual inspection with a microscope. Disruption was considered sufficient when a majority of the cells (ca. 70 to 90%) were broken. Cell debris was removed by centrifugation (15,000 × g, 15 min), and the supernatant was used in transformation experiments. Alternatively, cell extracts were prepared by chemical lysis by using the fast procedure for yeast DNA purification of Polaina and Adam (12), as modified by Rubio-Teixeira et al. (13). The DNA concentra-
ations in the extracts prepared by the two procedures were 10 to 50 μg/ml. These extracts were used in transformation experiments either directly or after precipitation of the DNA with ethanol and resuspension in 10% of the original volume.

DNA obtained from the yeast lysates was cut with excess HindIII (0.5 μg of MRY247 DNA and 20 U of enzyme in a 20-μl [final volume] mixture). The effectiveness of the digestion procedure was monitored by gel electrophoresis. Ligation of HindIII-digested DNA was carried out with 0.5 μg of DNA and 1 U of T4 DNA ligase (Amersham Iberica, Madrid, Spain) in 30 μl of 1× ligation buffer. After overnight incubation at 16°C, the DNA was ethanol precipitated and resuspended in TE buffer at a final concentration of 50 μg/ml.

**Transformation of E. coli.** Transformation by DNA from cell lysates of *S. cerevisiae* was assayed by using competent cells of *E. coli* DH5α prepared by the following two procedures: treatment with rubidium chloride (RbCl) (6) and electroporation (5, 20) with a Gene Pulser electroporation system (Bio-Rad Laboratories, Hercules, Calif.). Ten-microliter aliquots of the yeast lysate were used in transformation experiments either directly or after precipitation of DNA (fragments more than 20 kb long) were mixed with untreated cells from bacterial cultures. No transformation was observed regardless of the amount of DNA used (up to 50 μg/ml) or the physiological state of the culture (exponential- or stationary-phase cells grown in LB medium). Therefore, we assayed transformation of highly competent *E. coli* cells. Several experiments were carried out with cell extracts containing different concentrations of DNA (10 to 500 μg of DNA/ml) prepared by mechanical cell disruption and different transformation procedures (RbCl or electroporation). No transformants were obtained in these experiments. A similar set of experiments was carried out with extracts prepared by chemical cell disruption. In this case, transformation was observed when high concentrations of DNA (100 to 500 μg/ml) were used and the DNA was introduced by electroporation (Table 1). Preparation of cell extracts by the chemical procedure involved partial deproteinization and yielded cleaner DNA. This probably resulted in more efficient transformation.

The disrupted yeast cells released high-molecular-weight DNA. We digested the cell extracts with HindIII, an endonuclease that cuts pUC18 once. The digested DNA (5 to 10 kb; 10 to 50 mg/ml) was used to transform competent *E. coli* cells, but no transformants were recovered.

Circular plasmids are much more efficient for transformation than linear forms are (4). Therefore, we circularized the linear fragments generated with HindIII by treating them with DNA ligase, and the transformation frequencies increased (Table 1). This result shows that functional bacterial DNA that could be rearranged to transform *E. coli* was present in the recombinant yeast.

**Analysis of plasmid DNA recovered from E. coli transformants.** We analyzed plasmids recovered from the Amp<sup>r</sup> transformants (Table 1 and Fig. 2). The physical maps of plasmids pAA11 and pMR4 are similar, but these two plasmids can be distinguished by two characteristic restriction sites (an XbaI site in pMR4 and an SmaI site in pAA11). All of the plasmids recovered could have arisen from pAA11, which may be attributable to the differences in the number of plasmid copies in MRY247.

The majority of the plasmids isolated from transformants obtained with untreated DNA (8 of 10 plasmids) exhibited the same restriction pattern as pAA11 (Fig. 2A). The physical maps of the remaining two plasmids could have originated by a deletion event (Fig. 2B).

When the maps of plasmids isolated from transformants obtained with DNA digested with HindIII and treated with ligase were analyzed, a wider range of patterns was observed. Of the 34 transformants analyzed, the members of the largest group (26 transformants) had plasmids whose maps corresponded totally (7 transformants) or partially (19 transformants) to the map of plasmid pAA11 (Fig. 2C and D). A second group, consisting of six clones, contained plasmids whose maps corresponded to the map of pAA11 plus a large

---

**RESULTS**

**Gene transfer from disrupted yeast cells to E. coli.** MRY274 lysates prepared by either mechanical or chemical cell disruption and concentrated DNA from the lysates (fragments more than 20 kb long) were mixed with untreated cells from bacterial cultures. No transformation was observed regardless of the amount of DNA used (up to 50 μg/ml) or the physiological state of the culture (exponential- or stationary-phase cells grown in LB medium). Therefore, we assayed transformation of highly competent *E. coli* cells. Several experiments were carried out with cell extracts containing different concentrations of DNA (10 to 500 μg of DNA/ml) prepared by mechanical cell disruption and different transformation procedures (RbCl or electroporation). No transformants were obtained in these experiments. A similar set of experiments was carried out with extracts prepared by chemical cell disruption. In this case, transformation was observed when high concentrations of DNA (100 to 500 μg/ml) were used and the DNA was introduced by electroporation (Table 1). Preparation of cell extracts by the chemical procedure involved partial deproteinization and yielded cleaner DNA. This probably resulted in more efficient transformation.

The disrupted yeast cells released high-molecular-weight DNA. We digested the cell extracts with HindIII, an endonuclease that cuts pUC18 once. The digested DNA (5 to 10 kb; 10 to 50 mg/ml) was used to transform competent *E. coli* cells, but no transformants were recovered.

Circular plasmids are much more efficient for transformation than linear forms are (4). Therefore, we circularized the linear fragments generated with HindIII by treating them with DNA ligase, and the transformation frequencies increased (Table 1). This result shows that functional bacterial DNA that could be rearranged to transform *E. coli* was present in the recombinant yeast.

**Analysis of plasmid DNA recovered from E. coli transformants.** We analyzed plasmids recovered from the Amp<sup>r</sup> transformants (Table 1 and Fig. 2). The physical maps of plasmids pAA11 and pMR4 are similar, but these two plasmids can be distinguished by two characteristic restriction sites (an XbaI site in pMR4 and an SmaI site in pAA11). All of the plasmids recovered could have arisen from pAA11, which may be attributable to the differences in the number of plasmid copies in MRY247.

The majority of the plasmids isolated from transformants obtained with untreated DNA (8 of 10 plasmids) exhibited the same restriction pattern as pAA11 (Fig. 2A). The physical maps of the remaining two plasmids could have originated by a deletion event (Fig. 2B).

When the maps of plasmids isolated from transformants obtained with DNA digested with HindIII and treated with ligase were analyzed, a wider range of patterns was observed. Of the 34 transformants analyzed, the members of the largest group (26 transformants) had plasmids whose maps corresponded totally (7 transformants) or partially (19 transformants) to the map of plasmid pAA11 (Fig. 2C and D). A second group, consisting of six clones, contained plasmids whose maps corresponded to the map of pAA11 plus a large

---

**TABLE 1. Transformation of competent *E. coli* cells by cell extracts from a recombinant yeast culture**

<table>
<thead>
<tr>
<th>Source of DNA</th>
<th>DNA concn (μg/ml)</th>
<th>DNA preparation procedure</th>
<th>Transformation procedure</th>
<th>Transformation efficiency (no. of Amp&lt;sup&gt;r&lt;/sup&gt; colonies/μg of DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pUC18 (E. coli)</td>
<td>1</td>
<td>Chemical cell disruption</td>
<td>RbCl</td>
<td>3 × 10&lt;sup&gt;7&lt;/sup&gt;</td>
</tr>
<tr>
<td>pUC18 (E. coli)</td>
<td>1</td>
<td>Chemical cell disruption</td>
<td>Electroporation</td>
<td>6 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>MRY247</td>
<td>100–500</td>
<td>Chemical cell disruption</td>
<td>Electroporation</td>
<td>10, 15, 200&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>MRY247</td>
<td>10–50</td>
<td>Chemical cell disruption, HindIII digestion, ligation</td>
<td>RbCl</td>
<td>22, 200&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>MRY247</td>
<td>10–50</td>
<td>Chemical cell disruption, HindIII digestion, ligation</td>
<td>Electroporation</td>
<td>1,900, 900, 200&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>+</sup> Values obtained in independent experiments.
regulations that prohibit episomal plasmids in commercial GMOs.

When *E. coli* is transformed with a linear plasmid, the majority of the transformants contain perfectly recircularized molecules of the plasmid (4). The genome of MRY247 has multiple copies of pAA11 in a tandem array. Therefore, linear fragments of DNA from MRY247 extracts able to transform *E. coli* give rise to circular plasmids with the physical pattern of pAA11. An interesting possibility is the existence in the yeast nucleus of circular molecules of pAA11 generated as loop-outs by homologous recombination. Digestion of MRY247 DNA with HindIII followed by ligation leads to the formation of plasmid molecules whose maps are shown in Fig. 2C and D. As expected, these patterns are the ones most frequently recovered. Ligation of heterogeneous DNA fragments and in vivo recombination events explain the generation of the other forms isolated.

Even though horizontal transfer of chromosome-integrated sequences is rare, our results indicate that under certain circumstances it is possible. The nature of the plasmids recovered from *E. coli*, which have a bacterial origin of replication and a gene that confers a selective advantage under certain circumstances, means that a single transfer event could change an entire population and lead to unpredictable consequences. Therefore, it is advisable to prevent the presence in GMOs of genetic elements that could help spread recombinant DNA sequences.

**ACKNOWLEDGMENT**

This work was supported by grant ALI-0362-97 from CICYT.

**REFERENCES**


**FIG. 2.** Physical maps of pMR4, pAA11, and plasmids recovered from *E. coli* clones transformed by DNA of *S. cerevisiae* MRY247. (A and B) Plasmids recovered from clones transformed by untreated DNA. (C through G) Plasmids recovered from clones transformed by DNA treated with HindIII and ligase. Only restriction sites relevant for characterization of the plasmids are shown. Open and solid arrowheads indicate restriction sites that are critical for identification (XbaI in pMR4 and Smal in pAA11, respectively). The solid bars in maps B, E, F, and G represent DNA regions that were not characterized.

**DISCUSSION**

Our results suggest that spontaneous transfer of chromosome-integrated sequences is a rare event. Although *E. coli* can develop natural competence (3), the conditions under which we detected transformation (high concentration of DNA, high degree of cell competence, and use of electroporation) are very different from the conditions found in natural habitats. In addition, our results agree with the general observation that transformation is a rather inefficient mechanism of exchange for nonplasmidic genes (18). The increase in transformation frequency observed after DNA circularization supports safety