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Detailed Characterization and Comparison of Four Lactic Streptococcal Bacteriophages Based on Morphology, Restriction Mapping, DNA Homology, and Structural Protein Analysis

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Bacteriophages uc1001 and uc1002, which are lytic for *Streptococcus cremoris* UC501 and UC502, respectively, were characterized in detail. Comparisons were made with a previously characterized phage, P008, which is lytic for *Streptococcus lactis* subsp. *diacetylactis* F7/2, and uc3001, which is a lytic phage for *S. cremoris* UC503. Phages uc1001 and uc1002 had small isometric heads (diameters, 52 and 50 nm, respectively) and noncontractile tails (lengths, 152 and 136 nm, respectively), and uc1002 also had a collar. Both had 30.1 ± 0.6 kilobase pairs (kbp) of DNA with cross-complementary cohesive ends. Restriction endonuclease maps made with seven endonucleases showed no common fragments. Despite this there was a very high level of homology between uc1001 and uc1002, and results of cross-hybridization experiments showed that the organization of both phage genomes was similar. Heteroduplex analysis confirmed this and quantified the level of homology at 83%. The regions of nonhomology comprised 2.1-, 1.1-, and 1.0-kbp deletion loops and 13 smaller loops and bubbles. The sodium dodecyl sulfate-polyacrylamide gel electrophoretic structural protein profiles were related, with a major band of about 40,000 molecular weight and minor bands of 35,000 and 34,000 molecular weight in common. There were also differences, however, in that uc1001 had a second major band of 68,000 molecular weight and two extra minor bands. Except for the restriction maps, which were strain specific, phages uc1001, uc1002, and P008 were closely related by all the criteria listed above. Their DNAs also showed a very significant bias against the cleavage sites of 9 of 11 restriction endonucleases. Phage uc3001 was unrelated to uc1001, uc1002, or P008 in that it had a prolate head (53 by 39 nm) and a shorter tail (105 nm), contained approximately 22 kbp of DNA, had unrelated cohesive ends, showed no DNA homology with the isometric-headed phages, and displayed a very different structural protein profile.

Lytic bacteriophage infection of lactic streptococci, which are used for manufacturing fermented milk products, is the principal cause of culture failure in commercial practice. There have been extensive studies of these phages based on morphology, serology, and host range (32; for reviews see references 7, 19, 35). The vast majority belong to group B, which has been described by Bradley (3) and designated as the *Styloviridae* by Ackerman (1), i.e., with prolate or isometric heads and long, noncontractile tails. Different morphological groups are generally serologically unrelated, but no correlation has been observed with host range groups.

Analyses of lactic streptococcal phages at the genetic and molecular levels have only recently been undertaken, but despite this, there have been significant advances in the understanding of their origins, evolution, relationships, and genome structures. On the basis of DNA homology, serology, and genome size, Jarvis (14) classified 25 lytic phages into four morphological groups: small isometric headed, comprising collared and uncollared subgroups; short-tailed small isometric headed; large isometric headed; and prolate headed. Phages conforming to the first group are the most common, while the prolate-headed phages are also significant because of their wide host ranges (13). The mean genome sizes of the phage groups were 30, 35, 20, and 51 kilobase pairs (kbp), respectively, and these sizes are consistent with those of isometric- and prolate-headed phages reported previously (23, 25, 30). Restriction mapping of the small isometric-headed phage P008 (23) and the prolate-headed phage c6A (30) showed that they had cohesive ends and that c6A had a marked paucity of sites for several type II restriction endonucleases (31). Restriction mapping combined with heteroduplex analysis of small isometric-headed phages has shown possible evolutionary pathways, the probable location of genes coding for collar and whisker proteins, and comparable conserved and variable regions (17, 24). In a homology study between the DNAs of lytic and temperate phages, however, only a low degree of relatedness was found, indicating that, in this case at least, lytic phages are not derived from temperate phages (15). In contrast to the genomic studies, the proteins of lactic streptococcal phages have received little attention except for the preparation of structural protein profiles of c6A (30) and φMU1, a temperate phage of *Streptococcus cremoris* RI (12).

Our studies on lactic streptococcal phages to date have been principally concerned with phage insensitivity mechanisms (2, 4, 5, 11). Successful genetic manipulation of phage insensitivity in the host bacteria, however, will require more knowledge of the phages themselves at the genomic level. In this report we describe the detailed characterization of two *S. cremoris* phages, uc1001 and uc1002, under the following headings: morphology and host range, genome size and structure, restriction maps, extent of DNA homology, and structural protein profiles. Both phages had small isometric heads, and detailed comparisons with the *Streptococcus lactis* subsp. *diacetylactis* phage P008 (23, 24) showed that the three phages were closely related, except for their individual restriction maps. In contrast, a *S. cremoris* phage, uc3001, which had a prolate head, was unrelated by these criteria.

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MATERIALS AND METHODS

Bacterial strains, phages, and media. The bacterial strains *S. cremoris* UC501, UC502, and UC503 and their lytic phages uc1001, uc1002, and uc3001, respectively, were obtained from the collection at University College, Cork, Ireland. *S. cremoris* UC503 and phage uc3001 were previously designated *S. cremoris* F and phage f (4, 11). The phage P008 (23) and its host *S. lactis* subsp. *diacetylactis* F7/2 were obtained from M. Teuber, Bundesanstalt für Milchforschung, Kiel, Federal Republic of Germany. Cultures were grown by the methods described by Fitzgerald et al. (11). Phage and culture stocks were stored in broth containing 40% glycerol at −80°C.

Preparation of phages and isolation of phage DNA. Protocols for the preparation of phages and the isolation of phage DNA were essentially as outlined previously (11), except that the phages were pelleted from lysates at 13,000 rpm (28,000 × *g*) for 4 h to allow the use of higher capacity (6 by 250 ml) centrifuge rotors.

Electron microscopy of phages. Phages were pelleted as described above, suspended in 0.1 M ammonium acetate, and dialyzed overnight at 4°C against 1,000 volumes of the same solution. Copper grids (3.05 mm, 400 mesh; Agar Aids, Stansted, England) were coated with 0.4% Formvar in chloroform. The phages were absorbed to the coated grids and negatively stained (2% phosphotungstic acid, 2% ammonium molybdate [pH 5.3]). Observations were made with a transmission electron microscope (Corinth 500) at an accelerating voltage of 80 kV, and photographs were taken at magnifications of 50,000 and 80,000. A grating replica (Agar Aids) with 2,160 lines per mm at right angles to one another was used as a size standard. Between 15 and 30 measurements of the phage dimensions were made, and the means and 99% *t*-confidence intervals were calculated.

Enzyme analysis and agarose gel electrophoresis of phage DNA. Digestions with restriction endonucleases (Boehringer Corp., Dublin, Ireland, and Pharmacia, Milton Keynes, England) and S1 nuclease (Boehringer Corp.) and DNA ligations with T4 ligase (Boehringer Corp.) were done by the method described by Maniatis et al. (27). Restriction digests were stopped by adding 20% of a solution containing 4.0 M urea, 50% sucrose, 0.2 M disodium EDTA, and 0.1% bromophenol blue. They were heated (unless otherwise stated) to 70°C for 10 min, held in ice, and loaded onto horizontal 0.7% agarose (Sigma Chemical Co., Poole, England) gels containing ethidium bromide (0.5 μg/ml) and were electrophoresed at 40 V (constant voltage) for 14 h in half-strength Tris-borate buffer (27). Digests of lambda DNA (Boehringer Corp.) were used as size standards (18). The gels were photographed with film types 52 or 667 (Polaroid; Miniphoto, Dublin, Ireland). Restriction fragments for digestion with a second enzyme were isolated from low-melting-temperature agarose (Sigma) gels by the method described by Maniatis et al. (27). Fragments for labeling with either ^{32}P or biotin were purified from agarose gels by the partitioning procedures described by Langridge et al. (22) or by electrophoresis into DEAE-cellulose paper (DE81; Whatman, Inc., Clifton, N.J.) (9).

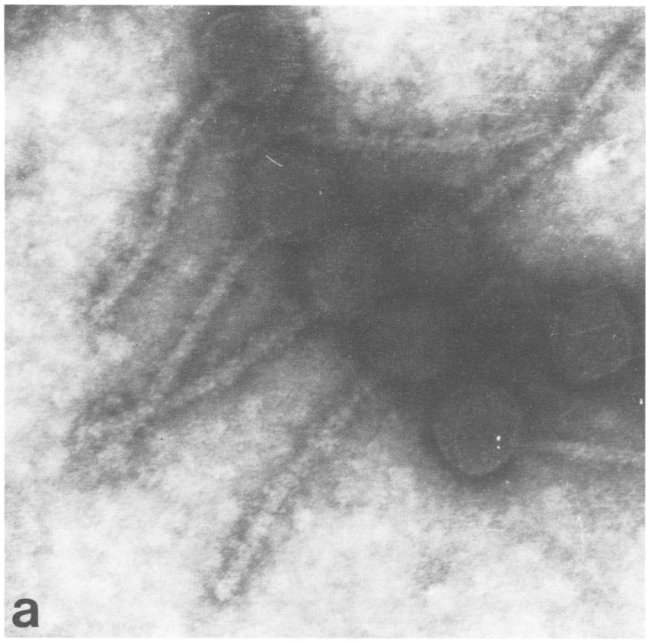
Detection of homologous DNA sequences. Phage DNA was labeled with [α-^{32}P]dCTP or biotin-11-dUTP by using nick translation kits (Amersham International, Buckinghamshire, England, and Bethesda Research Laboratories, Paisley, Scotland, respectively). After electrophoresis, restriction digests were transferred to Schleicher membrane filters (BA85; Schleicher & Schüll, Dassel, Federal Republic of Germany) by Southern (34) blotting. In high-stringency (T_m = 70°C) (29) experiments, filters were hybridized with labeled DNA at 68°C and washed twice at 68°C in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.5% sodium dodecyl sulfate (SDS) (27), whereas low-stringency (T_m = 92°C) (29) experiments were carried out at 60°C with three washes at 60°C in 2× SSC (14). Binding to homologous sequences by [^{32}P]DNA was detected by autoradiography (27) with X-OmatS film (Kodak, Dublin, Ireland). Hybridization with biotinylated DNA was detected with a DNA detection system (Bethesda Research Laboratories).

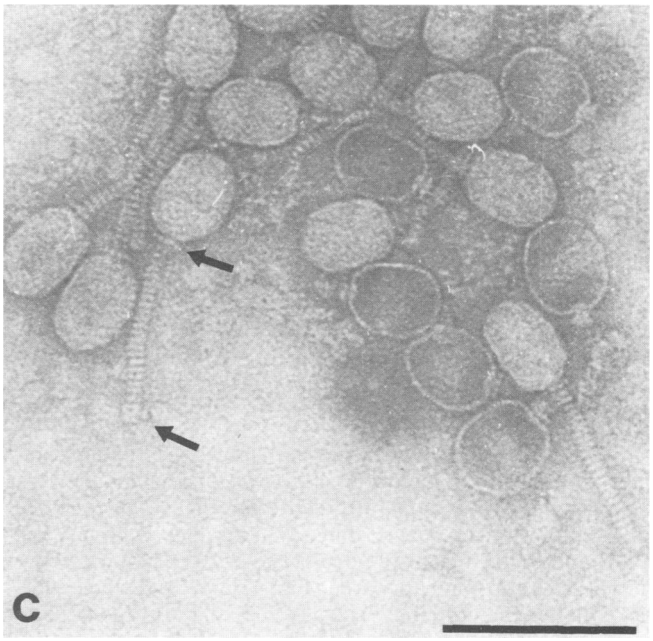
Size determination of phage genomes and restriction fragments. The genome size of uc1001 was taken as the average of the totals from 15 gels containing various single and multiple digests. Digests with restriction fragments larger than 13.2 kbp (i.e., equal to the *Pvu*II A fragment) were not used. The average of the totals from 13 *Eco*RI digests (largest fragment, 8.8 kbp) was taken as the genome size of uc1002, while that of uc3001 was calculated from *Eco*RI and *Hin*dIII digests.

The sizes of the uc1001 restriction fragments larger than *Pvu*II-A were calculated by subtracting the combined sizes of the remaining fragments, for the particular enzyme, from the total size of uc1001. Phage uc1002 fragment sizes larger than that of *Eco*RI-A were calculated in terms of their component *Eco*RI fragments. The sizes of the remainder of the uc1001 and uc1002 fragments, except those less than 0.5 kbp, for which data were limited, were the averages of between 5 and 14 measurements. The 99% *t*-confidence intervals of the measurements of the genome sizes and of all except the smallest restriction fragments were calculated.

Heteroduplex analysis. Heteroduplex molecules were prepared by alkali denaturation of phage uc1001 and uc1002 DNAs (8) followed by renaturation for 30 min in 50% formamide (nucleic acid grade; Bethesda Research Laboratories). The preparations were spread from a solution containing 40% formamide, 0.01 M Tris hydrochloride (pH 8.5) 0.001 M disodium EDTA, 0.01% cytochrome *c* (type VI; Sigma), and approximately 0.5 μg of phage DNA per ml onto a hypophase consisting of 10% formamide–0.01 M Tris hydrochloride (pH 8.5)–0.001 M disodium EDTA. The plasmids pAT153 (3.6 kbp) (36) or pVA838 (9.2 kbp) (26) were included as size standards. Samples were picked up on grids coated with 2% Parlodian (Mallinckrod, Inc., St. Louis, Mo.) in amyl acetate and negatively stained with 90% ethanol containing 0.1% phosphotungstic acid and 1% sulfuric acid. Observations were made on an electron microscope (Corinth 500) at an accelerating voltage of 60 kV, and photographs were taken at magnifications of 12,000 and 20,000. Contour length measurements were made with an image analyzer (MOP-AMO3; Kontron). Six measurements were made of each heteroduplex between uc1001 and uc1002 DNAs.

SDS-polyacrylamide gel electrophoretic profiles of phage structural proteins. After CsCl gradient centrifugation the phage band was removed in a volume of 0.75 ml and dialyzed against 2,000 volumes of buffer (0.01 M Tris hydrochloride, 0.001 M disodium EDTA [pH 7.5]). After the addition of 5% β-mercaptoethanol, samples were boiled for 5 min with an equal volume (100 to 150 μl) of sample buffer (0.075 M Tris hydrochloride, 0.1% sulfuric acid, 15% glycerol, 2% SDS, 0.01% bromophenol blue [pH 6.8]). These were electrophoresed on 15% discontinuous gels (21) (15% total monomer, 0.5% cross-linking monomer) by using the Protean II system (Bio-Rad Laboratories, Watford, England) and stained with





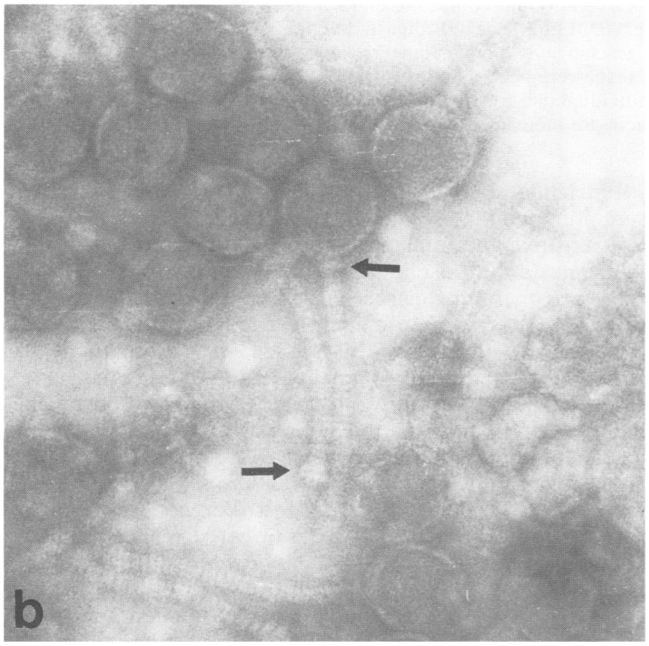


FIG. 1. Electron micrograph of phages. (a) uc1001; (b) uc1002; (c) uc3001. The collars and base plates of uc1002 and uc3001 are indicated by arrows. Bar, 100 nm.

0.1% Coomassie blue. High- (catalog no. SDS-6H) and low- (catalog no. SDS-7) molecular-weight standards (Sigma) were suspended in 1 ml of sample buffer, and fractions were stored at −20°C. When required they were prepared in the same manner as described above for the samples. Six or seven size measurements were made for each protein band, and 99% *t*-confidence intervals were calculated, except for faint bands which appeared only on overloaded gels.

RESULTS

Morphology and host range. Phages uc1001, uc1002, and uc3001 had icosahedral heads and noncontractile striated tails (Fig. 1) and were assigned to group B described by Bradley (3). Both uc1001 and uc1002 had isometric heads, but the latter had a collar and a significantly shorter tail of variable length (Table 1). The base plate of uc1002 was triangular with two short distal spikes, but the structure of the uc1001 base plate was not clearly resolved. Phage uc3001 had a prolate head and a collar, its tail was considerably shorter than those of uc1001 or uc1002, and its base plate was significantly smaller. Variable proportions of empty heads, recognized by their dark centers, were noted in all preparations of each phage.

Each of the phages was specific for its own host in this study.

Size and structure of the phage DNAs. The genome sizes of both uc1001 and uc1002 were calculated from restriction digests and estimated to be 30.1 ± 0.6 kbp. Likewise, uc3001 was found to have approximately 22 kbp of DNA.

The appearance of specific bands in the restriction digests of all four DNAs was variable after electrophoresis. However, these bands appeared consistently when the digests were heated to 70°C for 10 min, immediately prior to loading of the gels. This is shown for uc1002 by the breakdown of a 2.05-kbp *Bst*EII band in an unheated digest (Fig. 2, lane 1) into two bands of 1.08 and 0.97 kbp in a heated digest (Fig. 2, lane 2). This effect could be reversed by allowing a heated digest to remain at room temperature for 6 h (reannealing) before electrophoresis (Fig. 2, lane 3). From these data it was concluded that the heat-dependent bands were end fragments that migrated as a composite when the digests were not heated and that the phage genomes were similar in structure to that of phage lambda (10), i.e., linear and nonpermuted with single-stranded complementary cohesive ends. This was confirmed by pretreating the DNA with T4 ligase, which rendered the composite fragment insensitive to heat (Fig. 2, lane 4), and S1 nuclease, which made the application of heat unnecessary to obtain the individual end

TABLE 1. Morphological details of phages uc1001, uc1002, and uc3001, with P008 included for comparison

Phage	Head		Tail		
	Morphological type	Size (nm)[a]	Length (nm)[a]	Collar[b]	Base plate[b]
uc1001	Isometric	51–53	149–155	−	+
uc1002	Isometric	49–51	127–145	+	+
P008[c]	Isometric	51–55	155–163	+	−
uc3001	Prolate	52–54 × 37–41	102–108	+	+

[a] *t*-Confidence intervals were 99%.
[b] Key: +, Presence; −, absence.
[c] From Loof et al. (23).

fragments (Fig. 2, lane 5) and prevented their reannealing (Fig. 2, lane 6).

Possible complementarity between the cohesive ends of different phages was determined in a qualitative manner by heating a mixture of separate restriction digests of the two DNAs and allowing reannealing. When this was done with *Eco*RI and *Pvu*II digests of uc1001 and uc1002, respectively, a novel 2.52-kbp band appeared (Fig. 3, lane 2). Because this was equal to the combined sizes of the 2.05-kbp *Eco*RI end fragment of uc1001 and the 0.47-kbp *Pvu*II end fragment of uc1002, it implies that these reannealed to form a hybrid composite end fragment. The use of different enzymes and the fact that no other combination of fragments sizes could give a sum of 2.52 kbp excluded the possibility that the hybrid was the result of reannealing of other fragments caused by restriction endonuclease-generated sticky ends (this was never observed in reannealing experiments in any case). The size consideration also eliminated the possibility that the hybrid was a partial digest band. Therefore, it was concluded that this pair of cohesive ends were complementary to each other. Similarly, the opposite pair of uc1001 and uc1002 ends and those of uc1001 and P008, but not those of uc1001 and uc3001, were found to be cross-complementary (data not shown).

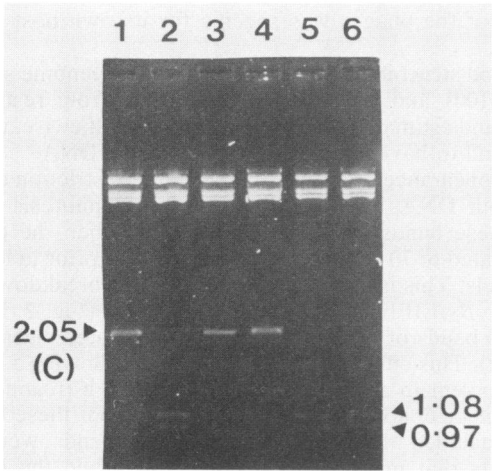


FIG. 2. Agarose gel (0.7%) electrophoresis showing the effect of heat treatment (70°C for 10 min), reannealing (room temperature for 6 h), and pretreatment with T4 ligase or S1 nuclease on uc1002 *Bst*EII digests. Lane 1, unheated; lane 2, heated; lane 3, heated and reannealed; lane 4, treated with T4 ligase and heated; lane 5, treated with S1 nuclease; lane 6, treated with S1 nuclease and reannealed. The sizes (in kilobase pairs) of the composite (C) and individual end fragments are indicated.

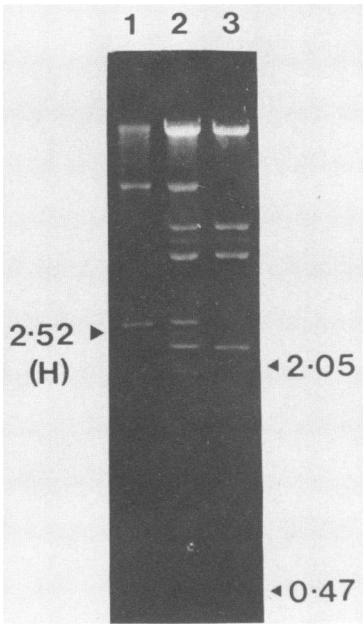


FIG. 3. Agarose gel (0.7%) electrophoresis showing the appearance of a hybrid composite end fragment derived from uc1001 and uc1002. Lane 1, uc1001 digested with *Eco*RI; lane 2, mixture of the digests in lanes 1 and 3; lane 3, uc1002 digested with *Pvu*II. All three digests were heated and reannealed. The sizes (in kilobase pairs) of individual end fragments and the hybrid fragment (H) derived from them are indicated.

Restriction maps. Restriction maps of uc1001 and uc1002 were prepared for seven endonucleases by (i) identifying the heat-sensitive end fragments, (ii) comparing single and multiple digests, (iii) isolating specific fragments from low-melting-temperature agarose gels for digestion with a second enzyme, and (iv) hybridizing restriction digests at high stringencies with specific ^{32}P-labeled restriction fragments (Fig. 4). No restriction fragments common to both phages were found by using the criteria of (i) comigration on the same gel, (ii) mapping in the same position, and (iii) having the same internal restriction sites. Different restriction patterns were also observed for five additional endonucleases; *Cla*I, *Hae*III, *Pst*I, *Scr*FI, and *Taq*I (data not shown). The maps of uc1001 and uc1002 were oriented relative to each other and to the map of P008 (24) by using the results of the complementarity studies between the cohesive ends of the phage DNAs (see above). No restriction fragments common to uc1001 and P008 were observed, but the left-hand ends of uc1002 and P008 were similar because the uc1002 fragments *Eco*RI-G and *Pvu*II-E comigrated with the P008 fragments *Eco*RI-D and *Pvu*II-E, respectively, and mapped in the same position. Also, the uc1002 *Pvu*II B and the P008 *Pvu*II B1 fragments mapped in the same position and were only slightly different in size. Otherwise, the restriction maps of these two phages were different. A map of uc3001 was not constructed, but its *Eco*RI (Fig. 5a) and *Hin*dIII restriction patterns were different from those of uc1001, uc1002, and P008.

In the course of the mapping studies a paucity of restriction sites was noted for some endonucleases. Therefore, the expected number of sites for 12 restriction enzymes in the DNAs of uc1001, uc1002, and P008 were calculated by the method described by Powell and Davidson (31) and compared with experimental findings (Table 2). There was a very

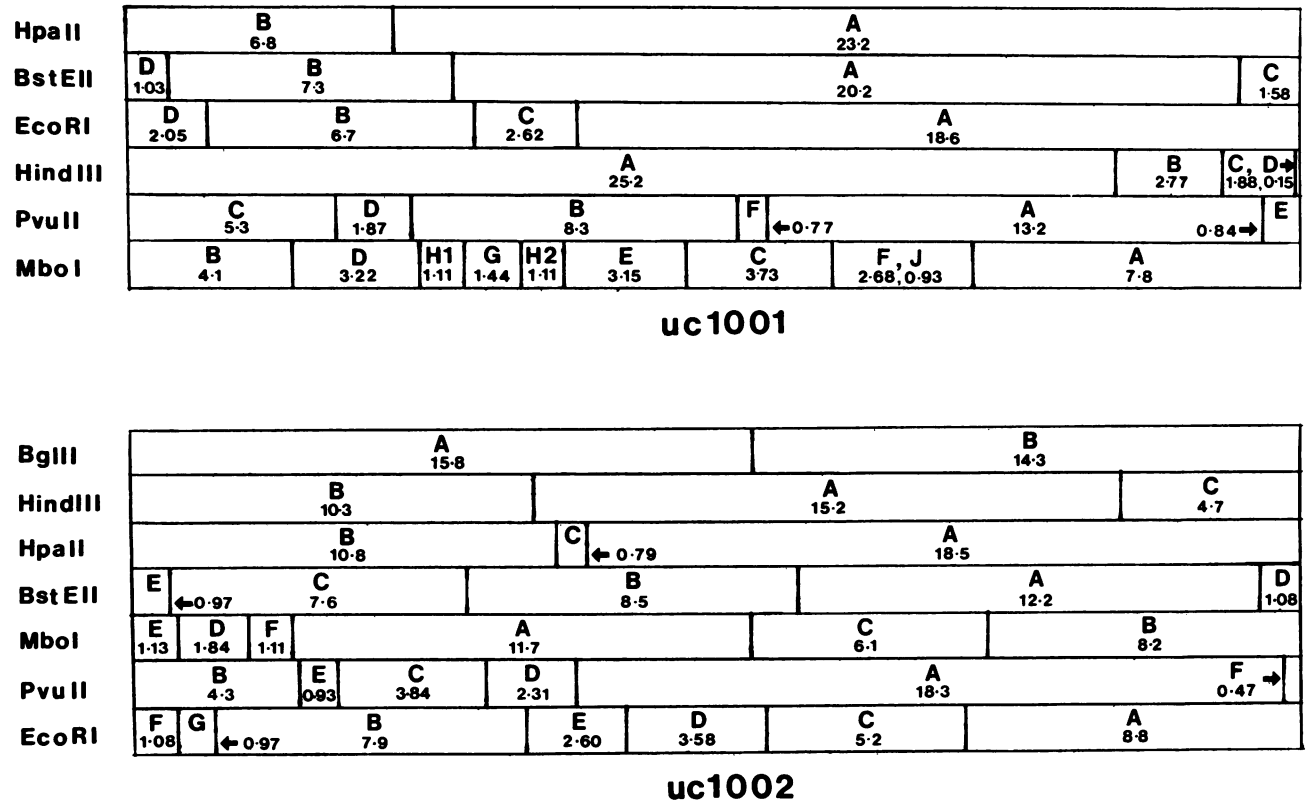


FIG. 4. Restriction maps of uc1001 and uc1002 DNAs. The 99% *t*-confidence intervals of the mean sizes (in kilobase pairs) for most of the fragments were less than 6% of the mean values. They were not calculated for the fragments of less than 0.5 kbp and were between 9.0 and 9.5% of the means for the *Hpa*II B and *Mbo*I A and B fragments of uc1001. The *Hin*dIII D fragment of uc1001 was not visible on the gels; its presence and size was determined indirectly as a component of the composite end fragment in unheated *Eco*RI-*Hin*dIII double digests. Numbered fragments comigrated on 0.7% agarose gels.

significant bias against the 4-base recognition sequences of the five enzymes *Mbo*I, *Taq*I, *Hae*III, *Hpa*II, and *Scr*FI ($P < 0.003\%$ of a number of cleavage sites equal to or lower than that observed, assuming a random sequence). The bias against the 6-base recognition sequences of *Cla*I, *Eco*RI, *Hin*dIII, and *Pst*I was also significant ($0.07\% < P < 2.9\%$, except for *Pst*I and *Eco*RI in uc1002, where $P = 6.68$ and 10.38%, respectively) but not as marked, while about the expected numbers of *Bst*EII and *Pvu*II (6-base recognition sequences) sites were observed. The results for *Bgl*II were not included in Table 2 because the central portion of its recognition sequence was the same as that of *Mbo*I. No sites for the endonuclease *Dpn*I, which recognizes a methylated *Mbo*I site (GA^mTC), were observed in uc1001 or uc1002, indicating that the paucity of *Mbo*I sites was not due to methylation.

Phage DNA homology. By using whole uc1001 DNA as a probe and low-stringency conditions of hybridization and filter washing, a very high level of homology between the isometric-headed phages uc1001, uc1002, and P008 was observed; but there was no detectable homology between uc1001 and the prolate-headed phage uc3001 (Fig. 5a and b). A more-detailed study of the extent of homology between uc1001 and uc1002 under high-stringency conditions involved labeling of restriction fragments spanning the entire genome of uc1001 and 65% of the uc1002 genome. When these fragments were used in cross-hybridization experiments to probe the two phage DNAs, all the labeled fragments showed strong homology only with fragments in the

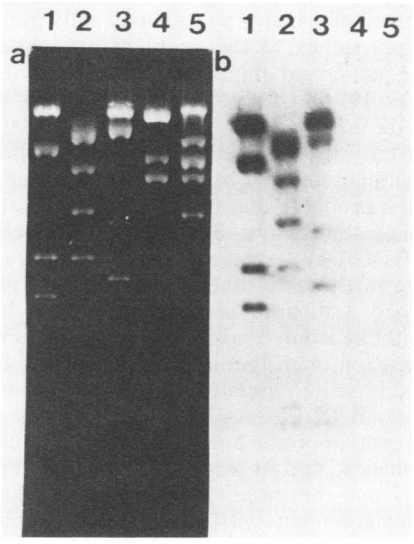


FIG. 5. (a) Agarose gel (0.7%) electrophoretic patterns of *Eco*RI restriction digests of phage DNAs. Lanes 1, uc1001; lanes 2, uc1002; lanes 3, P008; lanes 4, uc3001; lanes 5, lambda. (b) Hybridization of biotinylated uc1001 DNA to a nitrocellulose filter containing the phage DNAs described for panel a.

corresponding location on the restriction map of the other phage. The level of homology between uc1001 and uc1002 was quantified by using heteroduplex analysis (Fig. 6). In independent experiments only three incomplete but overlapping heteroduplex molecules were obtained. These, however, were sufficient to establish a value of 83% (24.9 kbp) homology. Principal features of the heteroduplex were three deletion loops of 2.1, 1.0, and 1.1 kbp at 7.5, 16.0, and 23.4 kbp, respectively, from the right-hand end. The 1.1-kbp loop appeared to have a stem at the base. The remaining areas of nonhomology were 12 loops and bubbles ranging from 0.7 kbp down to the limit of resolution of the technique (0.05 to 0.1 kbp).

Phage structural protein profiles. Phage structural protein profiles were examined by SDS-polyacrylamide gel electrophoresis (Fig. 7). The profiles of the isometric-headed phages uc1001, uc1002, and P008 were similar, with a large band in the molecular weight range of 68,000 to 76,000, two faint bands between 42,000 and 67,000, a major band between 40,000 and 41,000, and up to four bands between 30,000 and 35,000. Phage uc1001 differed because the intensity of its large band was equal to that of the band at a molecular weight of 40,000, while uc1002 differed in that it had only two bands, instead of four, in the 30,000 to 35,000 range. The profile of the prolate-headed phage uc3001 was very different, with three major protein bands with molecular weights of 176,000, 76,000, and 30,000, as well as seven minor bands. The bands in lanes 1, 3, and 4 (Fig. 7) below a molecular weight of 24,000 were of variable occurrence for all phages and identical for prolate- and isometric-headed phages and therefore were assumed to be contamination products, breakdown products, or both. A large band of 300,000 molecular weight in the profile of uc3001 was presumed to be undissociated material.

DISCUSSION

Infection of mesophilic streptococci by lytic bacteriophage can inflict considerable economic loss on the dairy fermentation industry. Although this destructive effect has been recognized for many years, it is only recently that traditional approaches to the problem have been supplemented by genetic and molecular analyses of the starter cultures (5, 6, 19) and their phages. Results of this study showed that three isometric-headed phages, uc1001, uc1002, and P008, were closely related, with largely homologous genomes of similar size that had cross-complementary cohesive ends. It is probable that they are related to the a and b groups of small isometric-headed phages described by Jarvis (14). Phage uc3001 appeared to have a similar genome size and structure to those of other prolate-headed phages (14, 30), but it had a distinct restriction pattern. Its lack of homology with the isometric-headed phages, examined here, was also consistent with the findings of Jarvis (14), and this was reinforced by the unrelatedness of its cohesive ends to those of the isometric-headed phages.

Structural protein profile analysis provides another way of comparing phages, and in this study major differences between uc3001 and the three isometric-headed phages were observed, in agreement with morphological and genomic comparisons. Differences between the profiles of the isometric-headed phages were also observed, however. The profile of uc1002 was missing two minor protein bands, while that of uc1001 showed two major bands, as opposed to the single major band consistently observed for uc1002 and P008. However, the profiles of the last two phages showed relatively faint bands in the same region as the larger major band of uc1001, and two major bands have been found in the profile of P008 (35). These observations indicate that there may be a second major band in uc1002 and P008 that is prone to degradation. More detailed studies of further isometric-headed phages will be needed to clarify the significance of these differences. In contrast, the protein profiles of uc3001 and that of the prolate-headed phage c6A (30) were very similar, indicating that these two phages are closely related.

Although a high level of DNA homology was observed among the three isometric-headed phages, the restriction maps of each were very different from each other and from published maps of morphologically similar phages (17, 24), except for the partially similar left-hand ends of uc1002 and P008. This is consistent with results of recent studies of lactic streptococcal (14) and *Lactobacillus* (28) phages, and it is clear that within a DNA homology grouping of phages, restriction patterns may be identical, share some bands, or may be unrelated. The cross-complementarity studies between the cohesive ends of different phage DNAs allowed the orientation of the maps; and in the case of uc1001 and uc1002, when individual restriction fragments were used as probes, they always hybridized with the corresponding region of the other phage genome. This indicates that the overall organization of the two phage genomes was similar. This was confirmed by the high level of homology observed by heteroduplex analysis. The differences in the maps in homoduplex areas were, presumably, due to the presence of many additional regions of nonhomology that were too small to be detected. The overall pattern of the uc1001-uc1002 heteroduplex was similar to some of those described previously for isometric-headed phages (17, 24). This indicates that there are shared conserved and variable regions which could be of significance in locating genes involved in phage-host interactions. Loof and Teuber (24) also have suggested that a deletion loop in the otherwise homologous P008-P113G heteroduplex contained genes for the collar and whisker proteins of P008. Deletion loops of approximately similar size and location in the uc1001-uc1002 heteroduplex and those between collared and uncollared phages described by Jarvis and Meyer (17) are consistent with this interpretation.

TABLE 2. Comparison of the predicted and observed numbers of restriction sites in the DNAs of uc1001, uc1002, and P008

Restriction endonuclease	Recognition sequence	Predicted no. of sites[a]	Observed no. of sites in:		
			uc1001	uc1002	P008
*Mbo*I	GATC	103	10	6	14
*Taq*I	TCGA	103	14	13	14
*Hae*III	GGCC	37	3	2	6[b]
*Hpa*II	CCGG	37	1	2	11
*Scr*FI	CCNGG	37	4	3	6
*Cla*I	ATCGAT	10	1	0	2[b]
*Eco*RI	GAATTC	10	3	6	4[b]
*Hin*dIII	AAGCTT	10	3	2	1[b]
*Pst*I	CTGCAG	4	0	1	0[b]
*Bst*EII	GGTNACC	4	3	4	6
*Pvu*II	CAGCTG	4	5	5	5[b]
*Dpn*I	GA^{m}TC	—[c]	0	0	ND[d]

[a] The 37.5% G + C content of P008 (23) was assumed for uc1001 and uc1002. Values are rounded to the nearest integer.
[b] Data from Loof et al. (23).
[c] —, Cannot be determined.
[d] ND, Not determined.

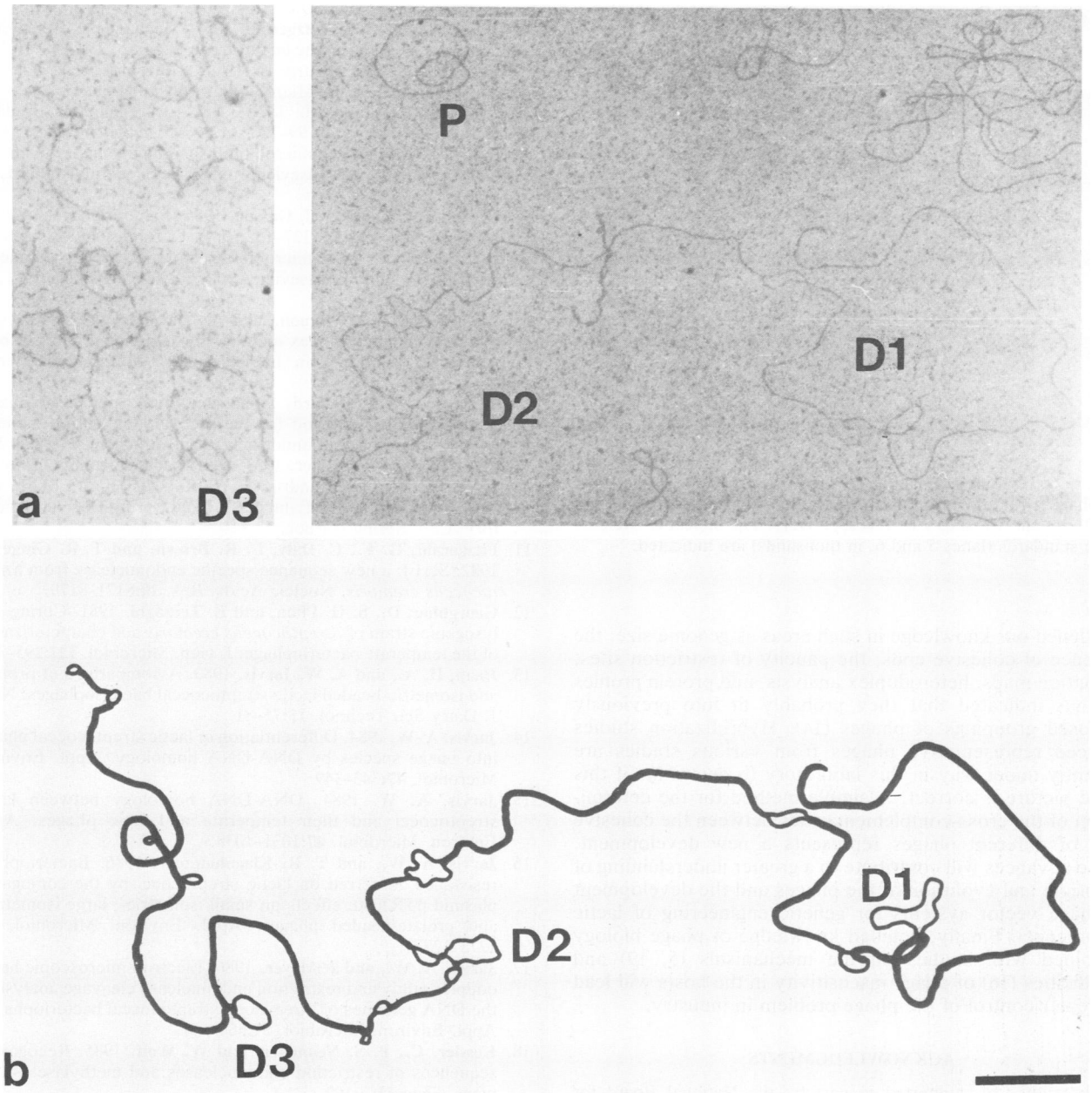


FIG. 6. (a) Composite photographs showing heteroduplex between phage uc1001 and uc1002 DNAs. The 2.1-, 1.0-, and 1.1-kbp deletion loops (D1, D2, and D3, respectively) are indicated. The circular molecule (P) is pAT153. (b) Interpretive drawing of the heteroduplex between uc1001 and uc1002. Bar, 1 kbp.

The restriction mapping studies highlighted a marked paucity of restriction sites, particularly for endonucleases with 4-base recognition sequences, in the genomes of uc1001, uc1002, and P008. This phenomenon was described recently for phage c6A (31) and also was noted by Jarvis and Meyer (17). The lack of sites for *Dpn*I, which recognizes a methylated *Mbo*I site, concurs with the conclusion of Powell and Davidson (30, 31) that the general resistance to digestion by a range of endonucleases is due to the absence of recognition sequences rather than site methylation. In certain *Bacillus* phages the paucity or lack of recognition sites for specific endonucleases has been linked to the presence of the corresponding enzymes in the bacterial host (20). In a detailed analysis of the sequences of eight coliphages and of the *Bacillus subtilis* phage φ29, Sharp (33) has shown that the lack of sites for host restriction enzymes is due to counterselection rather than other nonrandom features of their sequences. Its significance in lactic streptococcal bacteriophages is not clear because only one type II restriction endonuclease, *Scr*FI, has been found in the host bacteria (11), but it is noteworthy that this phenomenon occurs in both prolate- and isometric-headed phages. This would have to be considered in any future attempt to introduce novel restriction activities into starter strains.

In this study a detailed analysis of four phages, three isometric headed and one prolate headed, has significantly

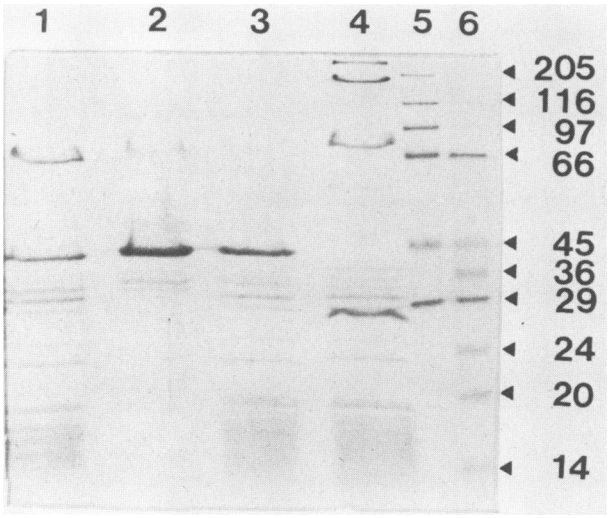


FIG. 7. SDS-polyacrylamide gel (15%) electrophoretic profiles of the phage structural proteins. Phages uc1001, uc1002, P008, and uc3001 are shown lanes 1 to 4, respectively. The molecular weights of the standards (lanes 5 and 6; in thousands) are indicated.

broadened our knowledge in such areas as genome size, the presence of cohesive ends, the paucity of restriction sites, restriction maps, heteroduplex analysis, and protein profiles and has indicated that they probably fit into previously proposed groupings of phages (14). Hybridization studies between representative phages from various studies are currently under way in this laboratory to determine if this broad picture is correct. A simple method for the determination of the cross-complementarities between the cohesive ends of different phages represents a new development. These advances will contribute to a greater understanding of the origin and evolution of the phages and the development of phage vector systems for genetic engineering of lactic streptococci. Finally, detailed knowledge of phage biology combined with studies on the mechanisms (5, 19) and specificities (16) of phage insensitivity in the hosts will lead to greater control of the phage problem in industry.

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