JOURNAL OF BACTERIOLOGY, Dec. 1981, p. 988-990
0021-9193/81/120988-03$02.00/0

Vol. 148, No. 3

Curing of the Killer Deoxyribonucleic Acid Plasmids of *Kluyveromyces lactis*

OSAMI NIWA,* KENJI SAKAGUCHI, AND NORIO GUNGE

Mitsubishi-Kasei Institute of Life Sciences, Tokyo 194, Japan

Received 23 February 1981/Accepted 9 August 1981

Ultraviolet irradiation gave rise to frequent curing of killer plasmids pGKl1 and pGKl2 of *Kluyveromyces lactis*. Almost all of the nonkillers obtained lost both plasmids, but one of them lost only pGKl1. The disappearance of pGKl1 was accompanied by the simultaneous loss of the killer activity and of the resistance to the killer factor. A new plasmid, pGKl1S, was obtained, which arose from a deletion in the central region of pGKl1. Genetic analysis suggested that pGKl1S has the killer gene lost by the deletion and the resistance gene intact and that pGKl1S shares the same replication control with pGKl1.

Kluyveromyces lactis strain IFO 1267 contains linear DNA plasmids, accompanied by the killer phenotype, which show non-Mendelian segregation. The strain contains no RNA plasmids such as are found in the killer *Saccharomyces* strain (3). The killer activity of *K. lactis* is distinguishable from that of the *Saccharomyces* strain by its pH optimum and by the range of sensitive strains. Thus, it appears that the killer of *K. lactis* consists of a new class of killer factor (1). To ensure that the linear DNA plasmids are responsible for the killer activity, we attempted to cure the plasmids. We used a killer strain, 2105-1D, which was obtained from a cross between IFO 1267 and W600B (described by Gunge et al. [1]), for the curing experiment. An overnight culture of the cells was irradiated with UV light (254 nm) for 2 min at a dose of 1,100 ergs s^{-1} cm^{-2}. Appropriate dilutions of the culture were plated on rich agar plates immediately or after incubation in a rich medium for several hours. About 70% of the cells survived the irradiation. Approximately 10% of the surviving colonies lost the killer activity, irrespective of the postirradiation incubation, whereas spontaneous nonkillers appeared at a frequency of less than 1% (2 of 300) without irradiation. It should be noted that the frequency of UV curing varied among experiments. In one experiment, for example, about 90% of the surviving colonies were nonkillers, although we did not check the survival ratio. We do not know the reason for this fluctuation. We analyzed DNA of each of the nonkillers by agarose gel electrophoresis according to Struhl et al. (2). The parent strain 2105-1D contained two kinds of plasmids, pGKl1 and pGKl2 (Fig. 1a). Of 127 nonkillers tested, 126 lost both plasmids; a representative strain of this class of nonkiller, NK1, is shown in Fig. 1d. One nonkiller strain, NK40, however, apparently lost only the shorter plasmid pGKl1 but retained pGKl2 (Fig. 1b). All of the nonkillers, including NK40, were sensitive to the killer factor. Therefore, we concluded that pGKl1 was involved in both the killer activity and the resistance to the killer. The effect of UV in curing the killer plasmids was not because of a selective killing of the killer cells, since the irradiation condition gave only a 30% loss of colony-forming units. In fact, NK1 had the same sensitivity to UV as did the parent strain (data not shown). We do not know why almost all of the nonkillers simultaneously lost both plasmids. Since there occurred one strain with pGKl2 alone, it is unlikely that each of the plasmids has a complementary function which is required for the maintenance of the plasmids. Simultaneous loss of the plasmids might be explained if pGKl2 is necessary for the maintenance of pGKl1 and much more susceptible to the effect of UV. In this context, it may be worth noting that NK40, the strain with pGKl2 alone, had about the same stability as did the parent strain 2105-1D; that is, 32 of 32 colonies without UV irradiation retained pGKl2, and 10 of 32 colonies from irradiated culture lost the plasmid. The simultaneous loss was not specifically obtained by UV irradiation. More than 50 spontaneously obtained nonkillers lost both plasmids (data not shown).

During the course of the study, we obtained an interesting nonkiller derivative from the original killer strain IFO 1267. Strain IFO 1267K^--1 contained pGKl2 but lost pGKl1; instead it had a new plasmid which migrated faster than pGKl1 in the gel. We designed the plasmid as pGKl1S. Restriction analysis revealed that pGKl1S arose from a deletion in pGKl1. The size of pGKl1S was 3.8×10^6 daltons, which was

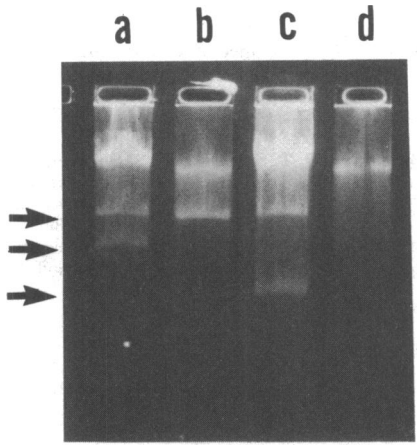

FIG. 1. *Gel electrophoresis in 0.7% agarose. The arrows indicate the positions of pGKl2, pGKl1, and pGKl1S from top to bottom, respectively. (a) DNA of 2105-1D, (b) NK40, (c) 2142-1C (this strain was made from cross IFO 1267K⁻-1 × L3α [1]), and (d) NK1.*

about 70% of pGKl1 in length. The deletion eliminated both *Eco*RI and *Bam*HI restriction sites which resided in the central region of pGKl1, whereas all of the *Pst*I and *Hin*dIII sites remained intact (Fig. 2). IFO 1267K⁻-1 was irradiated by UV at the same dose as described above, and surviving colonies were tested for their sensitivity to the killer factor. Of 45 colonies, 23 were sensitive to the killer factor, whereas all of the 45 colonies from unirradiated culture remained resistant. DNA content was analyzed. All of the sensitive derivatives of IFO 1267K⁻-1 (23 colonies) lost both of the plasmids, and the resistant colonies from the irradiated culture retained the plasmids (6 colonies were tested). As a control, when killer strain IFO 1267 was irradiated by UV, 67% of surviving colonies were nonkillers. These nonkillers were sensitive to the killer factor and contained no plasmids (three colonies were tested). Those results indicate that the resistance phenotype of IFO 1267K⁻-1 is associated with the presence of pGKl plasmids. Because pGKl1 is responsible for both the killer and the resistance, we suggest that pGKl1S has the killer gene lost by the deletion and the resistance gene intact. It has been shown that some nonkiller *K. lactis* strains are resistant to the killer, under the control of a chromosomal locus (or loci) (1). Thus, it appears that the resistance may be controlled both cytoplasmically and chromosomally.

We crossed IFO 1267K⁻-1 and 2105-1D, and the resultant tetrads were analyzed. All of the tetrads tested (14 of 14) had killer activity. However, the killer phenotype was not maintained stably; when colonies of siblings of each spore were tested for the killer, some of them no longer had killer activity. Twelve colonies from each spore were tested. No killer colonies were found among the siblings of 4 of 56 spores, whereas among the other 52 spores, 1 to 11 killers were found for each spore. No killer was recovered upon further subcloning of the nonkiller segregants, indicating that the nonkiller phenotype was stable. On the other hand, some killer segregants became stable for their killer phenotype, but others remained unstable (27% of all killers). When we analyzed DNA of the colonies from the original spores and from their siblings, we found that the original spores contained three kinds of plasmids (pGKl1, pGKl1S, and pGKl2). For the colonies of siblings, some contained pGKl1 and pGKl2, some contained pGKl1S and pGKl2, and others contained all three plasmids, corresponding to the phenotypes stable killer, nonkiller, and unstable killer, respectively. Representative results are shown in Fig. 3. We noted

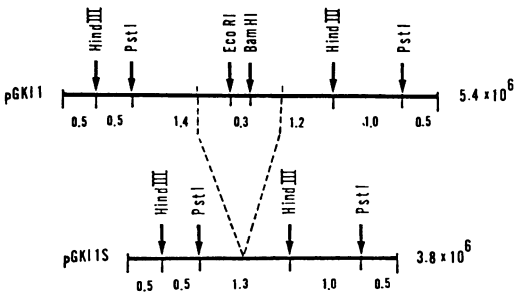

FIG. 2. *Physical structures of pGKl1 and pGKl1S. Numerals under the lines represent sizes of the fragments in megadaltons. Numerals at the right side represent daltons.*

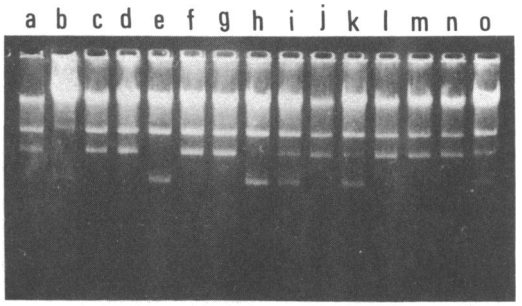

FIG. 3. *DNA analysis of siblings of 2144-8A, a spore obtained from a cross between IFO 1267K⁻-1 and 2105-1D. (a) 2105-1D, (b) IFO 1267K⁻-1, (c to n) siblings of 2144-8A, and (o) DNA of the colony from the original 2144-8A spore. (c, d, f, g, l, m, and n) DNA from stable killer colonies, (e and h) DNA from stable nonkillers, and (i, j, and k) DNA from unstable killer segregants. See the text for details.*

that the relative amount of pGKl1 and pGKl1S varied among the colonies of the original spores and that the smaller the relative amount of pGKl1 the more frequently nonkillers segregated out (data not shown). Therefore, it is conceivable that pGKl1S shares the same replication control with pGKl1, and during cell proliferation, either plasmid could be diluted out, presumably because neither plasmid has exclusive advantage in both replication and transfer to the progeny cell.

LITERATURE CITED

1. **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.
2. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. U.S.A. **76**:1035–1039.
3. **Wickner, R. B.** 1976. Killer of *Saccharomyces cerevisiae:* a double-stranded ribonucleic acid plasmid. Bacteriol. Rev. **40**:757–773.