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Induction of Prophage in *Streptococcus lactis* C2 by Ultraviolet Irradiation[1]

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Streptococcus lactis C2 has been used extensively by many laboratories in studies on the metabolism of lactic streptococci. By using ultraviolet irradiation as the inducing agent, this organism was shown to release a small bacteriophage, indicating that it is a lysogenic strain. The induced phage had a head approximately 40 nm in diameter and a tail length and width of about 180 and 6 nm, respectively. A structure resembling a collar was observed. Attempts to isolate a sensitive indicator strain for the virus were unsuccessful.

Lysogenic strains of lactic streptococci were first demonstrated by Reiter (10). He found three strains which spontaneously released phages that infected and lysed an appropriate indicator strain. Sandine et al. (11) examined several strains from his stock collection for possible lysogeny and found that *Streptococcus cremoris* W produced a phage which lysed *S. cremoris* 3. More recently, Keogh and Shimmin (7) observed that ultraviolet (UV) irradiation of *S. cremoris* C 11-56 was accompanied by cell lysis. Electron micrographs revealed the presence of particles resembling phage heads, most of which were empty. Only a few intact phage-like particles were observed. The authors suggested that the strain possessed a defective phage with bacteriocin-like activity.

During studies of the role lysogenization or phage conversion may have on lactose metabolism in lactic streptococci (manuscript in preparation), we discovered that *S. lactis* C2 harbors a latent or lysogenic virus. This paper describes the induction of this bacteriophage and shows electron micrographs revealing its morphology.

MATERIALS AND METHODS

Cultures. All lactic streptococci used in this study are maintained in our stock culture collection. Their maintenance and original source were described earlier (8). The following organisms were examined as possible indicator strains: *S. lactis* C2, b, E, C10, 11454, 7962, UN, 11955a, ML 3; *S. cremoris* B_1, Wg_2, C3, W, C 11-56, 144F, 3, M4W4-4-3, 9596, 9625, HP, ML_1, EB_7, AM_2; and *S. diacetilactis* 18-16, 11007, and DRC-3.

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Growth and irradiation. *S. lactis* C2 was grown in lactic broth (3) at 32 C for 10 h. The cells were centrifuged at 5,000 × *g* for 10 min and suspended in 0.85% saline so that a 1/20 dilution yielded an optical density of approximately 0.05 at 650 nm. Ten milliliters of the adjusted cell suspension was transferred to a sterile glass petri dish and the suspension was irradiated for 25 s during constant swirling (160 rpm). The UV source was 15-W General Electric germicidal lamp held 38.5 cm above the cell suspension.

To demonstrate the induction of lysis, the UV-treated cells were inoculated into 10 ml of broth containing: tryptone, 20 g; glucose, 5 g; NaCl, 4 g; sodium acetate, 1.5 g; $CaCl_2 \cdot 2H_2O$, 150 mg; $MgSO_4 \cdot 7\ H_2O$, 200 mg; and $MnSO_4 \cdot H_2O$, 50 mg per liter of deionized water. The pH of this medium was adjusted to 7.0 prior to autoclaving. Growth and lysis of the cells at 32 C were determined by measuring the change in absorbancy at 650 nm. Unirradiated cultures served as the control.

Assay for bacteriophage. Tests for bacteriophage were made by spotting 0.05 ml of the UV-induced lysate on a semisolid lactic agar overlay (6) seeded with about 10^7 host cells. The plates were incubated overnight at about 23 C, and the area where lysate had been placed was examined for a phage plaque. The host cells included strains of *S. lactis*, *S. cremoris*, and *S. diacetilactis*.

Electron microscopy. A 100-ml amount of lysate from UV-induced cultures of *S. lactis* C2 was centrifuged at 5,000 × *g* for 15 min in a Sorvall centrifuge. The supernatant fluid was filtered through a Millipore filter (0.8 μm) and then recentrifuged at 54,500 × *g* for 1 h in a Beckman ultracentrifuge. The supernatant fluids were discarded, and 0.1 ml of a phage diluent containing 0.05 M potassium phosphate buffer (pH 7.0), 0.1 M NaCl, and 0.05 M $MgSO_4$ was added to the pellets (not visible). The centrifuge tubes were gently shaken at 4 C for 3 h to resuspend any bacteriophage, and then the contents were pooled. One drop of the bacterio-

phage suspension was placed on a Formvar-coated copper grid and negatively stained with 2% Na phosphotungstate at pH 7. Grids were examined in a Philips 300 electron microscope.

To determine the approximate size of the lactic phage particles, tobacco mosaic viruses of known dimensions were added to the phage preparation and electron micrographs were prepared as described above.

RESULTS AND DISCUSSION

Exposure of lysogenic bacterial cells to UV irradiation or other inducing agents may result in induction of the prophage and subsequent lysis of the cells (5). A typical growth experiment of *S. lactis* C2 is shown in Fig. 1. Lysis of the UV-irradiated culture occurred after 2 h at 32 C, as evidenced by the decrease in optical density. Spotting of the lysate from the UV-irradiated culture on a lawn of *S. lactis* C2 did not result in lysis of the cells, indicating that the host cell was immune to lytic infection if a phage were present in the lysate. Streaking and purifying *S. lactis* C2 did not alter the induction of lysis by UV irradiation.

Attempts to isolate a sensitive indicator strain on which a bacteriophage in the UV-irradiated lysate would form plaques were unsuccessful. Nine strains of *S. lactis*, 14 of *S. cremoris*, and 3 *S. diacetilactis* strains were examined.

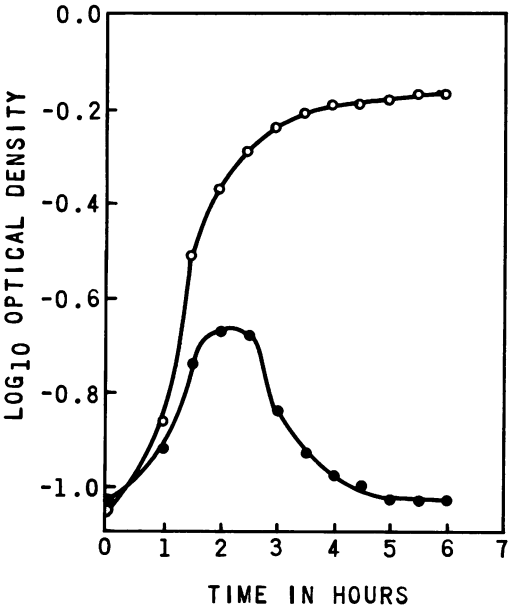

FIG. 1. *Induction of lysis in S. lactis C2 by UV irradiation. An exponentially growing culture was harvested, washed, and exposed to UV irradiation. The irradiated suspension was then inoculated into broth and the change in absorbancy was measured* (●). *Unirradiated cells served as the control* (○).

Examination of the UV lysate under the electron microscope, however, did reveal the presence of bacteriophages (Fig. 2). The head appeared to be hexagonal and had a diameter of about 40 nm. The tail was about 6 nm wide and about 180 nm in length. The tail, like that described for the d_4 lactic bacteriophage (1), was flexible and was generally observed in a curved position. The most striking feature of the induced phage was the presence of a structure resembling a collar. This type of structure, to our knowledge, has not previously been observed in lactic streptococcal bacteriophages.

Parmelee et al. (9), Sandine et al. (11), and Williamson and Bertaud (12) have examined electron micrographs of lactic streptococcal bacteriophages. The bacteriophages they observed were similar in shape and size and could not be clearly differentiated. More recently, Bauer et al. (1) concluded that, in spite of the similar ultrastructure of all examined *Streptococcus* phages, eight different bacteriophages could be distinguished morphologically. This was possible due to different particle sizes, tail lengths and widths, and morphology of the tail end plates. The presence of a structure resembling a collar in the phage described above indicates an additional type of lactic streptococcal bacteriophage. The induction of prophage in *S. lactis* C2 illustrates lysogeny in another strain of lactic streptococci.

The occurrence of lysogenic streptococci which spontaneously release bacteriophages that may infect other strains in a mixed-strain starter culture was first reported by Reiter in 1949 (10). Graham et al. (4) failed to demonstrate lysogenic strains among isolates from commercial cultures in the United States, although they observed that certain of these cultures did carry bacteriophages. The origin of phage in the cheese plant is not definitely known, but, as suggested by Czulak and Naylor (2), the lactic streptococci themselves must be considered as a possible reservoir. The use of inducing agents such as UV irradiation would provide a screening test for the lysogenic strains. Recently, by using this test, we examined strains of lactic streptococci isolated from commercial dairy starter cultures and found that some strains exhibited a growth response as illustrated in Fig. 1 (unpublished results). This suggests the presence of lysogenic strains in commercial dairy cultures used in the United States. Such strains should be avoided,

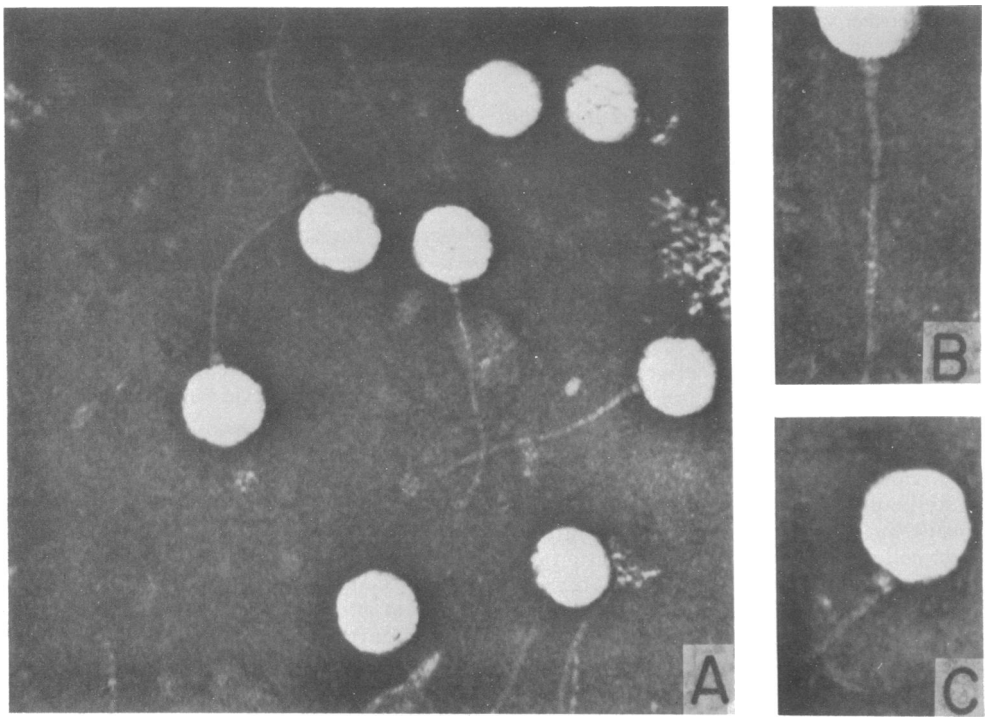

FIG. 2. *Electron micrographs of the latent bacteriophage induced from S. lactis C2 showing the general morphology (A, ×125,000) and the collar-like structure (B and C, ×170,000).*

if possible, in the selection of lactic streptococci to be incorporated into commercial starter cultures, as they may be a source of phage in the cheese plant.

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