Comparison of Mycoplasmatales Virus
MV-Lg-pS2-L172 With Plasmavirus MV-L2 and the Other Mycoplasma Viruses

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Summary
Mycoplasma virus MV-Lg-pS2-L172 was sensitive to heat (56 °C/30 minutes), Nonidet-P40 and ether. In these respects it resembled Plasmavirus MV-L2. However, it differed from MV-L2 (and the other mycoplasma viruses, MV-L1, MV-L3 and BN1 virus) in reciprocal plaque inhibition and serum neutralization tests (MV-L2 only). By plaque formation on host lawns resistant to the different mycoplasma viruses, including MV-Lg-pS2-L172, this latter virus was shown to be distinct from the other viruses, including MV-L2. Both MV-Lg-pS2-L172 and MV-L2 possessed one polypeptide band (out of 10) that was not common to the heterologous virus.

Introduction
Three morphologically distinct mycoplasma viruses have so far been isolated. These are Mycoplasmatales virus-laidlawii 1 (MV-L1), MV-L2 and MV-L3 (4). Morphologically, these viruses are respectively, rods, spheres and polyhedrons with short tails. They all contain DNA. A fourth virus, BN1 virus, that is serologically distinct but morphologically resembles MV-L3 has also been described (7). In 1972 LISKA (10) isolated a virus, designated MV-Lg-pS2-L172. By electron microscopy it was spherical, enveloped, variable in size (50—90 nm) (11) and protuberances 25—35 nm long and 12—20 nm thick were reported on many of the virus particles. By ultrafiltration the size of the virus was estimated as between 62 and 96 nm (8). The virus contained double stranded DNA (3).

This paper describes studies on the relationship of Liska's virus to MV-L2 and the other mycoplasma viruses.
Materials and Methods

Strains of Microorganisms

The viruses used were MV-L1, MV-L2, MV-L3 (4), MV-Lg-pS2-L172 (MV-L172) (10) and BN1 virus (7). Acholeplasma laidlawii strain M1305/68 was used as host for all the viruses except for BN1 virus, for which the BN1 strain of A. laidlawii was used.

A. laidlawii M1305/68 clones resistant to MV-L172 were selected from colonies that grew when virus wash preparations were diluted on GS solid medium plates. Three clones of M1305/68 resistant to MV-L1, MV-L2 and MV-L3 respectively (4) selected in a similar manner were also used.

Virus Production and Assay

The Acholeplasma strains were grown and the viruses produced and assayed as previously described (5). Incubation at 37°C was carried out for 48 hours, except for MV-L1 (24 hours).

MV-L172 Stock

MV-L172, obtained from the Czechoslovak Collection of Microorganisms, was cloned by picking a single plaque on 3 separate occasions using A. laidlawii strain S2 (10) as host. Cloned virus was then titrated simultaneously on the S2 and M1305/68 strains of A. laidlawii. The titre on the S2 lawn was $1 \times 10^7$ PFU/ml and on the M1305/68 lawn was $1.75 \times 10^4$ PFU/ml. The plaques on the latter lawn were washed in phosphate buffered saline (pH 7.3) for 6 hours. This virus wash (titre $5 \times 10^5$ PFU/ml) was subsequently passaged once more on the M1305/68 lawn and the second wash was used for the various studies.

Purification of MV-L172

The virus was purified by the method described previously for MV-L2 (5). To facilitate location of the virions during gradient centrifugation, the virus was labelled by incorporation of $\left(3^H\right)$-methyl thymidine ($0.03 \mu$g; sp. act. = 1 Ci/mmole) into the solid medium on which the host cells were grown.

Antisera

Antisera to MV-L172 was prepared in rabbits by the method used previously (5). The titre of purified virus used for each injection of each rabbit was $1 \times 10^9$ PFU/ml. Antisera to the other mycoplasma viruses have been referred to previously (4).

Serological Tests

All antisera were inactivated at 56°C for 30 minutes before use. All tests were performed at least three times.

Plaque Inhibition Test

Lawns of A. laidlawii M1305/68 or BN1 were allowed to dry and then 20 µl of a virus dilution, selected as the highest dilution that would give coalescent plaques (about $1 \times 10^6$ PFU/ml) was dropped on the lawns and allowed to dry. When dry 5 µl of antisera dilutions were dropped in the centre of the area of the virus drops. After incubation at 37°C plates were examined for areas of inhibition of plaque formation at the sites of the antisera drops. The plaque inhibition titre of antisera was expressed as the highest dilution producing visible inhibition. Preinoculation serum from the same rabbit was used as control.

Serum Neutralization Test

This was performed as described by Cleyde (1). The antisera neutralisation titre was expressed as the highest dilution which produced plaque reduction of 60 per cent or more compared with virus controls.