Curing of a Lysogenic Strain of *Streptococcus cremoris* and Characterization of the Temperate Bacteriophage

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A derivative of *Streptococcus cremoris* MU0001 (R_1) has been isolated which has been cured of an inducible prophage. This isolate, its parent strain and a relysogenized derivative of the cured strain have been compared with respect to their ultraviolet inducibility, production of plaque-forming units, bacteriophage immunity and milk coagulation properties. The bacteriophage (ϕ MU1) harboured by strain MU0001 as a prophage has been characterized by isopycnic density gradient centrifugation, electron microscopy and sodium dodecyl sulphate gel electrophoresis. Antiserum raised against this bacteriophage was used to demonstrate true lysogeny in both the original strain and the relysogenized strain.

INTRODUCTION

The streptococci are a diverse group of bacteria, many of which are pathogenic, for which reason they have been relatively well characterized (Deibel & Seeley, 1974). Group N streptococci, commonly known as lactic streptococci, are chain-forming and non-pathogenic; they are used extensively in lactose fermentations, particularly in the production of a variety of cheeses. The literature regarding the dairy microbiology of these organisms is extensive, but little is known of their genetic systems (Lawrence *et al.*, 1976). The origins of some of the bacteriophage which infect these organisms, resulting in fermentation failures, can probably be attributed to widespread lysogeny in the starter bacteria themselves (Terzaghi & Sandine, 1981; Huggins & Sandine, 1977; Reiter & Kirikova, 1976; Lowrie, 1974), although other sorts of stable association of bacteria and bacteriophage have been suggested (Barksdale & Arden, 1974). However, further careful and systematic studies of the putative lysogeny have been hindered by a general inability to find indicator strains for the bacteriophage found in induced lysates or to isolate strains cured of their putative prophage. Accordingly, a strict demonstration of lysogeny by the three generally accepted criteria [inducibility, immunity and resistance of the lysogenic state to antiserum raised against the lysogenic bacteriophage (Barksdale & Arden, 1974)] has not previously been carried out. Furthermore, very little is known of the physiological effects accompanying the lysogenic state in lactic streptococci in terms of lysogenic conversion or immunity. Such information is vital both for understanding and controlling bacteriophage infections in dairy fermentations and for selection of improved strains.

To this end and in order to establish a firm base from which other lactic streptococcal strains and bacteriophages could be systematically studied, one presumptive lysogenic strain, MU0001 (Lowrie, 1974), was selected for curing and detailed characterization. By the above criteria we have confirmed true lysogeny in this system. In addition, the bacteriophage produced by strain MU0001, which may be propagated either lytically or lysogenically, has been characterized with respect to morphology, buoyant density and sodium dodecyl sulphate gel electrophoresis.

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METHODS

Bacteria and bacteriophage. *Streptococcus cremoris* strains MU0001 and MU0002 were obtained from the New Zealand Dairy Research Institute as strains R₁ and 368, respectively. The latter was previously referred to as the indicator strain AM₁ (Lowrie, 1974) but was, in fact, a slow milk-coagulating derivative of that strain and has therefore been given a new designation. Strain MU0003 is a cured derivative of MU0001 described below, and MU0004 is a relysogenized derivative of MU0003.

Bacteriophage ϕ MU1 was obtained by u.v. irradiation of strain MU0001, as described below. This bacteriophage was propagated lytically on either strain MU0002 or strain MU0003 and designated, respectively, ϕ MU1/MU0002 and ϕ MU1/MU0003. A spontaneous clear plaque derivative of ϕ MU1/MU0003 was isolated and is designated ϕ MU1 (cl). This variant was used when higher titres of ϕ MU1 grown lytically on MU0003 were required. The wild-type ϕ MU1 generally yielded only very low titre stocks when grown lytically on MU0003.

The coliphage λ and the λ indicator *Escherichia coli* strain AB3013 were used in the density gradient analyses.

Media and diluent. M17 broth and agar media were used for growth as previously described (Terzaghi & Sandine, 1975). Titrations of bacteria and bacteriophage were done in standard saline citrate which contained 8.8 g NaCl and 4.4 g Na₂citrate. 2H₂O in 1 l water. Prior to use this diluent was supplemented with 1 mM-MgSO₄. Phosphate buffer (pH 7.2), used in antiserum reactions, contained 17.9 g Na₂HPO₄. 12H₂O (0.05 M), 3.4 g KH₂PO₄ (0.025 M), 4 g NaCl and 1 g gelatin in 1 l water.

For growth of strain AB3013 and titration of λ , 2% (w/v) Brain Heart Infusion (BHI) broth and BHI agar overlay plates were used.

Preparation of indicators. For titrating ϕ MU1 on its homologous host (Terzaghi & Terzaghi, 1978), a stationary culture grown in M17 broth was used. For titration on a heterologous host, the bacteria were grown to stationary phase in M17 plus 1% (w/v) lactose and then allowed to age for 10 d at 4 °C. The λ indicator, strain AB3013, was prepared by growth of a culture in 2% BHI broth to a titre of approximately 10⁹, sedimenting, resuspending in 1 mM-Mg²⁺ and incubating with aeration at 37 °C for 30 min.

Ultraviolet induction. Cells were grown in M17 broth to an A₂₅₄ of 0.1 to 0.2 at 30 °C, chilled, centrifuged for 15 min at 6000 g, and resuspended in 80% of the original volume of chilled 1 mM-MgSO₄. Resuspended cells (3 ml) were irradiated for 15 s with a 15 W Philips TUV P/40 bactericidal lamp of wavelength 243.7 nm which delivered approximately 1 phage T4 hit per 3 s. After irradiation, 6 ml M17 broth was added and incubation was continued at 30 °C in the dark in order to minimize photoreactivation. To determine induction, the absorbance at 600 nm was read at intervals on a Bausch & Lomb Spectronic 20 spectrophotometer.

Prophage curing. Cells were grown to approximately 2 × 10⁸ chains ml⁻¹ (as determined in a Petroff-Hauser counting chamber), washed twice in chilled 1% peptone, and treated for 2 min at high speed in an AtoMix blender. After centrifugation the cells were resuspended in phosphate buffer and irradiated to approximately 10⁻⁵ colony-forming units (c.f.u.) survival. The irradiated cells were then plated (0.1 ml) on M17 plates without the usual Ca²⁺ supplementation and incubated overnight at 30 °C. Individual colonies were picked into 0.1 ml broth in microtitre dishes (Linbro Multidish Dispo-trays) and grown for 3 h at 30 °C. They were then spotted (0.05 ml) on to a master plate to test for viability and to provide an untreated sample of each isolate; the remaining 0.05 ml aliquots were diluted with 0.2 ml phosphate buffer and the whole tray was u.v.-irradiated for 15 s. After 3 h incubation at 30 °C, each isolate was spotted on a lawn of sensitive MU0002 indicator bacteria (with Ca²⁺ supplementation) to test for the presence of induced bacteriophage by the criterion of production of a zone of lysis. Those irradiated isolates not producing zones of lysis after 16 h incubation were streaked from the master plate for single colonies, reisolated twice, and then tested for u.v. inducibility and the ability to support the growth of ϕ MU1.

Isolation of relysogenized bacteria. Bacteria were picked from the centre of turbid plaques produced when the MU0001 induced lysate was plated on MU0003 bacteria; they were purified by two sequential single colony isolations.

Bacteriophage adsorption. Stationary phase cells were centrifuged and resuspended to the same cell density in fresh M17 broth. Equal volumes of bacteria and bacteriophage were mixed, incubated for 10 min at 30 °C and centrifuged for 15 min at 6000 g. A portion of the supernatant was carefully removed and assayed for unadsorbed bacteriophage.

Isopycnic density gradient analysis. Bacteriophage preparations to be analysed in CsCl density gradients were first clarified by centrifuging for 10 min at 6000 g and then concentrated (along with portions of the coliphage λ) by sedimentation at 120 000 g for 3 h. The pellets were resuspended gently at 4 °C in CsCl (BDH, AnalaR) made up to a density of 1.48 g ml⁻¹ in 0.05 M-Tris/HCl buffer pH 7.2. The samples were then centrifuged for 36 to 48 h at 30 000 rev. min⁻¹ in the SW50.1 rotor of a Beckman ultracentrifuge. The resulting gradients were displaced by pumping Fluorinert into the bottom of the tube, scanned and fractionated with an ISCO UA-5 monitor fitted with a type 6 optical unit and an ISCO 640 density gradient fractionator. The refractive index of each fraction was measured on an Atago Abbe refractometer, then an equal volume of M17 broth was added and each fraction was assayed for bacteriophage titres (ϕ MU1 or λ) on the appropriate hosts.

For preparative purposes, preformed CsCl step gradients were constructed in Beckman SW41 tubes by adding

successive 1 ml portions of CsCl solutions (in 0.05 M-Tris/HCl pH 7.2) of densities 1.60, 1.50, 1.40 and 1.30 g ml⁻¹. The sample containing the bacteriophage was layered on top to fill the tube and the gradient was centrifuged at 30000 rev. min⁻¹ for 3 h. The gradient was fractionated and the bacteriophage were recovered from the region corresponding to the top of the 1.50 g ml⁻¹ zone.

Electron microscopy. Samples (5 ml) of bacteriophage preparations (either lytic stock or u.v.-induced lysates) from which bacterial debris had been removed by centrifuging at 6000 g for 10 min were sedimented at 105000 g for 2 h. The pellet was allowed to resuspend slowly in 0.5 ml 0.1 M-ammonium acetate. The Formvar/carbon-coated copper grids were floated, carbon side down, on a drop made of equal parts of bacteriophage suspension and a 1:1 mixture of 2% (w/v) potassium phosphotungstate (pH 4.0) and 2% (w/v) ammonium molybdate. After 3 to 10 min, the grids were rinsed with a drop of distilled water, drained, allowed to air dry and then examined in a Philips 200 electron microscope.

Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis. Analyses were performed on slab gels (120 × 100 × 1.5 mm) using a discontinuous buffer and gel system. The lower gel and buffer were 10% (w/v) acrylamide, 0.4% (w/v) methylene bisacrylamide, 0.375 M-Tris/HCl pH 8.8 and 0.1% (w/v) SDS. The upper gel and buffer were 4.5% acrylamide, 0.24% methylene bisacrylamide, 0.125 M-Tris/HCl pH 6.8 and 0.1% SDS. Polymerization of both gels was catalysed by 0.025% (w/v) ammonium persulphate and 0.05% (w/v) *N,N,N',N'*-tetramethylethylenediamine. The running buffer was 0.19 M-glycine, 0.025 M-Tris/HCl pH 8.3 and 0.1% SDS. Gels were fixed and stained in 0.04% Coomassie Brilliant Blue dissolved in 25% (v/v) propan-2-ol and 10% (v/v) glacial acetic acid. Destaining was done in 10% acetic acid. Molecular weight standards were provided by coliphage T4 purified on a CsCl step gradient (Vanderslice & Yegian, 1974). Samples for electrophoresis were dialysed exhaustively against distilled water, disrupted by nine cycles of freezing and thawing followed by digestion with a few micrograms of DNAase and finally lyophilized. The samples were then dissolved in a portion (generally 50 µl) of sample buffer, which contained, per 100 ml, 2.5 ml β-mercaptoethanol, 1.5 g SDS, 6.25 ml upper Tris buffer, 8 ml glycerol and 0.01 g bromophenol blue. Just prior to application, the samples were held at 100 °C for 1 to 2 min to ensure complete dissociation of protein complexes.

Antiserum preparation and use. Approximately 10¹² plaque-forming units (p.f.u.) of bacteriophage that had been purified on a sucrose gradient [10 to 30% (w/v) sucrose in 0.05 M-Tris/HCl pH 7.2, centrifuged for 1 h 40 min at 25000 rev. min⁻¹ in a Beckman SW27 rotor] were suspended in 0.6 ml 0.05 M-Tris/HCl pH 7.2 plus 0.6 ml Freund's complete adjuvant and shaken to yield an emulsion. Then 0.5 ml was injected intramuscularly into each hind leg of an immunologically fresh rabbit. The rabbits were reinoculated at 4 weeks, this time using incomplete adjuvant, and the serum was harvested 10 d afterwards. For determining the kinetics of bacteriophage inactivation 1.8 ml antiserum at the appropriate concentrations in phosphate buffer was equilibrated at 20 °C and then 0.2 ml bacteriophage in phosphate buffer (approximately 10⁶ p.f.u. ml⁻¹) was added. Samples were taken at appropriate times and titrated in the normal fashion.

RESULTS AND DISCUSSION

Bacterial strains

Streptococcus cremoris strain MU0001 was chosen for curing for three reasons. (1) *Streptococcus cremoris* strains are preferred to *S. lactis* strains for cheesemaking and strain MU0001 had been used as a cheese starter culture. (2) Strain MU0001 had previously been shown to be inducible by both mitomycin C and u.v. radiation (Lowrie, 1974). (3) An indicator strain (MU0002) had been found for the bacteriophage in the induced lysate (Lowrie, 1974). As evidence of curing, we chose the criteria of a lack of lytic response to a known inducing agent (u.v.), failure to detect bacteriophage particles in an induced lysate either by plaque production on the known indicator or by electron microscopic examination, and the ability to serve as an indicator for phage φMU1.

Isolation of a prophage-cured bacterium. Using the method described, approximately 2% of the u.v. survivors appeared to be cured of their prophage by the criteria of failure to lyse or to produce p.f.u. when spotted on strain MU0002 following an inducing dose of u.v. and, perhaps most importantly, by acquisition of bacteriophage indicator capacity as shown by the formation of turbid plaques when plated with a diluted u.v.-induced lysate of strain MU0001. One of these cured isolates (MU0003) was further purified.

Relysogenization. As a confirmation of curing, we wished to relysogenize strain MU0003 with the bacteriophage induced from MU0001 and thereby reconstruct the MU0001 strain. From the turbid plaques a bacterium (designated MU0004) was isolated which showed lytic

Fig. 1. Response of strains MU0001 (●), MU0003 (○) and MU0004 (□) to an inducing dose of u.v. radiation. Exponential phase cultures of these strains, which had been passaged through anti- ϕ MU1 serum, were irradiated in chilled 1 mM-MgSO₄, then allowed to grow in M17 broth at 30 °C. The absorbance was followed at 600 nm; the results are shown with all absorbances at time 0 (the end of irradiation) normalized to 0.1.

sensitivity to u.v., produced p.f.u. on MU0002 and MU0003 following u.v. induction and no longer served as an indicator for ϕ MU1.

Antiserum passage. To ensure that the bacterial strains which were to be examined with respect to their lysogenic property were free of externally carried bacteriophage, they were exhaustively treated with antiserum raised against purified ϕ MU1. Suspensions of strains MU0001 and MU0004, containing approximately 10^4 and 5×10^2 p.f.u. ml⁻¹, respectively, of spontaneously liberated bacteriophage, were prepared in M17 broth containing antiserum at a k value of 20 (Adams, 1950), which had been shown to inactivate the homologous bacteriophage to fewer than 10^{-3} survivors. These mixtures were incubated for 1 h at 30 °C and then streaked out for single colonies. The procedure was repeated and the final isolate was grown overnight in M17 broth in the presence of antiserum at a k value of 20. These overnight cultures were used in the following experiments to demonstrate the lysogenic state.

Ultraviolet induction. Antiserum-treated cultures of strains MU0001 and MU0004, as well as the cured strain MU0003, were u.v.-induced and their growth and lysis were followed spectrophotometrically (Fig. 1). The bacteriophage yields for both MU0001 and MU0004 following u.v. treatment were approximately 10^{10} p.f.u. ml⁻¹ titrated on MU0003 per 0.1 A_{600} unit measured at the time of irradiation. It is interesting to note that while the cured strain, MU0003, appears quite insensitive to a small dose of irradiation, both lysogenized strains are sensitive, with the original strain, MU0001, showing a distinctly greater sensitivity than the reconstructed lysogen MU0004. Although there is no further evidence which bears on this point, it is possible that the original strain was cured of more than just the prophage of ϕ MU1 in the process of generating strain MU0003. The observed differences in u.v. response were reproducible and identical to those observed with cultures that had not been exposed to anti- ϕ MU1 serum. This experiment demonstrates that the lysogenic state, as measured by the lytic response to u.v. irradiation and the production of p.f.u. by both the original and the reconstructed strains, persists in spite of treatment with antiserum specific for the induced bacteriophage, and demonstrates that bacteriophage, or prophage, are carried internally.

Bacteriophage adsorption and immunity. When the u.v.-induced lysates generated in the experiments described above were plated on MU0001 and MU0004, no plaques appeared, indicating an efficiency of plating (e.o.p.) lower than 10^{-9} relative to the titre on the cured strain MU0003. To ascertain that the inability to form plaques in MU0001 and MU0004 was due to immunity rather than resistance, strains MU0001, MU0002, MU0003 and MU0004 were compared for their ability to adsorb ϕ MU1. For this and most subsequent experiments, ϕ MU1 was propagated on either MU0002 or MU0003 and designated, respectively,

Table 1. Adsorption and efficiency of plating of ϕ MU1 on lysogenic, cured and original indicator strains of bacteria

Stationary phase cells were centrifuged and resuspended in an approximately equal volume of fresh M17 broth such that each preparation had a similar cell density, as measured spectrophotometrically. A known number of phage was added at a multiplicity of less than one and allowed to adsorb for 10 min at 30 °C. Remaining unadsorbed phage were measured by titrating the supernatant following centrifugation; the results are reported as percentage phage adsorbed (% ads.). The efficiency of plating (e.o.p.) of a phage on its homologous host is defined as 1.0.

Bacteriophage	Bacterial host							
	MU0001		MU0002		MU0003		MU0004	
	% ads.	e.o.p.	% ads.	e.o.p.	% ads.	e.o.p.	% ads.	e.o.p.
ϕ MU1/MU0002	94	<10 ⁻⁹	94	1.0	73	≈10 ⁻⁵	90	<10 ⁻⁹
ϕ MU1/MU0003	88	<10 ⁻⁹	97	≈10 ⁻⁶	64	1.0	96	<10 ⁻⁹

ϕ MU1/MU0002 and ϕ MU1/MU0003. As shown in Table 1, all four bacterial strains adsorbed both phage preparations in excess of 60%; strains MU0001 and MU0004, in particular, adsorbed phage very well. Thus, the inability of the latter two strains to support the growth of ϕ MU1 must reside elsewhere and is considered to be a manifestation of lysogenic immunity.

Examination of the plating efficiency of ϕ MU1/MU0002 and ϕ MU1/MU0003 on their respective homologous and heterologous hosts (as well as the two lysogenized strains) gave the results shown in Table 1. As expected, neither lysogenized strain served as a host for these bacteriophage stocks, while the cured strain and original indicator did, but at dramatically different efficiencies, depending on the identity of the indicating host and the immediately preceding propagating host. When the indicating strain and immediately preceding propagating strain were the same (homologous host) there was a high e.o.p. (defined as 1.0); when the indicating strain and immediately preceding propagating strain were different (heterologous host) there was a very low e.o.p. Although the results are formally reminiscent of the classical *Escherichia coli* modification/restriction system, it was found (Terzaghi & Terzaghi, 1978) that the degree of restriction manifested in the heterologous host varied over many orders of magnitude according to the physiological state of the host cells.

Physiological characterization. Strains MU0001 and MU0003 were compared for their acid production in milk, their speed of milk coagulation, their ability to utilize various sugars (lactose, glucose, galactose and sucrose) and their inability to deaminate arginine. In all of these respects, the two strains were identical, which suggests that lysogenization with ϕ MU1 does not alter any of the gross physiological features of the bacteria that may be of commercial importance (Georghiou, 1976).

Bacteriophage ϕ MU1

To ascertain that the bacteriophage liberated by strains MU0001 and MU0004 by u.v. irradiation are not only identical with one another but are also the same as that propagated on strain MU0002 and MU0003, a detailed examination of the physical characteristics of this bacteriophage was undertaken.

Electron microscopy. Electron micrographs of the four bacteriophage preparations showed them to be morphologically identical, within the limits of resolution afforded by this technique (Fig. 2). The isometric head is approximately 50 nm in diameter and the striated tail with about 30 annuli is approximately 120 nm long. In some of these micrographs there is a hint of a collar structure at the junction of the head and tail, which is occasionally clear but often is not visible. As the apparent presence or absence of the collar is not convincingly correlated with the host used in the bacteriophage preparation we tentatively conclude that this

Fig. 2. Electron micrographs of bacteriophage ϕ MU1 negatively stained with ammonium molybdate and potassium phosphotungstate. (a) Coliphage T4 used as an internal calibration standard; (b) ϕ MU1 recovered directly from a u.v.-induced lysate of MU0001; (c) ϕ MU1 recovered directly from a u.v.-induced lysate of MU0004; (d) ϕ MU1/MU0002; (e) ϕ MU1/MU0003. Bar markers represent 100 nm as measured by the spacings of the annuli of the bacteriophage T4 tail, which agreed well with the known instrument magnification factor.

Fig. 3. CsCl density gradient analysis of a u.v.-induced lysate of strain MU0004. Bacteriophage that had been freed of bacterial debris by low-speed sedimentation were sedimented at high speed and then resuspended in CsCl solution at a density of 1.48 g ml^{-1} . This preparation was centrifuged at $30000 \text{ rev. min}^{-1}$ in the SW50.1 rotor of a Beckman ultracentrifuge for 36 h. The gradient was displaced with Fluorinert through a u.v. monitor and 0.1 ml fractions were collected. The gradient profile is shown in terms of ϕ MU1 p.f.u. on MU0002 (O), ϕ MU1 p.f.u. on MU0003 (●), the A_{254} trace (---) and the CsCl density ($\square$) as measured by refractive index and normalized to a density of 1.500 g ml^{-1} for λ .

bacteriophage does have a collar which is either difficult to visualize with the ammonium molybdate/potassium phosphotungstate negative stain employed in this study, or is fragile and often lost in the course of specimen preparation.

CsCl density gradient analysis. Preparations of u.v.-induced lysates of MU0001 and

Fig. 4. SDS gel electrophoresis of ϕ MU1. Each preparation of ϕ MU1 (50 ml, approximately 10^9 p.f.u. ml $^{-1}$) was partially purified on a CsCl step gradient, exhaustively dialysed against distilled water, disrupted by repeated cycles of freezing and thawing followed by DNAase digestion and lyophilized. The samples were resuspended in 50 μ l SDS sample buffer, heated to 100 °C for several minutes and 20 μ l portions were layered in the sample wells. Gels were run for 1 h at 12 mA and then for approximately 4 h at 20 mA. Staining was done with Coomassie Brilliant Blue and destaining in 10% acetic acid. The T4 samples have been included to provide approximate molecular weight estimates (Vanderslice & Yegian, 1974). A, ϕ MU1 induced from MU0001; B, ϕ MU1 induced from MU0004; C, ϕ MU1/MU0003; D, ϕ MU1/MU0002.

MU0004 and lytic stocks of ϕ MU1/MU0002 and ϕ MU1/MU0003 were analysed by isopycnic density gradient centrifugation. The absorbance and p.f.u. profiles of one of these (MU0004 induced lysate) are shown in Fig. 3. Profiles of the other preparations were virtually identical in all respects. The main band of absorbance and p.f.u. in each case equilibrated at the same position in the gradient relative to λ at a buoyant density of approximately 1.485 g ml $^{-1}$ (assigning a value of 1.500 to λ). While the profiles of the bacteriophage stocks and the u.v.-induced lysate of MU0004 show a single main peak of absorbance and p.f.u., the profile of the u.v.-induced lysate of MU0001 usually showed several additional small satellite peaks of p.f.u. Sampling of these satellite peaks and either re-running directly on a fresh gradient, or propagating these bacteriophage of apparently aberrant density, followed by a gradient analysis, invariably yielded single bacteriophage bands in the correct position relative to λ . We conclude that these satellite peaks are artefacts connected somehow with u.v. induction of MU0001, and have not pursued the matter. It thus

Fig. 5. Antiserum inactivation of ϕ MU1. Antiserum was raised against ϕ MU1/MU0002 and the kinetics of inactivation by this antiserum of various preparations of ϕ MU1 were examined by mixing diluted antiserum and bacteriophage at approximately 10^6 p.f.u. ml $^{-1}$. The mixtures were incubated at 20 °C and samples were removed at the indicated times for titration. The surviving fraction is plotted (on a logarithmic scale) against time of incubation. In each case the inactivation of the homologous bacteriophage, ϕ MU1/MU0002, is shown (O). (a) Antiserum at a dilution of 10^{-4} incubated with ϕ MU1/MU0003 (●). (b) Antiserum at a dilution of 10^{-4} incubated with the bacteriophage induced from MU0004 (□). (c) Antiserum at a dilution of 5×10^{-5} incubated with the bacteriophage induced from MU0001 (■).

appears that the four different preparations of the bacteriophage yield particles of identical buoyant density.

SDS gel electrophoresis. The gel patterns of ϕ MU1 harvested from u.v.-induced lysates of MU0001 and MU0004, as well as from stocks grown on MU0002 and MU0003, are shown in Fig. 4. The patterns are substantially identical, differing primarily in the relative intensities of a few of the bands, which further supports the identity or near identity of the four preparations of ϕ MU1. A detailed comparison of the patterns, however, raises some puzzling points. In tracks A and B are shown the patterns of the bacteriophage purified from induced lysates of, respectively, MU0001 and MU0004. While the two patterns appear identical with respect to band positions, in the region of molecular weight 32 000 there is a reproducible reversal of band intensity. In tracks C and D are shown the patterns for the two lytically grown bacteriophage preparations (grown on, respectively, MU0003 and MU0002). Again, the patterns are grossly similar to each other and to the patterns produced by the phage purified from induced lysates. However, there are variations in band intensities in the regions of molecular weight 23 000 and 30 000, and the cluster of three bands at about 47 000 shows a slightly different spacing and relative intensity in the two types of bacteriophage preparations.

Recent work in our laboratory (unpublished) indicates that many of the strains of bacteria are lysogenic, or defectively lysogenic, for a number of very closely related phage, and that some phage stocks, hitherto regarded as homogeneous by the criteria used in this paper, are in fact subtly heterogeneous. Accordingly, we suspect that at least some of the variability in band density and position is due either to admixture of very closely related phage or to the acquisition by phage, perhaps by a process akin to 'marker rescue', of new structural genes from the host.

Antigenic properties. Antiserum was prepared against ϕ MU1/MU0002 and used to determine the kinetics of inactivation of the p.f.u. in lytic preparations of ϕ MU1/MU0002 and of ϕ MU1/MU0003 as well as in u.v.-induced lysates of MU0004 and MU0001 (Fig. 5). The two stocks ϕ MU1/MU0002 and ϕ MU1/MU0003 and the induced lysates were inactivated with very similar kinetics. A parallel series of experiments with antiserum raised against ϕ MU1/MU0003 (cl) yielded identical results. Thus, it appears that, at least in respect to those antigenic sites which are related to the infection process, the four different preparations of ϕ MU1 are identical.

Conclusion

The true lysogenic condition of *S. cremoris* strain MU0001 has been demonstrated (i) by virtue of the observation that lysogeny persists in the presence of antiserum specific for the bacteriophage concerned, and (ii) by the isolation of a strain that is cured of the lysogenic bacteriophage. As predicted on the basis of observations on other lysogenic systems, the original lysogen appears to be immune to the bacteriophage, while the cured strain serves as a host, forming turbid plaques when a bacterial lawn is seeded with the bacteriophage. A relysogenized strain was easily isolated from a turbid plaque and shown to have lysogenic and immunity properties substantially the same as those of the original lysogenic strain.

Bacteriophages liberated by u.v. induction of both the original lysogen MU0001 and the relysogenized derivative strain MU0004 were shown to be identical by the criteria of gross morphology, buoyant density, SDS gel electrophoresis pattern and antigenic specificity. By the same criteria, bacteriophages resulting from propagation of ϕ MU1 on MU0002 or MU0003 are identical. Arising out of these studies, however, there is the problem of the nature of the physiologically labile e.o.p. depression of ϕ MU1 when plated on its heterologous host which demands clarification.

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