Notes

Plasmid Distribution and Evidence for a Proteinase Plasmid in Streptococcus lactis C2

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Received for publication 19 December 1974

Five plasmids, distinguishable by their molecular weights (10^6, 2 \times 10^6, 5 \times 10^6, 10^7, 3 \times 10^7, respectively) were isolated from Streptococcus lactis C2. A spontaneous proteinase-negative derivative of this strain lacked the 10^7 plasmid.

Proteinase-deficient (prt⁻) mutants of lactic streptococci have long been known to arise spontaneously (1, 4, 9, 11), but the reason for their occurrence remains unknown. The high frequency (1 to 3%) of their appearance and their irreversible nature suggest that the deficiency could be due to loss of a plasmid (8). Two recent reports (7, 10) have indicated that certain proteolytic enzymes of these organisms are carried on plasmids. However, the high incidence of the spontaneous loss of proteinase activity and the effects of acriflavine as well as elevated temperature are only presumptive or indirect evidence for a plasmid being responsible for proteinase activity. To obtain further evidence, we examined the plasmid distribution in Streptococcus lactis C2 (lac⁺prt⁺) and its spontaneous prt⁻ derivative S. lactis C2S (lac⁺prt⁻).

A description of the organisms and their maintenance was reported previously (6). The labeling and extraction of deoxyribonucleic acid (DNA), preparation of cesium chloride (CsCl)-ethidium bromide gradients, and electron microscopy of plasmid DNA were described in an earlier paper (3). Molecular weights were calculated from the equivalence: 1.0 \mu m = 2.07 \times 10^4 DNA (5).

We recently demonstrated the existence of plasmid DNA in S. lactis C2 and showed that at least three plasmid species were present (3). Further electron microscope analyses of the DNA from the CsCl-ethidium bromide satellite band of S. lactis C2 have established five size classes of molecules which are readily distinguishable (Fig. 1). Contour length measurements of open circular molecules indicated molecular weights of about 10^6, 2 \times 10^6, 5 \times 10^6, 10^7, and 3 \times 10^7. Figure 2 shows electron micrographs of the 10^7 and 3 \times 10^7 plasmids isolated from S. lactis C2. The three smaller plasmids were illustrated in an earlier report (3).

To determine whether the instability of proteinase activity in S. lactis C2 was due to loss of a plasmid, cells of S. lactis C2S (a spontaneous proteinase-negative derivative of S. lactis C2 having a phenotype of Lac⁺Prt⁻) were labeled with [³H]thymine. The cells were harvested, lysed, and centrifuged to equilibrium in a CsCl-ethidium bromide density gradient. S. lactis C2S contains plasmid DNA, as evidenced by the existence of a dense peak separate from the chromosomal DNA (Fig. 3). To determine which, if any, of the five plasmid species were missing from this peak, electron microscope analyses of the DNA were performed as with the satellite band from the parent culture S. lactis C2. These results revealed molecules of a size

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1 Scientific Journal Series Paper No. 8945, Minnesota Agricultural Experiment Station, St. Paul, Minn.
FIG. 2. Open circular forms of the $10^7$ (A) and $3 \times 10^7$ (B) plasmids isolated from lac$^{+}$prt$^{+}$ S. lactis C2. Magnification: $\times 49,000$ for (A) and $\times 29,000$ for (B).

FIG. 3. Elution profile of CsCl-ethidium bromide gradients of DNA from cleared lysate material of S. lactis C2S.

FIG. 4. Distribution of contour lengths of circular molecules of DNA from lac$^{+}$prt$^{+}$ S. lactis C2S.

class corresponding to the $10^7$, $2 \times 10^7$, $5 \times 10^7$, and $3 \times 10^7$ molecular weight plasmids (Fig. 4); however, extensive searching of the grids failed to reveal molecules corresponding to the $10^7$ plasmid. These results, along with recent reports by McKay and Baldwin (6), Molskness et al. (7), and Pearce et al. (10), clearly suggest that certain proteolytic enzymes of lactic streptococci are carried on plasmids. In S. lactis C2, the determinant for proteinase production appears to be borne on the $10^7$ plasmid. The nature of the lost proteinase responsible for the Lac$^{-}$Prt$^{-}$ phenotype found in S. lactis C2S is not known, but presumably it is the surface-bound activity described by Pearce et al. (10) for S. lactis C10 and S. lactis H1.

We recently proposed that plasmid participation provided a mechanism for explaining the spontaneous loss of the lac and prt genes or prt genes alone from S. lactis C2 as well as the appearance of lac$^{+}$prt$^{+}$ or lac$^{-}$prt$^{-}$ transductants of lac$^{-}$prt$^{-}$ S. lactis C2 (6). The observation at that time of the three smaller plasmids in lac$^{+}$prt$^{+}$ S. lactis C2, as well as in the lac$^{-}$prt$^{-}$ derivative of this strain, suggested against this model. However, this model is now feasible based on the finding of the two large plasmids and on the finding that the $10^7$ plasmid appears to be associated with proteinase activity; it is present in lac$^{+}$prt$^{+}$ S. lactis C2
and absent in its spontaneous proteinase-deficient mutant. Whether lactose metabolism is mediated through the $3 \times 10^7$ plasmid remains unclear (2, 3, 6).

It may be possible, by using the transducing phage from S. lactis C2 coupled with plasmid DNA, to genetically construct lactic streptococcal strains needed by the dairy and food industries. Molskness et al. (7) suggested that it may be possible to stabilize the Prt characteristic in lactic streptococci. Furthermore, it may be possible to develop plasmid-specific transformation systems in lactic streptococci and thus convert many slow variants to fast acid producers by transformation of the proteinase plasmid.

We are grateful to R. J. Zeyen (Department of Plant Pathology, University of Minnesota) for use of the electron microscope facilities and to S. Falkow (University of Washington, Seattle, Wash.) for sharing valuable electron microscope procedures with L. L. M. during a visit to his laboratory.

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