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Transductional Evidence for Plasmid Linkage of Lactose Metabolism in *Streptococcus lactis* C2[1]

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A lactose-negative (Lac^-), proteinase-negative (Prt^-) mutant, designated $C14_5$, was isolated from *Streptococcus lactis* C2 after treatment with nitrosoguanidine and ultraviolet irradiation. The mutant appeared to be cured of the prophage(s) present in *S. lactis* C2 based on non-inducibility by ultraviolet irradiation or mitomycin C. When cleared lysate material from $C14_5$ was subjected to cesium chloride-ethidium bromide (EB) density gradient centrifugation, no plasmid peak was observed, suggesting that $C14_5$ was cured of plasmid deoxyribonucleic acid (DNA). A histogram showing distribution of contour lengths of circular molecules of DNA from $C14_5$, however, revealed the presence of a greatly diminished number of DNA molecules as compared with the parent culture and indicated the absence of the 30×10^6 plasmid. Cesium chloride-ethidium bromide gradient profiles from Lac^+ Prt^- and Lac^+ Prt^+ transductants of $C14_5$ revealed no plasmid peak, but electron microscopy of the fractions normally possessing the satellite band of DNA showed the presence of a new plasmid species having a molecular weight from 20×10^6 to 22×10^6. This plasmid was lost when the transductants became Lac^-. Examination of a plasmid histogram from a spontaneous Lac^- Prt^- mutant of *S. lactis* C2 resembled that of $C14_5$, with the absence of the 30×10^6 plasmid and the presence of the 22×10^6 plasmid in Lac^+ Prt^+ transductants. The results suggest that lactose metabolism is mediated through the 30×10^6 plasmid in *S. lactis* C2 and that the transducing bacteriophage, which is too small to accommodate the entire plasmid, is transferring about two-thirds of the original plasmid through a process termed transductional shortening.

We recently proposed that plasmid deoxyribonucleic acid (DNA) provided a mechanism for explaining the spontaneous loss of lactose metabolism and proteinase activity observed in *Streptococcus lactis* C2 as well as the appearance of Lac^+ Prt^+ or Lac^+ Prt^- transductants of Lac^- Prt^- *S. lactis* C2 (3, 9-11). This model was based on finding five plasmid sizes in Lac^+ Prt^+ *S. lactis* C2 and on finding that the 10^7-dalton plasmid appeared to be associated with proteinase activity; it was present in Lac^+ Prt^+ *S. lactis* C2 and absent in a spontaneous Lac^+ Prt^- mutant. Whether lactose metabolism was mediated through the 3×10^7-dalton plasmid present in Lac^+ Prt^+ and Lac^+ Prt^- strains was unclear at that time.

In the present paper, the isolation of a Lac^- Prt^- *S. lactis* C2 mutant defective in prophage induction and deficient in plasmid DNA is reported. This mutant and a spontaneous Lac^- Prt^- *S. lactis* C2 variant were used to show that lactose-fermenting ability is linked to plasmid DNA in *S. lactis* C2.

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MATERIALS AND METHODS

Bacterial strains. *S. lactis* C2, its Lac^- mutants, and the transductants are maintained in our stock culture collection. Their propagation and maintenance were previously described (11).

Curing of lysogenic *S. lactis* C2. *S. lactis* C2 was treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine as previously described by McKay et al. (13). The treated cells were then plated on lactose indicator agar (11) to select for Lac^- clones. The Lac^- mutants were examined for prophage induction by exposure to ultraviolet (UV) irradiation as described by McKay and Baldwin (8). A mutant not exhibiting a 25-s UV induction response was further irradiated by using an 80-s exposure to UV. Five milliliters of the irradiated cells was added to 5 ml of lactic broth and incubated for 23 h at 32°C. The culture was diluted and plated on lactic agar to obtain isolated colonies, which were then picked into 1.0-ml quantities of glucose broth. The tubes were incubated at 21°C for 16 h. Portions of each culture were spotted on lactic agar (8 to 10 cultures per plate) and spotted with 0.05 ml of lysogenic phage induced from *S. lactis* C2

as described by McKay and Baldwin (8). Plates were incubated at 25°C, and cultures were observed for lysis by noting a clear zone appearing at the area spotted with phage. Plaquing of potential cured strains with the UV-induced phage from C2 was attempted. Mitomycin C induction of potential prophage was conducted as described by Park and McKay (15).

Transduction. Transduction of lactose metabolism in the isolated mutant $C14_5$ was accomplished as previously described (12) and by streaking the culture on lactose indicator agar, spotting with C2 lysogenic phage, and observing for Lac^+ transductants on the periphery of the cleared zone. Transductants were purified by streaking on lactose indicator agar and picking isolated colonies into lactose broth.

Plasmid analysis. The labeling and extraction of DNA, preparation of cesium chloride-ethidium bromide (CsCl-EB) gradients, and electron microscopy of plasmid DNA were described earlier (3). Molecular weights were calculated using the equivalence: 1.0 μm = 2.07×10^6 DNA (7).

RESULTS AND DISCUSSION

During attempts to demonstrate complementation between various Lac^- mutants isolated from *S. lactis* C2 by the integration of a specialized transducing phage, a Lac^- Prt^- mutant was isolated that failed to exhibit normal UV induction of the prophage. Growth responses for *S. lactis* C2 and for the mutant are shown in Fig. 1. Lysis of the 25-s UV-irradiated parent culture occurred after 1.5 h, but lysis did not occur in the 25-s UV-irradiated mutant cells. The mutant cells, however, could be induced to lyse by increasing the exposure time of UV irradiation. Figure 2 shows an induction of lysis after a 40-s exposure period. In an attempt to isolate a prophage-cured strain on which the bacteriophage induced from *S. lactis* C2 would form plaques, the mutant was treated with UV irradiation and cultured as described in Materials and Methods. Isolated colonies were then streaked on lactic agar and spotted with a UV-induced lysate from *S. lactis* C2. Of the cultures spotted with phage, only one showed clearing in the zone spotted with phage. This mutant was designated $C14_5$. Attempts to demonstrate prophage induction and subsequent lysis of the cells were unsuccessful using different dosages of UV irradiation (Fig. 3) or concentrations of mitomycin C (data not shown). Mutant $C14_5$, although apparently cured of prophage and

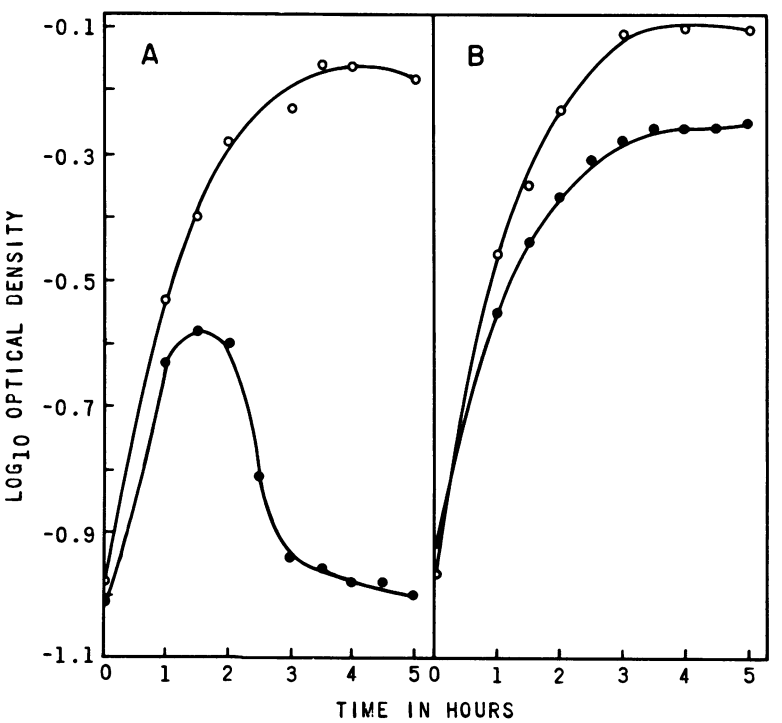

FIG. 1. *Exposure of S. lactis C2 (A) and an N-methyl-N'-nitro-N-nitrosoguanidine-induced Lac^- mutant (B) to UV irradiation. An exponentially growing culture was harvested, washed, and exposed to UV irradiation. The irradiated suspension was then inoculated into broth, and the change in absorbancy was measured (●). Unirradiated cells served as the control (○).*

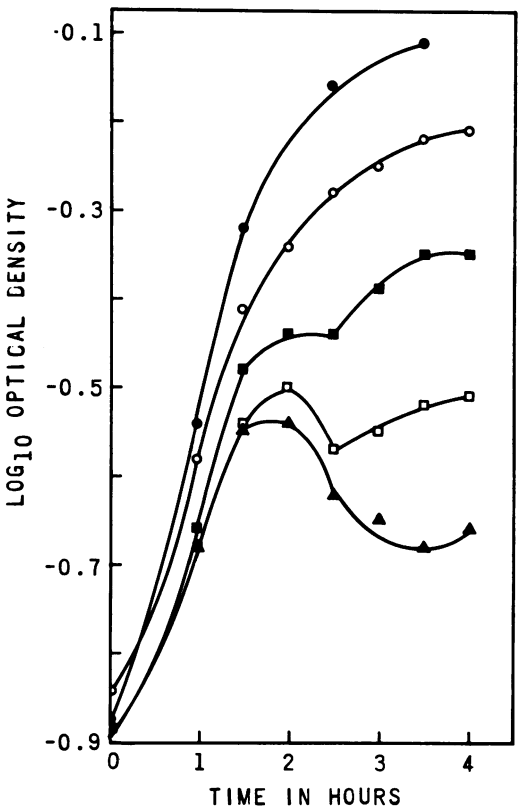

FIG. 2. *Exposure of an N-methyl-N'-nitro-N-nitrosoguanidine-induced Lac⁻ mutant of S. lactis C2 to UV irradiation. Seconds of irradiation: 0 (●), 25 (○), 30 (■), 35 (□), and 40 (▲).*

demonstrating spot lysis by the UV-induced prophage, did not serve as a sensitive indicator strain for the prophage. All attempts to demonstrate plaque formation were unsuccessful.

To determine whether $C14_5$ possessed plasmid DNA, cells were labeled with [^{3}H]thymine, harvested, lysed, and centrifuged to equilibrium in a CsCl-EB density gradient. *S. lactis* C2 contained plasmid DNA but $C14_5$ lacked a dense satellite peak separate from the chromosomal DNA, indicating that the cells contained very little, if any, plasmid DNA (Fig. 4). Electron microscope analysis for $C14_5$ plasmid DNA from the fractions normally containing the dense peak, however, revealed the presence of a very limited number of molecules. Extensive screening of grids was required from several CsCl-EB density gradients to reveal the presence of few small circular DNA molecules. The distribution of contour lengths of circular molecules of DNA found in $C14_5$ is shown in Fig. 5. It was previously observed that *S. lactis* C2 contained five plasmid size classes corresponding to 10^6, 2×10^6, 5×10^6, 10^7, and 3×10^7 daltons (10). No 3×10^7 plasmid was found in $C14_5$. Although plasmid sizes in $C14_5$ corresponding to the other four plasmids found in C2 appear to be present, the reason for the diminished number is not known.

McKay and Baldwin (9) have shown that proteinase activity and/or lactose metabolism can be transduced by a temperate phage from Lac⁺ Prt⁺ *S. lactis* C2 to spontaneous Lac⁻ Prt⁻ recipients of C2. The following experiments demonstrate that this transduction of lactose metabolism may involve the transduction of a portion of the 3×10^7 plasmid in Lac⁺ Prt⁺ *S. lactis* C2 to Lac⁻ Prt⁻ $C14_5$.

Lac⁻ Prt⁻ $C14_5$ was transduced to Lac⁺ Prt⁺ or Lac⁺ Prt⁻ derivatives. To determine whether plasmid DNA was involved in the acquisition of these genetic traits, the DNA was centrifuged in a CsCl-EB density gradient and examined

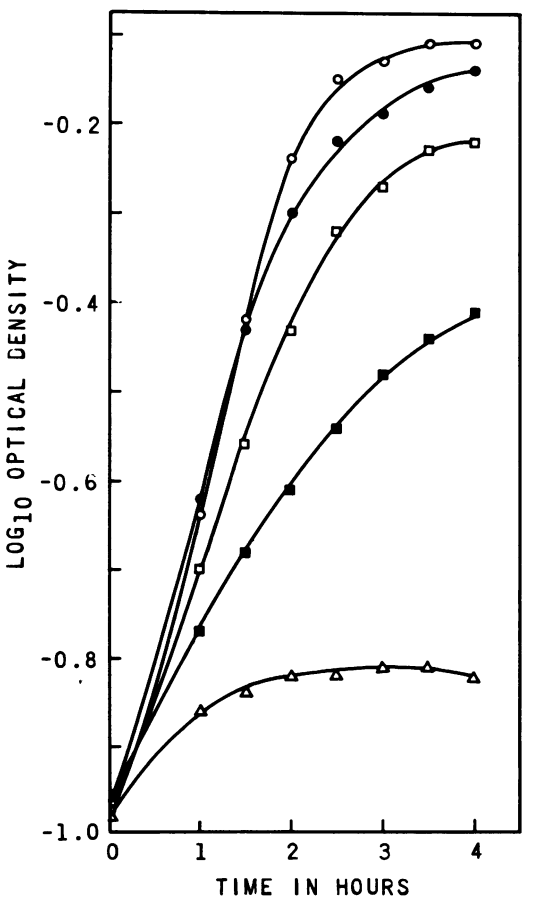

FIG. 3. *Exposure of $C14_5$ to UV irradiation. Seconds of irradiation: 0 (○), 25 (●), 40 (□), 60 (■), and 80 (△).*

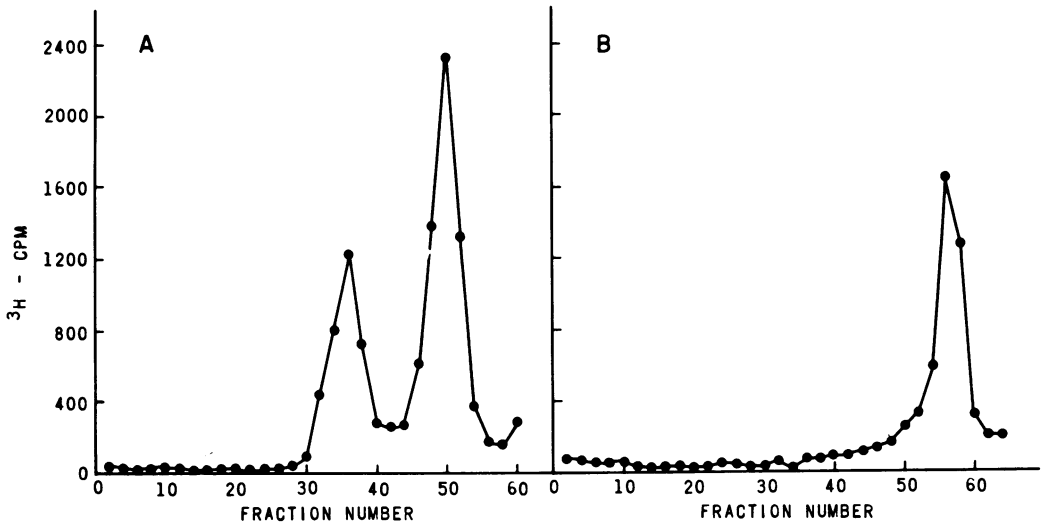

FIG. 4. *Elution profiles of CsCl-EB gradients of DNA from cleared lysate material of S. lactis C2 (A) and C14$_5$ (B).*

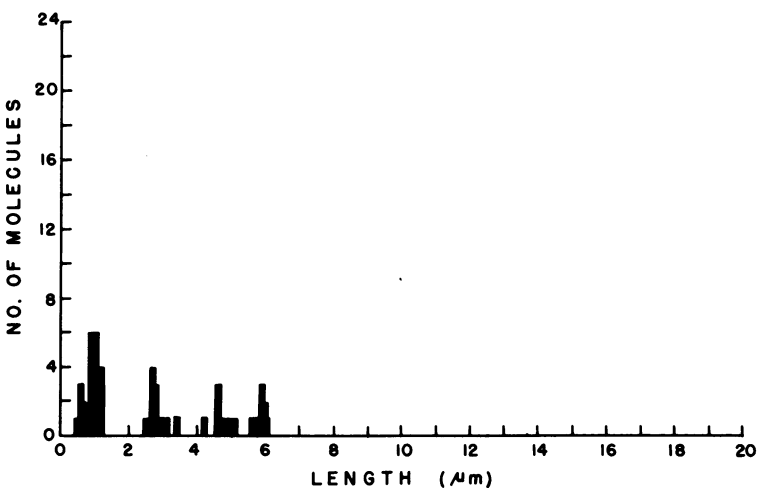

FIG. 5. *Distribution of contour lengths of circular molecules of DNA from C14$_5$.*

for a plasmid peak. No distinct satellite band of DNA was observed in either the Lac$^+$ Prt$^+$ or the Lac$^+$ Prt$^-$ transductants (Fig. 6). Presumably this was due to inefficiency in labeling and lysing of the cells since, when appropriate fractions were examined by electron microscopy, a distinct plasmid was observed in both transductants (Fig. 7 and 8). This plasmid was the predominate species of DNA and had a molecular weight of 20 × 10^6 to 21 × 10^6. A few small circular DNA molecules of various sizes were also observed but, in contrast to the 20 × 10^6 to 21 × 10^6 plasmid, required several preparations and extensive screening for their detection. The distribution of contour lengths of circular molecules of DNA shown on the histograms does not relate to the number of plasmid copies per cell. It is possible that these smaller plasmids represent catenated pieces of DNA, plasmid recombinants, or cast-off components of the larger plasmid since some plasmids are known to be comprised of smaller subunits, which, under certain conditions, segregate from the larger parent plasmid (14). The 20 × 10^6 to 21 × 10^6 plasmid was not found in Lac$^+$ Prt$^+$ or Lac$^+$ Prt$^-$ *S. lactis* C2 (10) and thus appears to be unique to the Lac$^+$ Prt$^+$ and Lac$^+$ Prt$^-$ transductants of Lac$^-$ Prt$^-$ C14$_5$. When the Lac$^+$

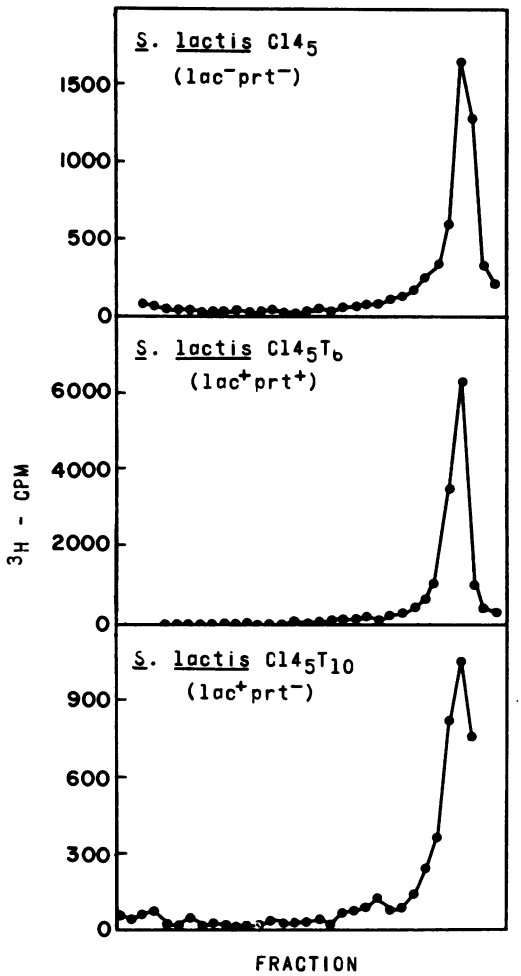

FIG. 6. *Elution profiles of CsCl-EB gradients of DNA from cleared lysate material of C14$_5$ and Lac$^+$ Prt$^+$ or Lac$^+$ Prt$^-$ transductants of C14$_5$.*

transductants of C14$_5$ became Lac$^-$ (spontaneous or acriflavine treatment), the 20 × 10^6 to 21 × 10^6 plasmid was no longer observed in electron micrographs. It thus appears that this plasmid is linked to lactose metabolism.

To obtain further evidence for this possibility, a spontaneous Lac$^-$ Prt$^-$ mutant of *S. lactis* C2, designated 25Sp, was examined. Cords et al. (3) had previously demonstrated the presence of a plasmid peak in elution profiles of CsCl-EB gradients of DNA from cleared lysate material of spontaneous Lac$^-$ Prt$^-$ *S. lactis* C2. They also observed the presence of three small plasmids having approximate molecular weights of 1, 2, and 5 million. The data in Fig. 9 confirm the presence of these three small plasmid species and indicate the presence of a 10^7 plasmid. No 3 × 10^7 plasmid was detected. The number of plasmids was greatly diminished compared to the parent culture. The reason for the presence of the 10^7 plasmid in 25Sp and C14$_5$ is not clear. In three other *S. lactis* strains examined by Efstathiou and McKay (4), it was found that Lac$^+$ Prt$^-$ mutants lacked an 8 × 10^6 to 10 × 10^6 plasmid and Lac$^-$ Prt$^-$ mutants lacked the 8 × 10^6 to 10 × 10^6 and an 18 × 10^6 to 22 × 10^6 plasmid. The Lac$^+$ Prt$^-$ derivative of *S. lactis* C2 lacked the 10^7 plasmid (10), and therefore one would assume it would also be absent in Lac$^-$ Prt$^-$ mutants. As mentioned earlier, one possible explanation for its presence in Lac$^-$ Prt$^-$ *S. lactis* C2 is that it represents catenated pieces of DNA, or, alternatively, the 10^7 plasmid found in Lac$^+$ Prt$^+$ *S. lactis* C2, but that some change has occurred in the cell such that proteinase activity cannot be expressed.

Since 25Sp, like C14$_5$, lacked the 3 × 10^7 plasmid, it was of interest to determine whether the 20 × 10^6 to 21 × 10^6 plasmid would appear in Lac$^+$ transductants of the Lac$^-$ Prt$^-$ mutant. Figure 10 shows the size distribution of plasmid molecules observed in a Lac$^+$ Prt$^+$ transductant of 25Sp. The number of copies of the 1-, 2-, 5-, and 10-million-dalton plasmids was greatly increased over that observed for 25Sp. No 3 × 10^7 plasmid was detected. However, a plasmid having a molecular weight of approximately 22 × 10^6 was observed. This species of plasmid DNA was not present in the parent culture.

The question arises as to the origin of a 20 × 10^6 to 22 × 10^6 plasmid in the transductants, since it is not observed in the parent culture. Klaenhammer and McKay have recently isolated and characterized two defective transducing phages induced by exposure of *S. lactis* C2 to UV irradiation (6). The molecular weights of the DNA from the two phages were 22.6 × 10^6 and 23.8 × 10^6, respectively. The transducing phages are thus too small to accommodate the 3 × 10^7 plasmid, which has been speculated to be linked to lactose metabolism.

Plasmid transduction has been described in *Escherichia coli* (1), *Salmonella typhimurium* (17), *Staphylococus aureus* (5), and *Proteus mirabilis* and *P. rettgeri* (2). Recently, Shipley and Olsen (16) described transduction with a phage that was too small to accommodate the entire plasmid genome, causing the formation of a smaller plasmid having only a portion of the genetic complement of the original parent plasmid. They termed the process transductional shortening and assumed that the process involved fragmentation of the plasmid DNA

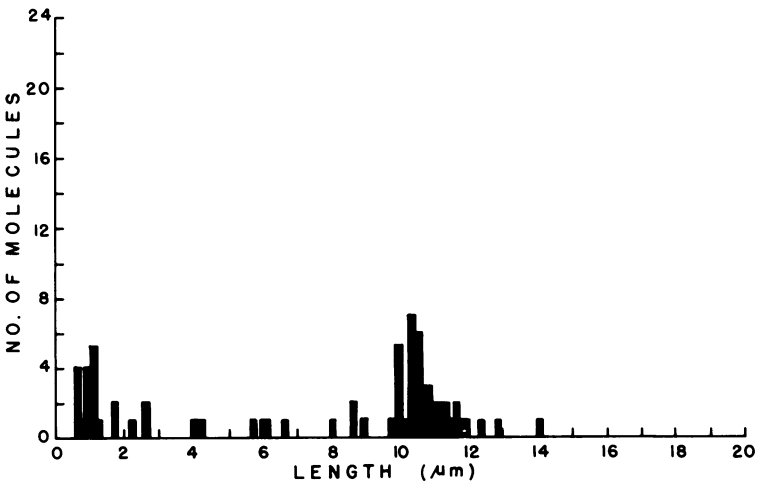

FIG. 7. *Distribution of contour lengths of circular molecules of DNA from a Lac$^+$ Prt$^+$ transductant of C14$_5$.*

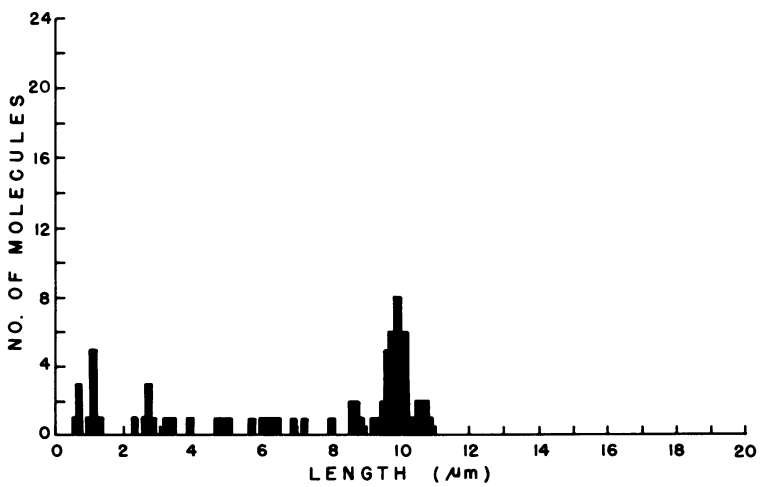

FIG. 8. *Distribution of contour lengths of circular molecules of DNA from a Lac$^+$ Prt$^-$ transductant of C14$_5$.*

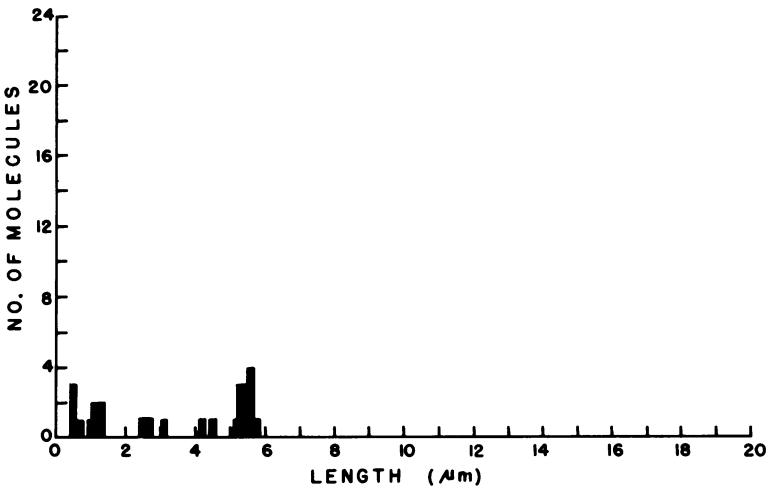

FIG. 9. *Distribution of contour lengths of circular molecules of DNA from 25Sp.*

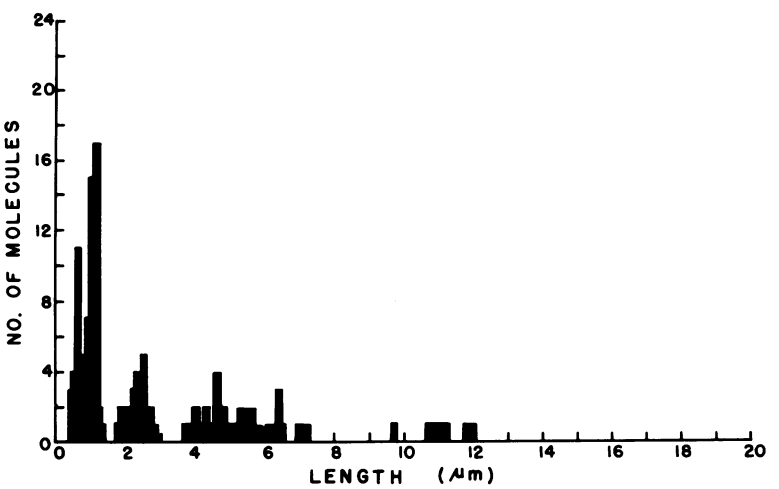

FIG. 10. *Distribution of contour lengths of circular molecules of DNA from a Lac$^+$ Prt$^+$ transductant of 25Sp.*

during packaging into the bacteriophage head followed by recirculation in the recipient to form a new autonomous replicating plasmid. From the data presented in this paper, a similar phenomenon of transductional shortening appears to be operating in *S. lactis* C2. Furthermore, it was established that lactose-fermenting ability is mediated through plasmid DNA in *S. lactis* C2 and that the plasmid does provide a mechanism for explaining the spontaneous loss of lactose metabolism observed in C2 as well as the appearance of Lac^+ transductants from Lac^- derivatives of C2.

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