Identification of an Enveloped Phage, Mycoplasma Virus L172, that Contains a 14-Kilobase Single-Stranded DNA Genome

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We have found that mycoplasma virus L172 is an enveloped globular virion containing circular, single-stranded DNA of 14.0 kilobases. L172 has been reported by other workers to have a double-stranded DNA genome of 13 to 17 kilobase pairs and has been classified as a plasmavirus, a group for which mycoplasma virus L2 is the type member. Mycoplasma viruses L172 and L2 differ in genome size and structure, DNA base composition, and protein composition, and they have no detectable DNA homology. As the only reported enveloped virion containing single-stranded DNA, L172 represents a new group of viruses.

Mycoplasma virus L2 is an enveloped virion containing superhelical, circular, double-stranded DNA of 11.8 kilobase pairs (kbp) or 7.8×10^6 daltons (reviewed in reference 16). The DNA base composition is 32% G+C (5). L2 is a temperate virus and infects strains of *Acholeplasma laidlawii*. The noncytotoxic, productive infectious cycle involves maturation by budding through the host cell membrane (14, 21), followed by establishment of lysogeny with a viral genome integrated into the cell chromosome (4, 22).

The initial L2 isolation was by Gourlay (7) in England. Subsequently, another virus, MV-Lg-p52-L 172 (designated L172 here), infecting *A. laidlawii*, was reported by Liska (10) in Czechoslovakia. Since early studies showed that L172 and L2 were both globular in shape, had similar size ranges, and contained DNA, it was assumed that L172 belonged to the group of plasmaviruses for which L2 is the type member (reviewed in reference 16). The plasmaviruses are enveloped, rounded, pleomorphic phages, about 80 nm in diameter, containing circular, supercoiled, double-stranded DNA of an approximate molecular weight of 7.6×10^6 (17).

In support of this classification, L2 and L172 virions were reported to have similar protein compositions (8). To compare viral genomes, the structure and size of L172 DNA were studied by electron microscopy (3) and sedimentation velocity analytical ultracentrifugation (2). L172 was reported to contain superhelical, circular, double-stranded DNA of 10×10^6 to 11×10^6 daltons (13 to 17 kbp). Doskar et al. (2) also determined the L172 DNA base composition to be 36.8% adenine, 35.8% thymine, 14.1% guanine, and 13.3% cytosine, in agreement with their value of 27% G+C as measured by equilibrium centrifugation. Therefore, it appeared that the L172 genome was similar in structure to that of L2 but was slightly larger and had a lower percentage of G+C.

We began experiments to investigate the homology between L2 and L172 DNAs. These led to studies reexamining the structure and size of L172 DNA. We report here that there is no homology between L2 and L172 DNAs and that their protein compositions are different. The L172 genome is circular, single-stranded DNA of 14.0 kilobases (kb) or 4.6×10^6 daltons. Hence, this is the first report of an enveloped virus containing single-stranded DNA, and L172 must represent a new virus group. These data allow us to consider why, in retrospect, previous studies of L172 were misinterpreted.

MATERIALS AND METHODS

Cells, viruses, and media. Host *A. laidlawii* strains JA1 (13), JA1-REP⁻ (18), and K2 (9) have been described previously. Unless explicitly stated otherwise, K2 cells were used for all experiments.

The L2 mycoplasma virus used in these studies was the original group L2 isolate of Gourlay (7). The L172 mycoplasma virus, previously designated MV-Lg-p52-L 172, was isolated by Liska (10), and a sample was obtained from Paul Smith (University of South Dakota). The L1 virus used in these studies was mycoplasma virus isolate L51 (13).

Tryptose broth, containing 1% glucose and 1% PPLO serum fraction, was used for cultivating cells (12). Cell CFUs were assayed on tryptose agar plates containing 1% agar in tryptose broth. Virus PFUs were assayed in soft agar overlays of *A. laidlawii* on tryptose agar plates (19).

Purification of viruses and DNAs. L2 and L172 viruses were purified by sedimentation velocity in a linear 15 to 30% (wt/vol) sucrose gradient (5). L1 virus was purified by centrifugation in a CsCl step gradient (25). Viral DNAs were prepared by phenol extraction of purified viruses (5). A similar protocol was used to isolate host cell chromosomal DNA (4).

Intracellular L172 double-stranded, replicative-form (RF) DNA was isolated as follows. A 2-liter K2 culture was infected with L172 virus at a multiplicity of infection of 1 to 10. At 4 h postinfection, cells were harvested by centrifugation (6,000 rpm for 10 min at 4°C in a Beckman JA-10 rotor). The cell pellet was washed in TES buffer (10 mM Tris-hydrochloride, 1 mM EDTA, and 100 mM NaCl; pH 8.0) and suspended in 20 ml of 50 mM Tris-hydrochloride containing 10% sucrose, pH 8.0. Cells were lysed by the addition of 10 ml of 5% sodium dodecyl sulfate (SDS) in 0.25 M EDTA. Chromosomal DNA was precipitated by adding 7.5 ml of 5 M NaCl and incubating overnight at 4°C. The mixture was centrifuged at 26,000 rpm for 45 min at 4°C in a Beckman SW27 rotor to pellet large chromosomal DNA. The supernatant was extracted three times with phenol saturated with TES buffer. The aqueous layer was extracted with ether and DNA precipitated with ethanol. This DNA preparation contained L172 double-stranded, RF DNA plus RNA and cellular chromosomal DNA. The RNA was removed by digestion with RNase A followed by phenol extraction and ethanol precipitation. Cellular DNA was then removed by acid phenol extraction (27). The yield of L172 RF DNA was ca. 100 µg/liter of infected cells. Isolated DNA was identified

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FIG. 1. SDS-polyacrylamide gel electrophoretic analysis of L2 and L172 virion proteins. Lanes: a, purified L2 virus grown on strain JA1; b, crude L2 pellet from which virions in lane a were purified; c, crude L172 pellet from which virions in lane d were purified; d, purified L172 virus grown on strain K2. On the left, the number at each L2 protein band is its molecular weight ($\times 10^3$), as determined by Putzrath et al. (21). On the right, the number at each L172 protein band is its molecular weight ($\times 10^3$), estimated by using the L2 proteins as standards.

as L172 DNA by its ability to produce L172 virions after transfection of K2 cells, with the transfection procedure described below.

Gel electrophoresis. For proteins, SDS-polyacrylamide gel electrophoresis was carried out in a 10% polyacrylamide slab gel; after electrophoresis, gels were stained with Coomassie blue (21).

DNAs were analyzed by agarose gel electrophoresis with 0.8% agarose horizontal slab gels. The electrophoresis buffer was 89 mM Tris-borate and 2 mM EDTA, pH 8.0. Electrophoresis was in the presence of 0.5 μ g of ethidium bromide per ml of buffer, and gels were photographed by using UV illumination.

Enzyme digestions and restriction endonuclease mapping. DNA was digested with restriction endonucleases as recommended by the suppliers. S1 nuclease (from *Aspergillus oryzae*) digestion was at 37°C for 30 min in buffer containing 200 mM NaCl, 50 mM sodium acetate (pH 4.5), 1 mM ZnSO₄, and 0.5% glycerol. Exonuclease VII (ExoVII) digestion was at a concentration of 6 U of enzyme per μ g of DNA at 37°C for 30 min in buffer containing 67 mM potassium phosphate buffer (pH 7.9), 8.3 mM EDTA, and 10 mM β -mercaptoethanol. Enzymes were obtained from either Bethesda Research Laboratories (Gaithersburg, Md.) or New England Biolabs (Beverly, Mass.).

DNA-DNA hybridization. DNA was transferred from agarose gels to nitrocellulose filters by the method of Southern (26). Filters were prepared by soaking overnight at 42°C in a solution containing 6 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0), 0.5% SDS, 5 $\times$ Denhardt solution (1 $\times$ Denhardt solution is 0.02% [wt/vol] each of Ficoll 400, polyvinyl pyrrolidone, and bovine serum albumin [1]), 100 μ g of denatured calf thymus DNA per ml, and 10% formamide. The filters were then exposed to denatured, ³²P-labeled L2 DNA overnight at 42°C in a solution containing 6 $\times$ SSC, 10 mM EDTA, 0.5% SDS, 5 $\times$

Denhardt solution, 100 μ g of denatured calf thymus DNA per ml, and 10% formamide. After hybridization, the filters were washed at room temperature for 30 min in 2 $\times$ SSC containing 0.5% SDS, followed by a wash at room temperature for 30 min in 2 $\times$ SSC containing 0.1% SDS. A final wash was at 49°C for 2 h in 0.1 $\times$ SSC containing 0.1% SDS. Filters were examined by autoradiography using Kodak X-ray film (DF-85).

L2 probe DNA was prepared by nick translation (23). The specific activity of the L2 [³²P]DNA was ca. 10⁸ cpm/ μ g of DNA.

DNA base composition analysis. The base composition of L172 DNA was determined by high-pressure liquid chromatography, as described previously (5).

Transfection and infection. A polyethylene glycol-dependent transfection system was used (24, 25). L172 DNA (10 μ g; isolated from virus propagated on JA1 cells) was used to transfect either 5.9 $\times 10^7$ CFU of strain JA1 or 4.9 $\times 10^7$ CFU of strain JA1-REP⁻; the transfection mixture contained 50 μ l of DNA, 250 μ l of cells, and 2 ml of polyethylene glycol. Infectious centers were assayed by plating on JA1 lawns.

For infection, 5 $\times 10^4$ PFU of L172 virus (propagated on JA1 cells) was added to either 2.4 $\times 10^8$ CFU of strain JA1 or 2.0 $\times 10^8$ CFU of strain JA1-REP⁻ (in a total volume of 1 ml). After 5 min at 37°C to allow adsorption, the mixture was centrifuged for 4 min in an Eppendorf microfuge no. 5412 (Brinkmann Instruments Inc., Westbury, N.Y.), and the supernatant containing unadsorbed virus was discarded. The cell pellet was washed once with fresh medium, suspended in medium, and plated for infectious centers on JA1 lawns.

Electron microscopy. Virus samples were adsorbed to carbon-coated, 400-mesh electron microscope grids and negatively stained with 1.5% (wt/vol) uranyl acetate or 2% phosphotungstic acid. Micrographs were taken with a Siemens Elmiskop IA electron microscope with a liquid nitrogen decontamination device, a 50- μ m objective aperture, and an 80-kV operating voltage.

L172 DNA was mixed with mycoplasma virus L1 single-stranded DNA in a solution containing 30% formamide and 70 μ g of cytochrome c per ml, and was spread on a hypophase containing 20% formamide. Samples were picked up on collodion-coated, 400-mesh electron microscope grids, stained with uranyl acetate, shadowed with tungsten, and examined in the electron microscope (20). Contour length measurements were made on projections of electron micrographs by using an electronic planimeter.

RESULTS

Relationship between L2 and L172. At the start of these studies, we assumed that L172, like L2, was a plasmavirus. To investigate their similarity, the protein compositions of L2 and L172 virions were compared by SDS-polyacrylamide gel electrophoresis (Fig. 1). The L2 protein pattern was the same as that previously reported for L2 virions (e.g., in reference 21). However, the L172 protein composition was different from that of L2.

Homology between L2 and L172 DNAs was examined directly by DNA-DNA hybridization with the Southern blot technique. ³²P-labeled L2 DNA was incubated with filter-bound L2 and L172 DNAs, by using low-stringency hybridization conditions (see above). The L2 [³²P]DNA probe hybridized to L2 DNA (Fig. 2, lane l) but not to L172 DNA (Fig. 2, lanes g and i to k). Hence, under these conditions there was no detectable homology between L2 and L172 DNAs.

FIG. 2. Analysis of L172 and L2 DNAs. Untreated and restriction endonuclease-treated DNAs were analyzed by agarose gel electrophoresis, stained with ethidium bromide (lanes a to f), transferred to nitrocellulose filters, hybridized against ^{32}P -labeled L2 DNA, and examined by autoradiography (lanes g to l). Lanes: a and g, L172 DNA; b and h, coliphage λ DNA; c and i, mixture of L172 and λ DNAs; d and j, *Clal*-treated L172 DNA; e and k, *Clal*-treated mixture of L172 and λ DNAs; f and l, L2 DNA. The bottom half of the ethidium bromide-stained gel (corresponding to linear DNAs smaller than about 1.5 kbp) is not shown (lanes a to f). The only bands in this half of the gel were in lane e, the small λ DNA fragments generated by the *Clal* digestion. The bottom three-quarters of the autoradiogram (corresponding to linear DNAs smaller than ca. 8.5 kbp) is not shown (lanes g to l). No bands were observed in this part of the autoradiogram.

Characterization of L172 DNA. L172 DNA was resistant to cleavage by most of the restriction endonucleases we examined (Fig. 2; Table 1). This was surprising since double-stranded DNA of 13 to 17 kbp (the reported size for L172 DNA [2, 3]) should contain sites for many of the nucleases tested. The activity of the enzymes was confirmed by showing that, in a mixture of L172 and coliphage λ DNAs,

TABLE 1. Action of restriction endonucleases on L172 virion and RF DNAs

Nuclease	Cleavage sites ^a on DNA from:	
	L172 virion	L172 RF
<i>AluI</i>	—	+
<i>BclI</i>	—	+
<i>BstEII</i>	—	1
<i>CfoI</i>	—	1
<i>ClalI</i>	—	2
<i>EcoRI</i>	—	—
<i>HaeIII</i>	—	3
<i>HindIII</i>	—	+
<i>HinfI</i>	+	+
<i>HpaI</i>	—	4
<i>HpaII</i>	—	+
<i>MboI</i>	+	+
<i>PstI</i>	—	+
<i>SstI</i>	—	—
<i>TaqI</i>	+	+
<i>XbaI</i>	—	+

^a For each nuclease, a numeral is the number of cleavage sites, + denotes that there were >4 cleavage sites, and — denotes that the enzyme did not cut the DNA.

the λ DNA was digested, but the L172 DNA was not (e.g., Fig. 2, lane e). A possible explanation for the resistance to nuclease cleavage is that L172 DNA might have contained a large fraction of modified bases. Therefore, the base composition of L172 DNA was analyzed by high-pressure liquid chromatography. These data, together with the reported base compositions of *A. laidlawii* strain K2 (the host used to grow L172 and L2 viruses for these studies) and L2 virus DNAs are shown in Table 2. L172 DNA had 1.4% of its cytosine methylated to 5-methylcytosine and 0.2% of its adenine methylated to *N*⁶-methyladenine. For comparison, K2 and L2 DNAs have 2.2 and 1.6%, respectively, of their cytosine methylated and 0.3% of their adenine methylated. This low level of methylated bases is not sufficient to account for the resistance of L172 DNA to restriction endonuclease cleavage, and no other modified bases could be detected.

The resistance of L172 DNA to restriction endonuclease cleavage and the observation that the electrophoretic bands of L172 DNA were more diffuse than expected led us to reexamine the structure of the L172 chromosome. In particular, the lack of cleavage and diffuse bands could be explained if the L172 genome was single- rather than double-stranded DNA as previously reported.

To investigate whether L172 DNA was single or double stranded, the L172 chromosome was treated with S1 nuclease, which is specific for single-stranded DNA, and analyzed by agarose gel electrophoresis. High concentrations of S1 nuclease (20 U per reaction mixture) completely digested

TABLE 2. Base compositions of strains K2, L2, and L172 DNAs

DNA source	Base composition (mol %) ^a					
	Thy	Gua	Cyt	m ⁵ Cyt	Ade	m ⁶ Ade
K2 cells ^b	36.2	15.0	15.9	0.36	33.2	0.10
L2 virus ^b	34.2	16.0	16.1	0.26	33.4	0.10
L172 virus	33.8	15.5	13.7	0.20	36.6	0.07

^a Thy, Thymine; Gua, guanine; Cyt, cytosine; m⁵ Cyt, 5-methylcytosine; Ade, adenine; m⁶ Ade, *N*⁶-methyladenine.

^b Data from Dybvig et al. (5).

FIG. 3. Agarose gel electrophoresis of DNAs after treatment with S1 nuclease. Lanes: a, untreated L172 DNA; b, L172 DNA treated with 0.2 U of S1.

L172 DNA, but only converted superhelical molecules of L2 DNA to relaxed circular and linear double-stranded molecules, without noticeable degradation (data not shown). Even at S1 nuclease concentrations so low (0.02 to 0.2 U per reaction mixture) that there was no effect on L2 DNA, L172 DNA was digested (Fig. 3, lane b). Hence, the L172 chromosome is single-stranded DNA.

We noted that L172 DNA produced two electrophoretic bands (Fig. 2 and 3, lanes a). Since both DNAs were digested by S1 nuclease (Fig 3, lane b), both must have been single stranded. It seemed most probable that one band was circular DNA and the other was linear DNA. To examine this possibility, we treated L172 DNA with ExoVII, which is a single-strand-specific exonuclease, and analyzed this

FIG. 4. Agarose gel electrophoresis of DNAs after treatment with ExoVII. Lanes: a, untreated L172 DNA; b, L172 DNA treated with ExoVII.

FIG. 5. Electron micrograph of L172 DNA. An area is shown containing two circular DNA molecules, a large molecule of single-stranded L172 viral DNA and a small molecule of double-stranded pUC9 DNA, which was included in the preparation as a size (2.7 kbp) and conformation marker.

treated DNA by agarose gel electrophoresis. ExoVII digested the faster migrating L172 DNA band, but not the slower migrating band (Fig. 4, lane b). Hence, the slower migrating L172 DNA band was circular, single-stranded DNA, and the faster-migrating band was linear, single-stranded DNA.

Electron microscope observations of L172 DNA confirmed that this DNA consisted of circular, single-stranded molecules (Fig. 5). Contour length measurements were made on a mixture of DNAs from L172 and L51 (a single-stranded DNA mycoplasma virus [20]). The L172/L51 contour length ratio was 3.21 ± 0.49 . Since L51 DNA is 4,520 nucleotides (20), we estimated the size of the L172 genome as 14.5 ± 2.2 kb.

We concluded that the circular form of L172 DNA was the genome conformation, because (i) whereas the ratio of circular to linear molecules varied in L172 DNA preparations, the linear form was generally the minor species and in some cases was almost completely absent, and (ii) during storage at 4°C, purified L172 DNA was slowly converted from circular to linear molecules. Therefore, the linear form must have been generated from circular DNA by single-strand breaks during L172 DNA preparation and storage.

The double-stranded RF of L172 DNA was isolated from L172-infected cells. RF molecules were in two forms: covalently closed, circular molecules (RFI), and a relaxed, open, circular form of RFI (RFII). Most of the restriction endonucleases that could not cleave L172 viral single-stranded DNA were able to cleave L172 RF DNA (Table 1). Restriction endonuclease digests of L172 RF DNA were analyzed on agarose gels, and a cleavage site map of the L172 genome was constructed (Fig. 6). From these data, we estimated the size of L172 DNA to be 14.0 kb, in agreement with the electron microscopic results. Therefore, the L172 genome is a 14.0-kb molecule of circular, single-stranded DNA.

L172 virion structure. The fact that L172 is an enveloped virion was confirmed by studies showing its sensitivity to nonionic detergents. The survival fraction of L172 in 1% octyl glucoside was $<10^{-9}$, and in 0.4% Triton X-100, the survival fraction was $<10^{-7}$. For comparison, the survival

FIG. 6. Restriction endonuclease cleavage map of L172 DNA. Cleavage sites were mapped by digestion of L172 RF DNA and agarose gel electrophoretic analysis of the DNA fragments. The circular L172 map was broken at the single *BstEII* site to allow a linear representation. The scale is in kb; the length of L172 DNA is 14.0 kb.

fraction of enveloped L2 virions was $<10^{-8}$ in both octyl glucoside and Triton X-100. The nonenveloped mycoplasma virus L51 was not inactivated by Triton X-100.

Electron microscopy of unfixed and glutaraldehyde-fixed L172 virions, negatively stained with uranyl acetate, showed globular particles about 60 to 80 nm in diameter, frequently with a protuberance (data not shown). However, no protuberance was seen in unfixed preparations negatively stained with phosphotungstic acid.

L172 growth characteristics. K2 cells lysogenic for L2 virus, designated K2(L2), are immune to superinfection by homologous (i.e., L2) virus but can be infected by heterologous mycoplasma virus (22). L172 plated with equal efficiency on both K2 and K2(L2) lawns (data not shown), indicating that cells lysogenic for L2 are not immune to L172 infection.

A. laidlawii strains K2 and JA1 have been shown to restrict and modify mycoplasma viruses (15). L172 also was found to be restricted and modified by these host cells; L172 virus grown on JA1 cells had an efficiency of plating of 0.06 on K2 relative to that of JA1 cells, and L172 virus grown on K2 cells had an efficiency of plating of 0.02 on JA1 relative to that of K2 cells. Similar restriction and modification frequencies were measured for transfection of L172 DNA and infection by L172 virions (data not shown), indicating that restriction and modification of L172 are mediated at the DNA level rather than at some other level (such as at viral adsorption).

JA1 cells can be infected by all three of the previously reported mycoplasma virus groups (L1, L2, and L3) as well as by L172. JA1-REP⁻ is a variant of JA1 that cannot propagate the single-stranded DNA mycoplasma virus group (L1) but can propagate double-stranded mycoplasma viruses (L2 and L3) (18). L172 virus also was able to grow on JA1 but not on JA1-REP⁻ cells (Table 3).

The experiment with JA1-REP⁻ cells suggests that the two single-stranded DNA mycoplasma viruses, L1 and L172,

have some common host-dependent replication steps, although their morphology and genome size are different. In JA1-REP⁻ cells, L1 single-stranded DNA has been shown to be converted to parental double-stranded, RF molecules, but these are not replicated further to produce progeny RF molecules (18). This block in L1 replication in JA1-REP⁻ cells can be bypassed by transfection with either L1 single-stranded or RF DNA (25). Therefore, transfection was carried out with L172 DNA. L172 DNA transfected JA1 and JA1-REP⁻ with equal efficiency (Table 3), in agreement with there being some common replication requirements, which can be bypassed by transfection, for the two single-stranded DNA mycoplasma viruses in JA1-REP⁻ cells.

During virus purification, we noted that L172 preparations were heterogeneous. Velocity sedimentation in 15 to 30% sucrose gradients showed at least three peaks of L172 particles (data not shown). Similar sedimentation profiles have been reported for mycoplasma virus L2 (14) and apparently represent heterogeneity in the viral assembly process (S. K. Poddar, S. P. Cadden, J. Das, and J. Maniloff, submitted for publication).

DISCUSSION

The data presented in this paper show that mycoplasma virus L172 is an enveloped globular virion containing a genome of circular, single-stranded DNA of 14.0 kb. This is the only reported enveloped virus containing single-stranded DNA (cf. reference 17). The other known enveloped phage containing DNA is also a mycoplasma virus; mycoplasma virus L2 is an enveloped globular virion containing circular, double-stranded DNA of 11.8 kbp (19). All other reported single-stranded DNA viruses have nucleocapsids with either icosahedral (e.g., parvovirus and phage ϕ X174) or helical symmetry (e.g., phages fd and L1), containing 4.5 to 6.0 kb of DNA (reviewed in reference 17). Hence, L172 represents a new virus group.

Since our findings contradict previous reports on L172 protein composition and genome size and structure, we had to verify that we were studying the same L172 virus used by other workers. The morphology of L172 that we observed by electron microscopy was similar to that found by others (8, 11). L172 was sensitive to mild detergent treatment, as previously reported (8), confirming the presence of an envelope. L172 DNA base composition (Table 2) was very close to the results of Doskar et al. (2). Finally, all of the previously reported conclusions on L172 could be shown to be consistent with our results.

In the initial report on L172 DNA, Drasil et al. (3) observed double-stranded molecules in their DNA samples for electron microscopy. Their stated preparative conditions did not include the use of formamide or a similar reagent to stabilize single-stranded DNA, so single-stranded molecules in their L172 DNA samples should have collapsed and not been measurable. We have observed that crude preparations of mycoplasma virus L1 single-stranded DNA are usually contaminated with L1 double-stranded-DNA RF molecules (25). The L172 DNA preparations of Drasil et al. (3) may have been similarly contaminated with L172 RF molecules. Although they included no internal DNA size standard, their value of 13 kbp is close to the expected size of L172 RF DNA. The fact that Drasil et al. (3) indeed were studying a single-stranded DNA preparation (probably containing a minor fraction of RF DNA) is indicated by two of their other observations: (i) they noted only one peak of viral DNA by

TABLE 3. L172 infection and transfection of JA1 and JA1-REP⁻ cells

Infection with	Host cells	Infectious centers ^a	PFU/CFU (Relative no.)
L172 virus	JA1	6.7×10^3	2.8×10^{-5} (1.0)
	JA1-REP ⁻	1.4×10^2	7.0×10^{-7} (0.025)
	None ^b	7.0×10	
L172 DNA	JA1	9.0×10^3	1.5×10^{-4} (1.0)
	JA1-REP ⁻	8.7×10^3	1.8×10^{-4} (1.2)

^a Infectious centers were assayed as PFU on JA1 lawns.

^b A low background for a mock infection was also observed with mycoplasma virus L1 (25) and is believed to be due to residual virions not removed by washing before plating.

sedimentation velocity in both neutral and alkaline sucrose gradients, and (ii) they found that L172 was inactivated by a gamma radiation dose sufficient to cause only single-stranded breaks.

Later analytical-centrifugation results of Doskar et al. (2) appeared to confirm the size of the double-stranded DNA, with a value of 17 kbp calculated from a 39.6S sedimentation coefficient. However, if instead of an equation for superhelical DNA, the equation for single-stranded DNA is used (6), a size of 9.7 kb is calculated. This value is lower than the actual 14.0-kb value, but not unreasonable in view of the lack of any correction for DNA concentration, the probability of intramolecular base pairing, all of the solvent corrections involved in the calculation, and the exponential relationship between sedimentation coefficient and molecular weight. The fact that a single-stranded DNA was being used in those studies is indicated by the observation of only a single peak of viral DNA by equilibrium centrifugation in neutral and alkaline CsCl gradients (2).

The report of Gourlay et al. (8) of similar protein compositions for L2 and L172 probably was due to cell membrane contamination in the virus preparations. Their gel pattern for L2 proteins was unlike those found for L2 virus (reviewed in reference 16). Cell membrane contamination has been shown to be a problem in purifying L2 and L172 virions (Fig. 1) (21). L2 and L172 have different protein compositions (Fig. 1), as expected in view of their lack of genetic homology (Fig. 2). The difference in virion composition is also seen in the finding of Gourlay et al. (8) of no detectable common antigens between L2 and L172.

As shown in the studies reported here, mycoplasma viruses L172 and L2 differ in their genome size and structure, DNA base composition, DNA homology, and protein composition. However, an interesting relationship is that both give rise to heterogeneous progeny viruses having similar sedimentation distributions. This may reflect some commonality in the budding mechanisms possible for assembly of enveloped viruses in mycoplasmas.

The two circular, single-stranded DNA mycoplasma viruses have different morphologies: L172 is an enveloped globular virion containing 14.0-kb DNA, and L1 is a naked, bullet-shaped virion containing 4.5-kb DNA (20). However, both single-stranded DNA viruses do not replicate in JA1-REP⁻ cells (Table 3); for L1, JA1-REP⁻ cells have been shown to have a block in the replication of double-stranded, RF DNA (18). This block can be circumvented by transfection, since JA1-REP⁻ cells can be transfected by L1 (25) and L172 DNAs (Table 3). These observations indicate that L1 and L172 have some common host-dependent replication steps.

In summary, mycoplasma virus L172 represents a new group of viruses, characterized by an enveloped virion containing 14.0-kb, single-stranded DNA. Morphologically (and perhaps in its assembly), L172 resembles the other enveloped mycoplasma virus group, L2. In terms of genome structure (and perhaps replication biochemistry), L172 appears similar to the single-stranded DNA mycoplasma virus group, L1.

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