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Transformation of *Saccharomyces cerevisiae* with Linear DNA Killer Plasmids from *Kluyveromyces lactis*

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Protoplasts of *Saccharomyces cerevisiae* were mixed with linear DNA plasmids, pGKl1 and pGKl2, isolated from a *Kluyveromyces lactis* killer strain and treated with polyethylene glycol. Out of 2,000 colonies regenerated on a nonselective medium, two killer transformants were obtained. The pGKl plasmids and the killer character were stably maintained in one (Pdh-1) of them. Another transformant, Pdl-1, was a weak killer, and the subclones consisted of a mixture of weak and nonkiller cells. The weak killers were characterized by the presence of pGKl1 in a decreased amount, and nonkillers were characterized by the absence of pGKl1. The occurrence of two new plasmids which migrated faster than pGKl1 in an agarose gel was observed in Pdl-1 and its subclones, whether weak or nonkillers. Staining with 4′,6-diamidino-2-phenylindole revealed that the pGKl plasmids exist in the cytosol of transformant cells with numerous copy numbers.

Killer strains of *Saccharomyces cerevisiae* are well known to harbor two linear double-stranded RNA plasmids, M and L, encapsulated in virus-like particles (1, 12). M double-stranded RNA codes for the production of a killer toxin and the immunity to the toxin, in analogy with the bacteriocin system of certain bacteria. L double-stranded RNA codes for a coat protein of virus-like particles and exists in most strains of *S. cerevisiae*, whether killers or not (1, 12). We have previously reported a novel killer system in *Kluyveromyces lactis*, which is encoded by two linear DNA plasmids, pGKl1 (5.4×10^6 daltons) and pGKl2 (8.4×10^6 daltons) (6). The function of pGKl plasmids differed from that of double-stranded RNA plasmids by the killing spectrum against various sensitive strains and by the pH range of killing (6). Cell fusion experiments revealed that the pGKl plasmids could replicate and express the killer function not only in *K. lactis* but also in *S. cerevisiae* (7). In this paper, we describe the transformation of *S. cerevisiae* with a mixture of pGKl1 and pGKl2 isolated and purified from *K. lactis*.

DNA was collected from a sodium dodecyl sulfate lysate of protoplasts of a *K. lactis* killer strain, 2132-2B (**a** *his leu ade*), carrying the pGKl1 and pGKl2 plasmids by alcohol precipitation. pGKl1 and pGKl2 were separated from the nuclear and mitochondrial DNAs by agarose gel electrophoresis, then individually extracted from the gel by an electrophoretic elution procedure (4) and purified by phenol treatment and alcohol precipitation. A mixture of pGKl1 and pGKl2 thus obtained was used to transform a nonkiller neutral petite mutant of *S. cerevisiae*, AH22 (**a** *leu2-3,2-112 his4-519 can1 [KIL-0]*ρ^0), as in the following. The recipient AH22 cells were grown in YEPD (1% yeast extract, 2% peptone, 2% glucose), harvested in the log phase, and converted into protoplasts with Zymolyase 60,000. The protoplasts were well washed with 0.6 M KCl and suspended in a buffer (0.6 M KCl, 10 mM Tris-hydrochloride, 50 mM $CaCl_2$, pH 7.6). To 10 μl of the suspension containing about 3×10^5 protoplasts we added a mixture of 10 μl of 3.6 M KCl and 40 μl of DNA solution containing approximately 1 μg each of pGKl1 and pGKl2. The whole (60 μl) was kept in ice for 30 min and mixed with 500 μl of 33% polyethylene glycol 4,000 containing 50 mM $CaCl_2$ and 10 mM Tris-hydrochloride (pH 7.6) and incubated at 30°C for 60 min. After cooling, it was suspended in 10 ml of 0.6 M KCl, and then 1-ml samples of the suspension or serial dilutions with 0.6 M KCl were individually mixed with 10 ml of a medium (YEPD, 0.6 M KCl, 3% agar), melted at 45°C, and immediately poured onto plates containing the same medium. After 4 days of incubation at 30°C, about 6,000 colonies were produced in total, indicating that the regeneration frequency of protoplasts was about 2%.

Two thousand colonies were randomly picked up and tested by the method of Somers and Bevan (10) for killing activity against an *S.*

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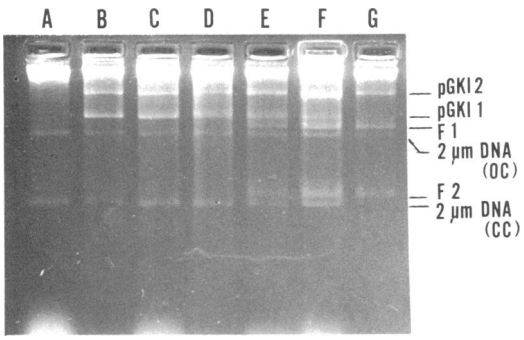

FIG. 1. Plasmid analysis by agarose gel electrophoresis. Electrophoresis was run from top to bottom. The photograph shows DNA extracts from AH22 (lane A), cell fusant F102-2-2 (6) (lane B), transformant Pdh-1 (lane C), a subclone of Pdh-1 (lane D), transformant Pdl-1 (lane E), a weak killer subclone of Pdl-1 (lane F), and a nonkiller subclone of Pdl-1 (lane G). F1 and F2 are the two new plasmids appearing in Pdl-1 and its subclones. The 2-μm DNA bands represent the open circular (OC) and closed circular (CC) forms.

cerevisiae strain, B511-4C (ATCC 38659), which is sensitive to pGKl-encoded killer strains (7). As a result, two colonies, Pdh-1 and Pdl-1, were found to kill the indicator B511-4C cells, although Pdl-1 showed a somewhat weak killing activity as compared with Pdh-1, in terms of the size of the killing zone against the indicator cells. The killing activity of Pdh-1 was comparable to that of the pGKl plasmid donor strain *K. lactis* 2132-2B. Both Pdh-1 and Pdl-1 carried the same genotype as AH22, except for the killer character.

DNA plasmids were analyzed by agarose gel electrophoresis according to Cameron et al. (3). As shown in Fig. 1, Pdh-1 and Pdl-1 contained both pGKl1 and pGKl2, in addition to the resident 2-μm DNA plasmid of the recipient cells, ensuring that they were killers transformed with the pGKl plasmids. Figure 1 also shows that the plasmid pattern of Pdh-1 was not different from the result of transfer of the pGKl plasmids by cell fusion (7). The weak killer Pdl-1, however, possessed pGKl1 at a decreased level and, instead, it carried new plasmids F1 and F2, which migrated faster than pGKl1 in the gel. The decrease in the amount of pGKl1 in the weak killer is interesting in light of the previous observation (8), which suggested that the killer gene is located on pGKl1 rather than on pGKl2. Single colonies were isolated from each transformant. All 54 subclones of Pdh-1 contained pGKl1 and pGKl2 at a normal level and retained the same killing activity as Pdh-1. Subclones of Pdl-1, however, consisted of weak killers (resistant to pGKl-encoded killers) and nonkillers (sensitive to pGKl-encoded killers); out of 56 colonies 17 were nonkillers. The plasmid pattern of the weak killer subclones was the same as that of Pdl-1. The nonkillers, however, lacked pGKl1, although pGKl2 and the two new plasmids existed as in Pdl-1. F1 and F2 were shown to possess sequence homology to pGKl1 by Southern blotting hybridization (11), using a ^{32}P-labeled pGKl1 restriction enzyme fragment as a probe (data not shown). Thus the new fragments must have been derived from pGKl1 and lost both the killer and resistance regions. The molecular weights of F1 and F2 are estimated to be 4.7×10^6 and 2.3×10^6, respectively, assuming that they are linear. The possible linearity of the DNA structure was supported by the fact that F1 and F2 could not be detected when the extraction of plasmids from cells was done by

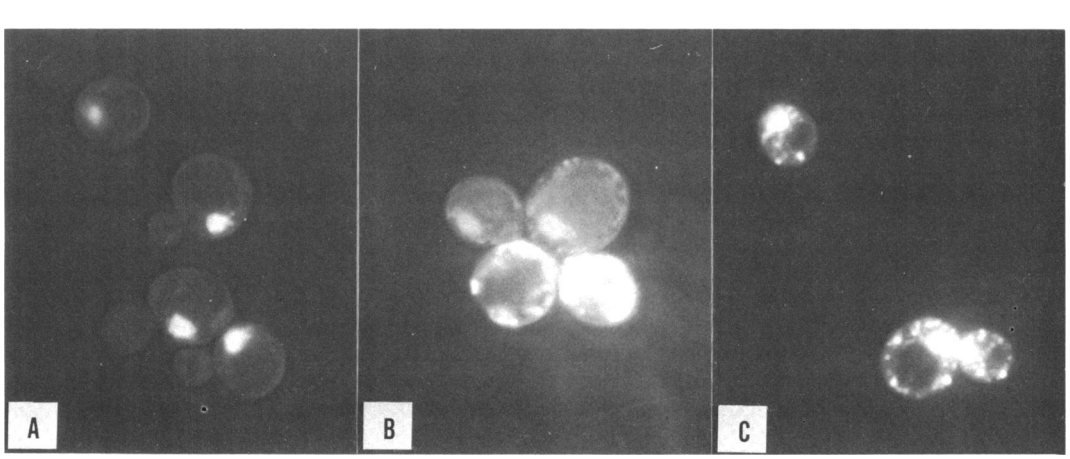

FIG. 2. Fluorescent photomicrographs of cells stained with DAPI. (A) AH22 (ρ^0). (B) G102D (ρ^+), and (C) transformant Pdh-1 (ρ^0).

the procedure reported by Bimboin and Doly (2), which causes alkaline denaturation of linear DNA and permits selective detection of covalently closed circular DNA.

The non-Mendelian inheritance of the pGKl plasmids associated with the killer character was demonstrated in *K. lactis* by tetrad analysis (6), indicating the cytoplasmic location of the plasmids. To confirm this, cells of AH22 and the killer transformants were vital-stained with a fluorescent dye, DAPI (4′,6-diamidino-2-phenylindole), by the method of Williamson and Fennell (13). The DAPI staining revealed the existence in each AH22 cell of a single large fluorescent body which corresponded to the nucleus (Fig. 2). No mitochondrial DNA bodies could be detected, in agreement with the neutral petite (suppressiveness zero) character of AH22, whereas they were conspicuously seen in ρ^+ cells of a control strain, G102D. The transformant Pdh-1 cell, which was also neutral petite, on the contrary, was shown to contain numerous small cytoplasmic bodies in addition to the nucleus. The result supports the view that the pGKl plasmids exist in the cytosol of transformed *S. cerevisiae* cells with a number of copies. Judging from a densitometric measurement of DNA amounts by agarose gel electrophoresis (data not shown), we estimate that the respective copy numbers of pGKl1 and pGKl2 per cell are comparable to that of the 2-μm DNA plasmid, which has been reported to exist with 50 to 100 copies per haploid cell (5). Although the existence of 2-μm DNA in AH22 cells was clearly demonstrated in an electrophoretic analysis (Fig. 1, lane A; see also reference 7), we failed to detect the plasmid with DAPI staining (Fig. 2A). The result may be explained if most or all of 2-μm DNA plasmid molecules are located within the nucleus rather than in the cytosol, as suggested by previous investigations (9).

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LITERATURE CITED

1. **Bevan, E. A., and D. J. Mitchell.** 1979. The killer system in yeast, p. 162–199. *In* P. A. Lemke (ed.), Viruses and plasmids in fungi. Marcel Dekker Inc., New York.
2. **Bimboin, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
3. **Cameron, J. R., P. Philippsen, and R. W. Davis.** 1977. Analysis of chromosomal integration and deletions of yeast plasmids. Nucleic Acids Res. **4**:1429–1448.
4. **Chouikh, Y., M. Volovitch, and P. Yot.** 1979. A simple and fast electrophoretic method for elution of nucleic acids from gels. Mol. Biol. Rep. **5**:237–239.
5. **Clark-Walker, G. D., and G. L. G. Miklos.** 1974. Localization and quantification of circular DNA in yeast. Eur. J. Biochem. **41**:356–365.
6. **Gunge, N., A. Tamaru, F. Ozawa, and K. Sakaguchi.** 1981. Isolation and characterization of linear deoxyribonucleic acid plasmids from *Kluyveromyces lactis* and the plasmid-associated killer character. J. Bacteriol. **145**:382–390.
7. **Gunge, N., and K. Sakaguchi.** 1981. Intergeneric transfer of deoxyribonucleic acid killer plasmids, pGKl1 and pGKl2, from *Kluyveromyces lactis* into *Saccharomyces cerevisiae* by cell fusion. J. Bacteriol. **147**:155–160.
8. **Niwa, O., K. Sakaguchi, and N. Gunge.** 1981. Curing of the killer deoxyribonucleic acid plasmids of *Kluyveromyces lactis*. J. Bacteriol. **148**:988–990.
9. **Petes, T. D.** 1980. Molecular genetics of yeast. Annu. Rev. Biochem. **49**:845–876.
10. **Somers, J. M., and E. A. Bevan.** 1969. The inheritance of the killer character in yeast. Genet. Res. **13**:71–83.
11. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
12. **Wickner, R. B.** 1979. The killer double-stranded RNA plasmids of yeast. Plasmid **2**:303–322.
13. **Williamson, D. H., and D. J. Fennell.** 1975. The use of fluorescent DNA-binding agent for detecting and separating yeast mitochondrial DNA. Methods Cell Biol. **12**:335–351.