Isolation and Characterization of a *Bacillus megaterium* QM B1551 Bacteriophage

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**SUMMARY**

A bacteriophage specific for *Bacillus megaterium* QM B1551 was isolated. This phage (MP-7) was a lytic phage of typical morphology and distinct stability properties. The DNA was double-stranded, with a mole % G+C of 38.9 ± 0.7 and a mol. wt. of 42 to 44 x 10^6.

*Bacillus megaterium* QM B1551 has been used extensively to study the properties of spores and the mechanism of germination. While there are considerable biochemical data and a variety of mutants available (Hyatt & Levinson, 1964; Vary, 1972; Setlow, 1975), there is no method to study the genetics of this strain (Vary, 1975). Towards this end, we have isolated a bacteriophage that is specific for *B. megaterium* QM B1551 which may be used (a) to identify this strain, (b) as a source of DNA for transfection studies, and (c) as a potential helper phage in attempts at isolating transducing particles. The properties of this bacteriophage are reported here.

Bacteriophage were isolated and purified from the soil by the method of Adams (1959). One of the clear plaques (MP-7) was arbitrarily chosen for further study. Lysates were made from single plaques and assayed for plaque-forming units (p.f.u.) by the overlay method of Adams (1959). The indicator cells were grown from *B. megaterium* QM B1551 by suspending lyophilized spores (approx. 10^8 spores) in 1 ml sterile H2O followed by heat activation at 60 °C for 10 min and dilution into 50 ml of supplemented nutrient broth (SNB; Vary, 1972). The culture was incubated on a rotary shaker at 300 rev/min and 30 °C until the extinction at 660 nm was about 0.5.

The characteristics of MP-7 were as follows. The phage produced clear plaques surrounded by a halo as has been reported for other *B. megaterium* phages (Murphy, 1957; Cooney, Jacob & Slepecky, 1975). Electron microscopy showed that MP-7 had a hexagonal head of 60 to 70 nm in diam. and a thin tail about 150 nm long, not unlike other bacillus lytic and transducing phages. Host range studies showed that MP-7 was specific for *B. megaterium* strains QM B1551 and ATCC 7051 and did not infect strains ATCC 9885 or ATCC 19213, *B. cereus* T, *B. licheniformis* A5 or *B. subtilis* 168 at either low (< 0.1) or high (> 1000) multiplicities of infection. With those strains of *B. megaterium* that were insensitive to MP-7, attempts at phage induction by ultraviolet light irradiation (Yelton & Thorne, 1970) were unsuccessful.

In a one-step growth curve by the method of Adams (1959), the latent period was 60 to 65 min, similar to other bacillus phages. However, the eclipse time was 50 min and the burst size was 1370 ± 190 (average ± standard deviation from 7 experiments). This large burst size was probably not a result of multiple infections because the multiplicities of infection were < 0.1. Some of the other properties of MP-7 (Table 1) were its stability to heating at 50 °C but not 60 °C, relatively high stability in organic solvents and sensitivity to salt. The inhibition by salt was a result of inhibition of adsorption similar to that described for *Escherichia coli* T phages and *B. subtilis* φ25 phages (Kozloff & Henderson, 1955; Doyle et al. 1974).
**Short communications**

Table 1. *Characteristics of MP-7 phage particles*

<table>
<thead>
<tr>
<th>Heat stability*</th>
<th>% decrease in viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 °C for 2 h</td>
<td>1</td>
</tr>
<tr>
<td>60 °C for 15 min</td>
<td>76</td>
</tr>
</tbody>
</table>

Sensitivity to organic solvents:†

- Chloroform: 0
- Toluene: 0
- Benzene: 30
- Hexane: 5

Sensitivity to 60 mM-salt:‡

- Na₃HPO₄: 90
- K₂HPO₄: 90
- K₂SO₄: 90
- KCl: 90

* Samples of a lysate containing 10¹⁰ p.f.u./ml were placed in tubes at the indicated temperature and time and then cooled on ice. Samples were assayed for phage viability by the agar overlay method.
† A mixture of 0.2 ml of each solvent was added to 1.0 ml of lysate containing approx. 10⁹ p.f.u./ml. The suspension was mixed for 15 s on a Vortex mixer, incubated for 30 min at 30 °C and the aqueous layer was assayed for phage viability by the agar overlay method.
‡ A lysate was assayed by the agar overlay technique on plates containing each of the above listed salts at a final concentration of 60 mM (pH 7).

Table 2. *Properties of MP-7 DNA*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Property</th>
<th>Mole % G+C</th>
<th>Reference for method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base composition by paper chromatography*</td>
<td>G = C</td>
<td>39.3 ± 0.6</td>
<td>Sinsheimer &amp; Koerner (1952)</td>
</tr>
<tr>
<td>Thermal denaturation in 0.015 M-NaCl to 0.015 M-sodium citrate (pH 7.2)*</td>
<td>Tₘ = 70 °C</td>
<td>39.3</td>
<td>Mandel &amp; Marmur (1968)</td>
</tr>
<tr>
<td>Analysis of hyperchromicity data</td>
<td>ρ = 1.6973</td>
<td>41</td>
<td>Hirschman &amp; Felsenfeld (1966)</td>
</tr>
<tr>
<td>Mol. wt. by chemical analysis†</td>
<td>43.9 × 10⁶</td>
<td>38.1</td>
<td>Schildkraut, Marmur &amp; Doty (1962)</td>
</tr>
<tr>
<td>Mol. wt. by velocity sedimentation‡</td>
<td>41.5 × 10⁶</td>
<td>41</td>
<td>Bruner &amp; Vinograd (1965)</td>
</tr>
</tbody>
</table>

* DNA was extracted from a high titre lysate by phenol extraction, dialysed against 0.15 M-NaCl-0.15 M-sodium citrate (pH 7.2) and used in the indicated experiments.
† A high titre lysate was assayed for the number of p.f.u. by the overlay method. The amount of DNA was estimated by the diphenylamine positive material that was soluble in hot 5% (v/v) perchloric acid. The mol. wt. was calculated from the µg DNA/p.f.u., assuming double-stranded DNA with an average mol. wt. of 625/nucleotide pair.
‡ The DNA was extracted from a high titre lysate with NaClO₄, dialysed against 0.15 M-NaCl-0.15 M-sodium citrate, treated with 250 µg Pronase/ml at 30 °C for 2 h, dialysed again and the S value determined.

In the course of these studies, we learned of the isolation and characterization of a lytic phage (CS-1) for *B. megaterium* ATCC 19213 (Cooney, Jacob & Slepecky, 1975). These two phages were similar in both morphology and the physicochemical properties of their DNA. However, the following properties distinguished the two phages. The CS-1 phage was sensitive to organic solvents whereas MP-7 was not; CS-1 was stable to heat at 60 °C whereas MP-7 was not. The host range specificity of MP-7 was limited to 2 strains whereas CS-1 infected all the *B. megaterium* strains tested. While the latent period of the two phages was the same, the eclipse period of MP-7 was longer than that of CS-1 and no burst size was measured for CS-1.
The phage DNA was extracted and characterized. In order to concentrate the phage, lysates were centrifuged at 59000 g for 3 h and the phage pellet was suspended in phage buffer (Romig, 1962). The DNA was extracted from this high titre lysate by either phenol (Epstein, 1967) or sodium perchlorate (Freifelder, 1966). The DNA sample was assayed for deoxyribose (Burton, 1956) and phosphate (Ames & Dubin, 1960) and found to contain an average of 0.989 μmol of phosphate/μmol of deoxyribose. The sample contained undetectable amounts of RNA and < 3% protein by the methods of Keleti & Lederer (1974) and Lowry et al. (1951). The phage DNA had a spectrum typical for DNA with a ratio of extinctions at 260 nm and 280 nm of 1.84 and a ratio of extinctions at 260 nm and 230 nm of 1.9. No unusual extinction peaks were observed. When phage DNA was denatured using alkali a typical melting profile was obtained for a double-stranded DNA. Table 2 summarizes the properties of MP-7 phage DNA. The mole % G + C for the phage DNA calculated from thermal denaturation, buoyant density in CsCl, and base composition agreed closely. These studies suggest that the phage has a double-stranded DNA with a mole % G + C of 38.9 ± 0.7 with no unusual bases.

Mol. wt. determinations were done by sedimentation velocity with sodium perchlorate extracted DNA. This DNA was first treated with pronase to digest the phage protein that tended to complex with the released DNA. The phage DNA had an S value of 39.95 corresponding to a mol. wt. of 41.5 x 10^{6}. This value agrees, within the error of the methods, with the mol. wt. obtained by chemical analysis of the amount of DNA per viable phage (Table 2).

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REFERENCES


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