NOTES

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

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Five plasmids, distinguishable by their molecular weights (10^4, 2 x 10^4, 5 x 10^4, 10^5, 3 x 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 μm = 2.07 x 10^6 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 10^4, 2 x 10^4, 5 x 10^4, 10^5, and 3 x 10^7. Figure 2 shows electron micrographs of the 10^7 and 3 x 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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class corresponding to the $10^8$, $2 \times 10^8$, $5 \times 10^8$, 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.

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**LITERATURE CITED**


