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Lysogenic Strains of Group N Lactic Streptococci

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A temperate bacteriophage, designated r_1t, was inducible from the group N lactic streptococcus, *Streptococcus cremoris* R_1, by ultraviolet irradiation or mitomycin C treatment. Induced lysates produced plaques on lawns of three closely related *S. cremoris* strains, AM_1, SK_{11}, and US_3. Strain SK_{11} was readily lysogenized. *S. cremoris* AM_1 was the most reliable indicator strain, although the age of the culture used for seeding plates was critical. Zones of lysis but no plaque formation were observed on lawns of nine additional *S. cremoris* strains. Phage r_1t could not be detected in filtrates of stationary-phase R_1 cultures and was near the limits of detection in logarithmically growing cultures. Phage levels were still very low (1 plaque-forming unit on AM_1 per 10 induced cells) in induced lysates of R_1 cultures. These low levels of detectable phage may be attributable to an inadequate indicator, lysogenization of the indicator, adsorption of induced phage to cellular debris, concurrent induction of other undetectable phages, or the production of high proportions of defective phages. Electron micrographs of induced R_1 lysates revealed a high incidence of incomplete phage particles, fragments, and ghosts.

Lysogeny is known to be widespread amongst streptococci of groups A and C (17). Some lysogenic strains have also been found in groups G (3) and H (11). Very recently lysogeny was demonstrated in strains of group N streptococci designated as *Streptococcus lactis* (7, 9) and *S. diacetilactis* (7), although lysogeny was not found in *S. cremoris* (7). Lysogeny in the *S. cremoris* strains of group N lactic streptococci, used as starters in cheesemaking, has been suspected for some years (4, 5, 12, 14), but in no case has the presumed lysogeny been rigorously demonstrated. Keogh and Shimmin (6) induced lysis of *S. cremoris* C 11-56 by ultraviolet (UV) irradiation but were unable to show the presence of a typical temperate phage. Lysates from strain C 11-56 produced clear zones on 9 of 12 strains of *S. cremoris* when spotted on appropriately seeded plates. Striking features of the lytic spectrum were that strain C 11-56 was itself sensitive to the lysate and that plaque formation was not observed. Electron micrographs showed that the lysate contained phage-like particles, mostly empty heads. The antibacterial activity of lysates was therefore attributed to the presence of an induced defective bacteriophage, or a lethal component of it.

This study (carried out in partial fulfillment of the requirements of the Ph.D. degree in Food Technology, Massey Univ. New Zealand) reports the induction of lysis in cultures of *S. cremoris* R_1 by UV irradiation or by mitomycin C (MC) treatment. These lysates produced zones of lysis on lawns of several *S. cremoris* strains, and plaque formation occurred on *S. cremoris* strains AM_1, SK_{11}, and US_3. Bacteriophage particles were seen in electron micrographs of these lysates, and some characteristics of the inducible phage were investigated using *S. cremoris* AM_1 as the indicator strain.

MATERIALS AND METHODS

Streptococcal strains. All of the cultures used in this study were cheese starter strains of *S. lactis* and *S. cremoris* from the collection of the New Zealand Dairy Research Institute.

Bacteriophages. Temperate bacteriophage, designated r_1t, was isolated from UV-induced lysates of *S. cremoris* R_1. The virulent bacteriophages, r_1v (NZDRI 652), am_1 (NZDRI 601), and sk_{11} (NZDRI 690), were drawn from the New Zealand Dairy Research Institute collection.

Media and growth of cultures. M16 broth and M16 agar were prepared as previously described (8). Streptococci were grown routinely at 22 or 30 C, without shaking, in M16 broth from a 2% inoculum of an overnight (22 C, 16 h) broth culture.

Optical density. Optical density (OD) of cultures was measured in a Bausch and Lomb Spectronic 20 colorimeter. An OD value of 0.2 at 580 nm represented approximately 10^8 colony-forming units (CFU)/ml.

Colony counts. Samples were first diluted with

chilled 0.14 M NaCl to a final volume of 100 ml and blended at 13,000 rpm for 1 min in an AtoMix blender (Measuring and Scientific Equipment Limited, Crawley, England) to reduce chains of lactic streptococci to an average CFU of 2.2 to 2.6 cocci. Appropriate dilutions were plated on M16 agar by soft agar overlay. Plates were incubated at 30 C for 16 to 24 h.

Assay of phage. Phage was assayed by the soft agar layer method (1) using M16 agar supplemented with calcium borogluconate (Veterinary grade, May and Baker Ltd., Dagenham, England) to a final concentration of 0.005 M (8). Plates were seeded with 0.1 ml of the required overnight (22 C, 16 h) culture. When *S. cremoris* AM_1 was used as an indicator for phage r_1t, plates were seeded with 0.1 ml of a 30 C, 24 h culture. The lytic spectrum of undiluted, diluted, and concentrated phage lysates was determined by spotting 10-μliter quantities of membrane-filtered preparations (HA membrane, 0.45-μm pore size, Millipore Corp., Bedford, Mass.) on plates previously seeded with the selected strains by soft agar overlay. Plates were incubated at 30 C for 16 h.

Induction of lysogens: MC treatment. Selected cultures were grown in M16 broth at 30 C to an OD_{580} of 0.1 when MC (Sigma Chemical Co., St. Louis, Mo.) was added, normally to a final concentration of 1 μg/ml. Incubation was continued at 30 C, and the OD_{580} was followed until completion of lysis.

UV irradiation. Mid-logarithmic-phase cultures in M16 broth at 30 C were harvested by centrifugation (10,000 $\times$ *g* for 10 min), resuspended in 100 ml of chilled phosphate-buffered saline (pH 6.5) and blended in an AtoMix blender as described above. After blending, cells were again centrifuged and resuspended in sufficient buffered saline to give an OD_{580} of 1.0 (5×10^8 CFU/ml). Quantities of 1 ml were UV irradiated in petri dishes (5.5 cm diameter) 29 cm from a Hanovia bactericidal UV lamp. UV irradiation dosages were operationally defined from their lethal effects on a typical group N lactic streptococcus, *S. lactis* ML_3 (NCDO 763; NZRCC 20030) which has a mean CFU of 2.64 cocci (15). UV exposures of 5, 10, 15, 20, and 30 s reduced survival of the noninducible strain ML_3 to 30, 12, 5, 1.5, and 0.2%, respectively. After UV irradiation, suspected lysogens were diluted 10-fold into M16 broth, incubated at 30 C, and examined at intervals for lysis.

Propagation of phage. Virulent phages were propagated on their homologous hosts in M16 broth, containing 0.005 M calcium borogluconate, by adding phage in a 1:100 ratio (plaque-forming units [PFU] to CFU) to logarithmic cultures (5×10^8 CFU/ml) which were incubated at 25 or 30 C until lysis occurred. Lysates were centrifuged (10,000 $\times$ *g* for 10 min) and filtered through an HA membrane.

MC- or UV-induced lysates of *S. cremoris* R_1 were plated on *S. cremoris* AM_1 to obtain phage r_1t. Single plaques were picked into 1-ml quantities of M16 broth from which 0.1 ml was inoculated into early logarithmic-phase cultures (about 10^5 CFU/ml) of *S. cremoris* AM_1. Infected cultures were incubated at 25 C for 16 h when the titers on strain AM_1 were 10^8 to 10^9 PFU/ml. Attempts to obtain high-titer preparations of phage r_1t by conventional methods from these stocks were unsuccessful.

Concentration of phage r_1t. A 2,000-ml logarithmic culture (OD_{580} of 0.1) of strain R_1 in M16 broth at 30 C was induced with MC (0.5 μg/ml). After lysis was complete, the induced phage was concentrated by the dextran sulfate-polyethylene glycol two-phase separation system as described for coliphage T2 by Albertsson(2). The crude phage concentrate was further purified and concentrated by two cycles of differential centrifugation. Approximately 1 ml of temperate phage r_1t at greater than 10^{10} PFU/ml on strain AM_1 was obtained per liter of R_1 lysate.

Electron microscopy. High-titer bacteriophage preparations were diluted 10-fold into 0.1% bovine serum albumin solution and negatively stained with an equal volume of 2% neutralized phosphotungstic acid. Aerosols of stained preparations were sprayed onto carbon film grids and examined using a Phillips EM200 eletron microscope.

RESULTS

Search for lysogens. Initially, filtrates from M16 broth cultures of 29 strains of lactic streptococci (22 *S. cremoris* and 7 *S. lactis*) were spotted on lawns of these 29 strains. No obvious signs of either phage reactions or of growth inhibition were observed. However, when MC was added to logarithmic (OD_{580} of 0.1) M16 broth cultures at 30 C, at final concentrations of 0.5 and 1.0 μg/ml, overt lysis resulting in complete clearing of the cultures was observed in 4 of 22 *S. cremoris* and 2 of 7 *S. lactis* strains tested. In preliminary lytic spectrum determinations, the lysate from *S. cremoris* R_1 showed plaque formation on some strains. *S. cremoris* R_1 was, therefore, selected for further study.

MC induction of lysis in S. cremoris R_1. The effects of MC additions to cultures of *S. cremoris* R_1 are shown in Fig. 1. Neither time of addition nor final concentration of MC were particularly critical factors in inducing lysis of cultures. Cultures incubated at 30 C were inducible by a range of MC concentrations from 0.1 to 4.0 μg/ml, provided that cultures were growing logarithmically and contained less than 10^8 to 2×10^8 CFU/ml. Induction of lysis by MC was most consistent when MC was added at a final concentration of 0.5 to 1.0 μg/ml to logarithmically growing cultures which had reached an OD_{580} of 0.1 ($\sim 5 \times 10^7$ CFU/ml after blending). Under these conditions there was little effect on growth for some 30 min; then there was a gradual slowing of increase in OD over the next 60 to 90 min, followed by marked and rapid lysis of the culture. Either higher or lower MC concentrations consistently gave less complete lysis of cultures. No visible lysis occurred if MC concentrations were lower than 0.05 or greater than 5.0 μg/ml.

UV induction of lysis in S. cremoris R_1. Irradiation for at least 5 s was required to give

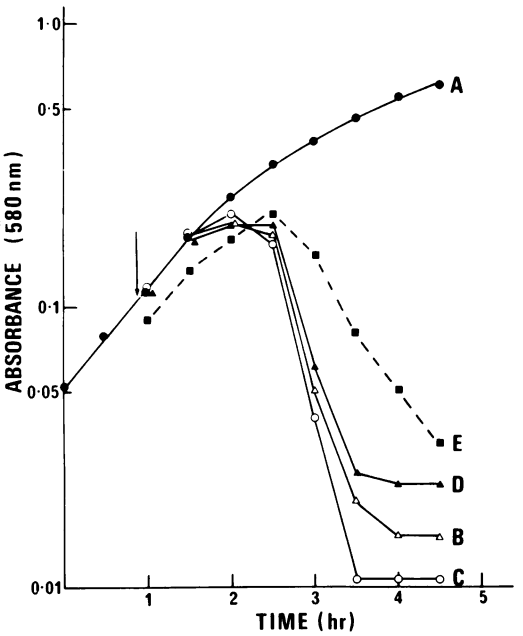

FIG. 1. *Induction of lysis in S. cremoris R_1. At the time indicated by the arrow a 1,000-ml M16 broth culture of strain R_1 at 30 C was divided into four cultures of 250 ml which received no additions (curve A), 0.5 μg of MC per ml (curve B), 1.0 μg of MC per ml (curve C), and 2.0 μg of MC per ml (curve D). Incubation was continued at 30 C, and 10-ml samples were withdrawn at 30-min intervals for OD readings. In a separate experiment, washed cells of strain R_1 were UV irradiated for 10 s and transferred to M16 broth, and the change in OD was followed during incubation at 30 C (curve E). A nonirradiated sample showed a growth pattern similar to curve A.*

visible lysis of strain R_1. Most pronounced lysis resulted from 10 to 15 s of UV irradiation. Longer UV exposures brought about progressively less lysis. After 30 s of UV irradiation, strain R_1 showed little growth and no lysis during 4 h of subsequent incubation in broth at 30 C. The OD readings showing growth and lysis of R_1 transferred to M16 broth after UV treatment for 10 s are included in Fig. 1. The pattern of induction after this exposure to UV irradiation is very similar to that brought about by MC addition.

Lytic spectrum. MC- and UV-induced lysates of strain R_1 were spotted, undiluted and at decimal dilutions, on lawns of 29 strains of *S. lactis* and *S. cremoris*. Twelve strains, all *S. cremoris*, which gave a positive response are listed in Table 1. Serial dilutions of the MC-induced R_1 lysate were plated by the soft agar overlay technique on all 12 strains which had shown either lytic zones or plaques in the spot tests. Plaque formation occurred on only three strains, AM_1, an AM_1 derivative SK_{11}, and the closely related US_3. The zones of lysis observed on the remaining nine strains were not caused by sensitivity of some strains to MC present in the induced lysates, because lysates obtained by UV treatment showed the same combination of plaquing and nonplaquing reactions in the lytic spectrum. The lytic spectrum of a concentrated MC-induced R_1 lysate was also tested. The concentration procedures raised PFU on strain AM_1 from ~10^7/ml to ~10^{10}/ml, but no changes were found in the range of strains showing zones of lysis, and there was no increase in the number of strains exhibiting plaque formation.

The nine strains showing a reaction to the lysates, but never exhibiting plaque formation, may have become lysogenized by phage in the lysates. Colonies isolated from lytic zones and turbid areas produced by R_1 lysates on all nine strains were tested as suspected lysogens, but the results were either negative or inconclusive.

The phage present in induced R_1 lysates was isolated from plaques produced on strain AM_1 and propagated on this strain. From Table 1 it can be seen that only two of the nine strains which had shown a positive but nonplaquing

TABLE 1. *Lytic spectrum[a] of temperate and virulent bacteriophages of S. cremoris R_1*

Strain of *S. cremoris*[b]	Lytic spectrum				
	R_1 culture filtrate[c]	MC-induced R_1 lysate	UV-induced R_1 lysate	$r_1t \cdot AM_1$[d]	$r_1v \cdot R_1$[e]
R_1	—	—	—	—	1.4×10^9[f]
AM_1	—	3.0×10^7	3.7×10^7	2.8×10^7	4.0×10^2
AM_2	—	±	±	+	+
BR_4	—	+	+	—	—
C_{18}	—	++	++	++	—
HP	—	++	++	—	—
KH	—	++	++	—	—
ML_1	—	+	±	—	—
P_1	—	++	++	—	—
P_2	—	++	++	—	—
R_6	—	+	+	—	5.3×10^2
SK_{11}	—	2.3×10^6	2.0×10^6	2.2×10^6	8.0×10^1
US_3	—	1.4×10^7	1.8×10^7	1.1×10^7	3.8×10^2

[a] Symbols: +, lysis; ++, pronounced lysis; ±, weak or inconsistent lysis; —, no lysis.

[b] No lysis was observed on 9 *S. lactis* and a further 11 *S. cremoris* strains tested.

[c] Phage preparation tested.

[d] Temperate phage from *S. cremoris* R_1 propagated on strain AM_1.

[e] Virulent phage of *S. cremoris* R_1 propagated on strain R_1.

[f] Plaque-forming units per milliliter in preparations which showed plaquing reaction in spot tests.

reaction to R_1 lysates were retained in the lytic spectrum after the phage had been propagated on AM_1. Plaque formation and relative efficiency of plating (EOP) on strains AM_1, SK_{11}, and US_3 were unchanged.

Indicator strain. *S. cremoris* AM_1 gave the highest plate counts for the phage present in induced R_1 lysates. Strain AM_1 was used, therefore, as the indicator for this phage which was designated r_1t. Initially, the plaque counts of phage r_1t on AM_1 showed considerable variation. It soon became apparent that the age of the culture used for seeding plates had a marked effect on plating efficiency. Highest EOP on AM_1 was found when plates were seeded from cultures grown well into the stationary phase by incubation at 30 C for 24 h. Plaque counts were always 10 to 100 times less if cultures grown at 22 C or still in logarithmic growth were used as host cells for plating r_1t phage. Indeed, no plaque formation occurred at any dilution if plates were seeded with early logarithmic AM_1 cultures. The age of seed culture also affected EOP of r_1t phage on strains SK_{11} and US_3, but the effects were not nearly as pronounced as with AM_1. These observations suggested that the indicator for r_1t phage was itself becoming lysogenized, but specific attempts to demonstrate lysogenization of AM_1 by phage r_1t were inconclusive.

Lysogenization of strain SK_{11}. Strain SK_{11}, which exhibits better growth characteristics on synthetic media than its parent strain AM_1, was tested for lysogenization by phage r_1t because EOP on this strain remained lowest regardless of the age of seed culture. Colonies of SK_{11} resistant to lysis by r_1t phage were isolated from the turbid areas formed when the phage was spotted on plates seeded with SK_{11}. After subcloning three times, the r_1t phage-resistant SK_{11} isolates and the appropriate control cultures were treated with MC. The induction of lysis was less obvious in these suspected lysogens than in strain R_1, and the level of spontaneously induced phage was higher. Nevertheless, the phage titer in MC-induced cultures increased to greater than 10^6 PFU/ml on AM_1. The increase observed in the untreated resistant isolates was only 10^2 PFU/ml. Cultures of SK_{11} colonies, sensitive to r_1t phage, that were isolated from the same turbid zone as the lysogenized colonies, as well as control stock SK_{11} cultures, sporadically and inconsistently showed lysis when treated with MC. The r_1t phage was never isolated from these lysates, and no indicator strain was found. Immunity to the lysogenizing phage was the only difference in phage susceptibility that was observed between strain SK_{11} and the r_1t phage-lysogenized isolates. Sensitivity to two serologically unrelated virulent phages, am_1 and sk_{11}, was unchanged.

Induction of phage r_1t. Once *S. cremoris* AM_1 was established as the indicator strain for phage r_1t, it was possible to reinvestigate the induction of strain R_1 and assay for the presence of this temperate phage in induced and noninduced cultures. It can be seen (Fig. 2) that there is a low but consistent rate of spontaneous induction during logarithmic growth of strain R_1 in M16 broth. Detectable phage r_1t was at a PFU to CFU ratio of 10^{-6} until mid-logarithmic growth. This ratio increased slightly as the culture entered late logarithmic growth, and then fell suddenly with the onset of stationary phase. Since the levels of r_1t phage detected in noninduced R_1 cultures never exceeded 5×10^2 PFU/ml at any time, and were much lower in stationary-phase cultures, it is not surprising that the lytic spectrum tests on culture filtrates

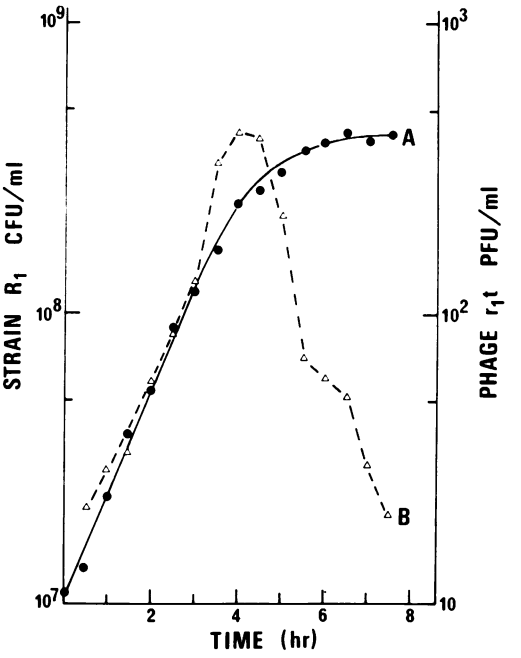

FIG. 2. *The presence of phage r_1t in an uninduced culture of S. cremoris R_1. M16 broth (500 ml) was inoculated (2%) from an overnight culture of strain R_1 and incubated at 30 C. Samples (10 ml) were withdrawn at 30-min intervals and colony-forming units per milliliter (curve A) were determined. The balance of the sample was chilled and centrifuged (10,000 × g for 10 min). Supernatants were assayed for phage r_1t using S. cremoris AM_1 as the indicator strain (curve B).*

gave negative results (Table 1). The increase in detectable r_1t phage in an MC-induced R_1 culture is shown in Fig. 3. This increase, some 10^4 PFU/ml over that found in untreated control cultures, coincided with the slowing of OD increase and onset of lysis. The induced culture reached a maximum OD_{580} equivalent to 1.7×10^8 CFU/ml (from 6×10^7 CFU/ml at MC addition) before lysis of 90% of the cells. The phage titer in this lysate of 5×10^6 PFU/ml on the indicator strain AM_1 represented a burst of less than 1 detectable phage per 10 induced cells.

Relationship between virulent and temperate phages of S. cremoris R_1. The host range of a virulent phage of strain R_1, r_1v propagated on R_1, is shown in Table 1. A low incidence of plaque formation on strain R_6 together with the expected virulence on strain R_1 were the only differences in the range of hosts on which the temperate and virulent phages could produce plaques. In routine propagation of phage r_1v on R_1, lysis normally occurs when cultures are in late logarithmic growth, the time when spontaneously induced temperate phage is highest (Fig. 2). Plaque formation on AM_1, SK_{11}, and US_3 would, therefore, appear to represent the extent of temperate phage contamination of the virulent phage preparation.

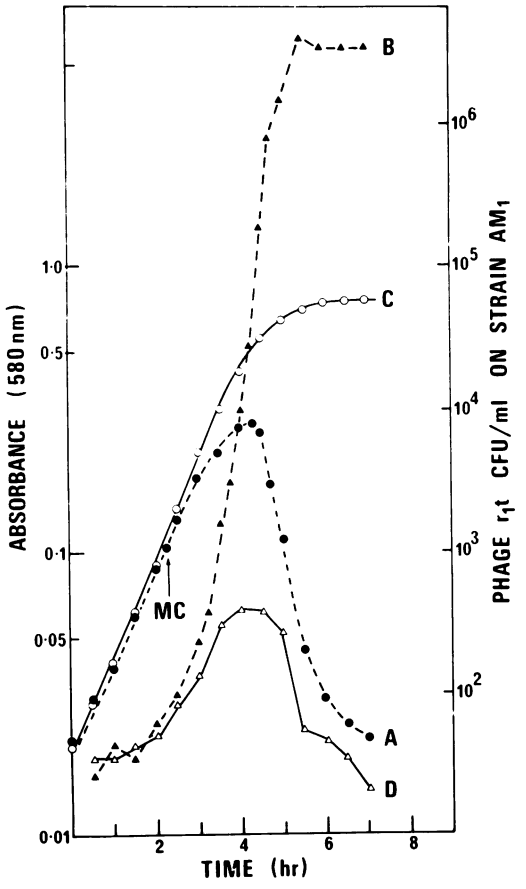

FIG. 3. *Phage r_1t in an MC-induced culture of S. cremoris R_1. An M16 broth culture of strain R_1, prepared as in Fig. 2, was incubated at 30 C. MC (1 μg/ml) was added when the culture reached an OD_{580} of 0.1 (arrow). Samples were withdrawn at intervals for OD readings (curve A). The balance of each sample was centrifuged and supernatants were assayed for phage r_1t as in Fig. 2 (curve B). The OD profile (curve C) and r_1t phage levels (curve D) of an untreated control culture are also shown.*

The similarity in host range between the virulent phage (originally isolated from cheese whey) and the induced phage suggested that the former might be a virulent mutant of the temperate phage. An examination of electron micrographs of the two phages showed that they were indeed very similar in appearance and dimensions (Fig. 4A and B). The most striking contrast between the virulent and temperate r_1 phages was the high amounts of incomplete phage particles, fragments, and ghosts that were seen in temperate phage preparations (Fig. 4C). Extensive searching of grids was necessary in order to find any fragments or ghosts in virulent phage preparations.

DISCUSSION

Lysogeny among strains of group N lactic streptococci has been suspected for some years (4, 5, 12, 14), and it is surprising, in view of the increasing commercial significance of *S. cremoris* strains, that this presumed lysogeny has not been confirmed much earlier. Certainly, the simple procedures of looking for phages in culture filtrates and cross-streaking of suspected lysogens have, in the main, been unsuccessful. Where reactions between strains have been observed (14) the possibilities of inhibition of growth by culture products, such as the antibiotic nisin, have not been eliminated. Strains of group N streptococci inducible by the two most frequently used inducing agents, UV irradiation and MC treatment, appear to be relatively widespread. Use of either of these agents caused unambiguous induction of lysis in 4 of 22 *S. cremoris* strains and 2 of 7 *S. lactis* strains that were investigated in this study. Kozak and his colleagues (7) found that 4 of 46 *S. lactis* and 2 of 24 *S. diacetilactis* strains were UV inducible. Induction by UV irradiation of *S. cremoris* strains has also been found by Keogh and Shimmin (6) and by Reiter (13). It may well

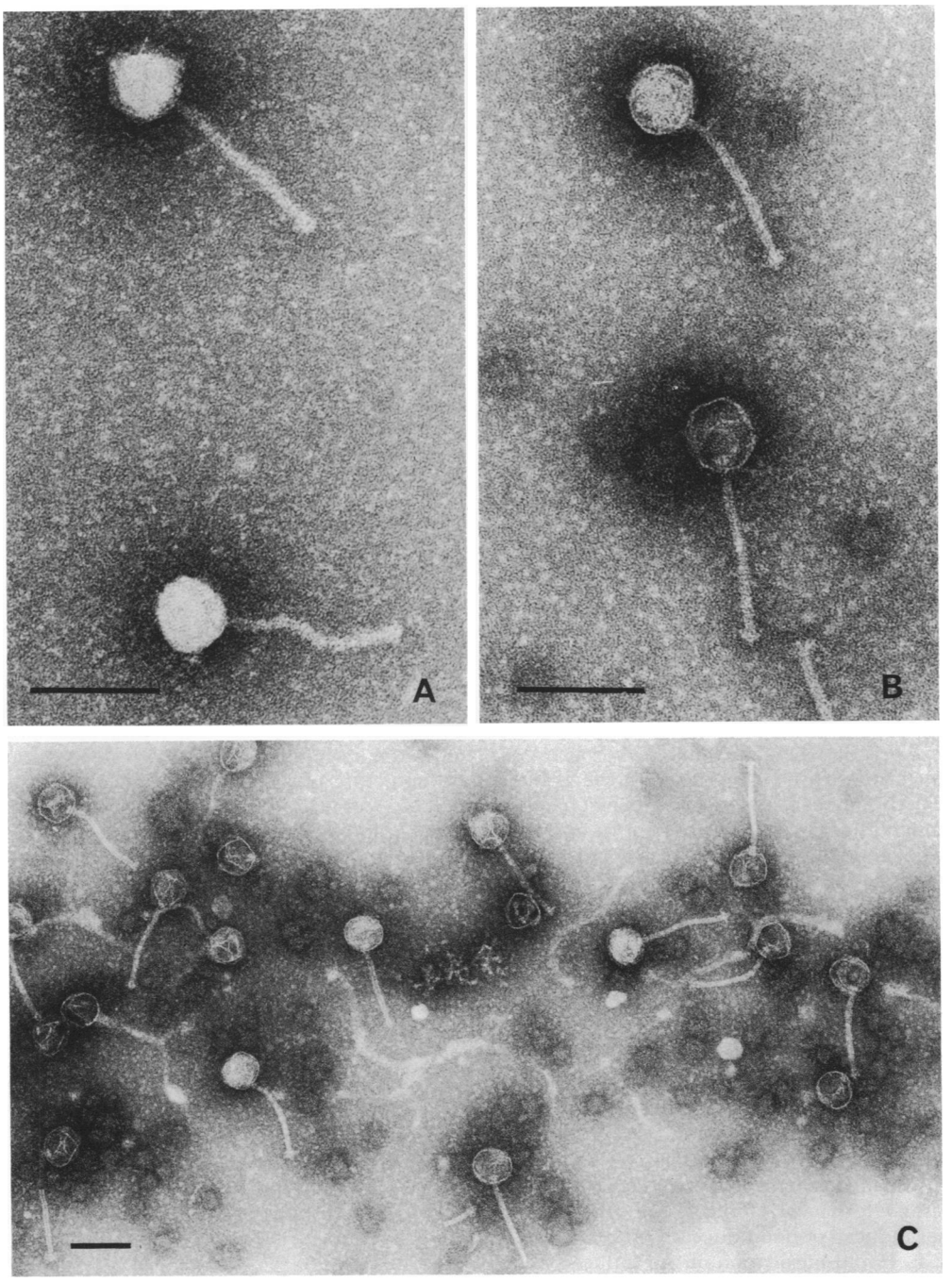

FIG. 4. *Phages of S. cremoris R_1 negatively stained with neutral 2% potassium phosphotungstate. A, Virulent phage r_1v. Magnification ×180,000. B, Temperate phage r_1t. Magnification ×180,000. C, Phage, phage fragments, and ghosts in an MC-induced lysate of S. cremoris R_1. Magnification ×85,000. Bar markers represent 100 nm.*

be that lysogeny, or defective lysogeny, is such a common feature of the group that the absence of indicator strains rather than of lysogens has impeded the characterization of temperate bacteriophages. McKay and Baldwin (9) failed to find an indicator for the phage revealed in

electron micrographs of lysates from UV-induced *S. lactis* C2.

Indicator strains, on which plaque formation occurred, were found for only one (*S. cremoris* R_1) of the six strains that showed overt lysis after UV irradiation or MC treatment. In addition, zones of lysis but no plaque formation were produced on lawns of several strains. Unlike the report of Keogh and Shimmin (6), no lysis was observed on lawns of the strain which gave rise to the lysate. Of the three closely related strains on which R_1 lysates produced plaques, *S. cremoris* AM_1 was selected as the routine indicator for the phage induced from R_1, although strain AM_1 is an unusual indicator in several respects. The history of the culture used for seeding plates was found to have a striking effect on the EOP of phage r_1t. Highest plating efficiencies were obtained with late stationary-phase cells that had been grown at 30 C. The reasons why these cultures gave highest plate counts are not understood and the finding was unexpected because the use of early to mid-logarithmic cultures for seeding plates was essential for highest EOP of some group H temperate phages on their indicator *S. sanguis* strain Wicky (11). No plaque formation whatsoever was observed if logarithmically growing AM_1 cultures were used in phage r_1t assays. It is not surprising, therefore, that AM_1 was a poor propagating strain for r_1t phage. It seems likely that AM_1 may become lysogenized by phage r_1t, although direct evidence could not be obtained. However, strain SK_{11}, which consistently showed the lowest EOP for phage r_1t (Table 1), did become lysogenized, since r_1t phage-resistant colonies from which the phage could be induced by MC treatment were readily isolated from the turbid areas formed on SK_{11} plates by lysates of strain R_1. The sensitivity of the SK_{11} lysogens to two serologically unrelated virulent phages was unchanged from that of the parent strain. Clearly the resistance of *S. cremoris* R_1 to these two virulent phages is not due to the presence of this temperate phage.

An inadequacy of the indicator strain alone seems insufficient reason to account for the generally low level of phage r_1t detectable in either induced or noninduced R_1 cultures (Fig. 2 and 3). Rapid adsorption of the induced phage to cell debris may cause the apparently low titers of phage, since losses as high as 99.9% of PFU have been attributed to such adsorption in a group H streptococcal temperate phage system (10, 11).

From Table 1 it can be seen that R_1 lysates caused lytic reactions but no plaque formation on several strains in addition to those that showed plaques. The possibilities of inhibitory substances or of some modification and restriction phenomenon have not been eliminated entirely. However, it would seem more likely that strain R_1 is lysogenized, perhaps defectively, by more than one phage, or, alternatively, that only a small proportion of the induced phage is infective. The high incidence of incomplete phage particles, fragments, and ghosts observed in electron micrographs of R_1 lysates (Fig. 4C) is compatible with either possibility. However, all but one of the non-plaquing reactions were lost from the lytic spectrum when phage r_1t was propagated on AM_1 (Table 1). Again, a host range modification of phage r_1t by AM_1 cannot be eliminated, but it is possible that R_1, unlike AM_1, carries in addition to phage r_1t one or more inducible defective phages similar to those found by Keogh and Shimmin (6) and by Reiter (personal communication), and that R_1 lysates must be treated as mixed phage preparations.

Virulent phages have been a major industrial problem in the manufacture of fermented dairy foods for many years (16). Virulent phages of newly isolated strains of lactic streptococci appear rapidly when these strains are used commercially. The possibility that these previously unknown phages arise as virulent mutants of temperate phage may account for their sudden appearance. Neither the electron micrographs (Fig. 4A and B) nor the host range data would be incompatible with this hypothesis in the case of the virulent and temperate phages of *S. cremoris* R_1. Experiments to artificially produce mutants of r_1t phage that are virulent for *S. cremoris* R_1 are in progress.

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