Properties of a new phage lytic for *Brucella suis*

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**SUMMARY**

The properties of a new brucella phage isolated from culture fluids of *Brucella suis* and designated the Weybridge phage, were examined. The phage was lytic for smooth *Br. abortus*, *Br. suis* and *Br. neotomae* cultures but there was no lysis of *Br. melitensis*, *Br. canis* or *Br. ovis* strains. The host range, adsorption pattern, chemical stability and particle morphology were similar to those reported for brucella phages M51 and S708. However, phage neutralization tests established that Weybridge phage was serologically distinct from M51 and S708 although the three phages possessed antigenic determinants in common with each other and with the Tbilisi reference phage. One-step growth experiments of Weybridge phage on *Br. abortus*, *Br. suis* and *Br. neotomae* are described. No evidence of lysogeny was found although the origins of the phage were suggestive of this.

Although a number of phages lytic for various *Brucella* species have been described, the majority of brucella phages will replicate only in *Brucella abortus* strains (Van Drimmelen, 1959; Jones, Merz & Wilson, 1968). Furthermore, most of these phages have been shown to resemble closely the Tbilisi (Tb) reference phage (Morgan, 1963; Parnas, 1971). Three new phage isolates showing marked differences in host range from Tb phage were described by Moreira-Jacob (1968). These were subsequently examined by Morris, Corbel & Phillip (1973) who confirmed that two, M51 and S708, underwent lytic cycles of replication in *Br. suis* and *Br. neotomae* strains as well as in *Br. abortus*. These phages were serologically distinct from each other and from Tb phage, although both were similar in host range, adsorption properties, morphology and physical and chemical stability.

A preliminary examination of a phage, designated the Weybridge phage, isolated at this laboratory from *Brucella suis* (K. Glowinska, unpublished observations) suggested that it shared a number of features in common with M51 and S708 phages. Clearly the relationship of the Weybridge phage to other brucella phages is of taxonomic significance. The present study was undertaken to characterize the Weybridge phage and to determine its relationship to phages M51, S708 and the Tb phage.

The Weybridge phage was isolated in 1969 by Mrs K. Glowinska. The method used was a modification of that described by Parnas (1963). However, precise details of the strain of *Brucella suis* from which the phage was isolated and the exact incubation conditions were not recorded.

In an attempt to propagate phage on various bacteria, unit vol. of 0.1 ml of a standard phage suspension containing $3 \times 10^4$ p.f.u./ml were used to inoculate 10 ml vol. of logarithmic phase cultures of bacteria grown in Albimi brucella broth to a concentration of $10^9$ viable organisms/ml. The cultures were all incubated at 37°C in a shaking water bath for 18 h and the number of p.f.u. determined by the agar double-layer method.

One-step growth experiments were performed on *Brucella abortus* 544, *Br. neotomae* 5K33 and *Br. suis* 1330 using early log phase cultures. The method of Jones *et al.* (1968) was employed in which, following adsorption, the phage+bacteria mixture was diluted
Table 1. One-step growth experiments

<table>
<thead>
<tr>
<th></th>
<th>Br. abortus 544</th>
<th>Br. neotomae 5K33</th>
<th>Br. suis 1330</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input count*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phage</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Bacteria</td>
<td>15,000</td>
<td>15,000</td>
<td>15,000</td>
</tr>
<tr>
<td>Counts after 30 min adsorption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total phage</td>
<td>15</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Free phage</td>
<td>11</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Infected bacteria</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Total phage count after shaking at 37 °C for</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 h</td>
<td>12</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>1.0 h</td>
<td>16</td>
<td>103</td>
<td>9</td>
</tr>
<tr>
<td>1.5 h</td>
<td>23</td>
<td>127</td>
<td>17</td>
</tr>
<tr>
<td>2.0 h</td>
<td>49</td>
<td>153</td>
<td>26</td>
</tr>
<tr>
<td>2.5 h</td>
<td>111</td>
<td>135</td>
<td>58</td>
</tr>
<tr>
<td>3.0 h</td>
<td>116</td>
<td>124</td>
<td>56</td>
</tr>
<tr>
<td>4.0 h</td>
<td>114</td>
<td>120</td>
<td>53</td>
</tr>
</tbody>
</table>

* All counts x 10⁵.

1 in 10⁴ to prevent further adsorption. In every one-step growth experiment the phage was assayed on lawns of the propagating strain used in that experiment.

Other methods used to characterize the Weybridge phage have been described by Morris et al. (1973).

Determination of host range: The Weybridge phage underwent replication in all of the 35 smooth cultures of Brucella abortus biotypes 1, 2, 3, 4, 5, 6, 7 and 9 tested. No strain of Br. abortus biotype 8 was available. Discrete plaques were produced in all strains at phage concentrations just below the routine test dilution (RTD) (Morris et al. 1973), whereas higher concentrations of phage produced areas of confluent lysis.

A total of 65 authenticated strains of smooth Brucella melitensis, representative of biotypes 1, 2 and 3 were examined but no evidence of lysis was observed at phage concentrations of RTD or 10⁴ RTD.

Discrete plaques were produced on 60 cultures of Brucella suis biotypes 1, 2, 3 and 4 at phage concentrations below RTD and confluent lysis at higher concentrations. The only authenticated strains of Br. suis which were not lysed by phage at RTD were six cultures of biotype 2 which exhibited the characteristics of rough variants.

Brucella neotomae was lysed by the Weybridge phage at RTD and 10⁴ RTD, with formation of isolated plaques at lower concentrations. There was no evidence of lysis of Br. canis or Br. ovis cultures by phage at concentrations of RTD or 10⁴ RTD.

Weybridge phage propagated on Brucella suis 1330 had a host range identical with that of phage propagated on Br. abortus 544.

Growth kinetics: Following incubation of Weybridge phage with cultures of Brucella abortus 544 or Br. suis 1330, the total phage yield was of the order of 10⁴ times greater than the phage input. Following incubation with Br. neotomae 5K33 the increase in total phage count was of the order of 10⁵.

There was no increase in p.f.u. after incubation with cultures of Brucella canis RM6-66, Br. melitensis 16M, Br. ovis 63/90 or Escherichia coli.

The results of typical one-step growth experiments are summarized in Table 1. The total
phage counts with *Brucella abortus* 544 as host strain increased after 1·0 to 1·5 h, whereas replication of phage on *Br. neotomae* was detectable within 30 to 60 min. In both cases the highest counts occurred after 2 to 3 h of incubation. With *Br. abortus* 544 as host, the mean burst size was 25; with *Br. neotomae* as host the mean burst size was 22.

Phage replication on *Brucella suis* 1330 could be detected after 30 to 60 min and reached a maximum between 2 and 3 h of incubation. In all of the experiments (8) with *Br. suis* 1330 the total phage count after 30 min incubation was always lower than the phage count immediately after adsorption. It was therefore impossible to determine a true burst size from these results.

Plaque morphology: No differences were observed between the plaque morphology on overlay plates of *Brucella abortus* 544, *Br. neotomae* 5K33 or *Br. suis* 1330. At 18 h two types of plaques were predominant; small turbid plaques, 0·1 to 0·5 mm in diam.; and large clear plaques, 0·5 to 2·5 mm in diam. The small turbid plaques, however, developed into the large clear type on prolonged incubation. At 48 h the plaques were mainly of the clear variety, often with a diam. of 3 mm or more (Fig. 1).

Phage adsorption tests: Weybridge phage adsorbed to smooth *Brucella abortus* strain 99 (80 % reduction in titre), but not to the rough strain, *Br. abortus* 45/20 (< 10 %). Phage was adsorbed by *Br. suis* (85 % reduction) and adsorbed most strongly by *Br. neotomae* (94 % reduction).

There was no adsorption to preparations of *Brucella melitensis*, *Br. canis*, *Br. ovis*, *Yersinia enterocolitica* IX, *Escherichia coli*, *Pseudomonas aeruginosa* or *Proteus vulgaris* (all < 5 % reduction).
Phage inactivation: The heat sensitivity of the Weybridge phage was similar to that reported for Tb, A422, M51 and S708 phages (Morris et al. 1973). Thus dialysed Weybridge phage heated at 60 °C for 1 h experienced a > 90% reduction in the number of p.f.u./ml. There was no inactivation on exposure to toluene or diethyl ether (< 5% reduction). Chloroform caused a notable loss of activity (26% reduction).

Of the surface-active agents tested the non-ionic detergent, Triton X-100, did not reduce the phage titre; the anionic detergent, sodium deoxycholate, had a significant effect (33% reduction) and alkyltrimethyl ammonium bromide, the cationic agent, caused complete inactivation.

DNase, RNase and EDTA each had little effect on phage titre (all < 10%) but the proteolytic enzymes, trypsin, chymotrypsin and ficin, produced substantial reductions in the number of the p.f.u. (all > 80%). Complete inactivation was produced on oxidation with M/50 periodate.

Phage morphology: Examination of negatively stained preparations of Weybridge phage revealed a hexagonal icosahedral head and a short tail (Fig. 2). The mean diam. of the head varied from 65 nm to 70 nm. The tail was 25 nm to 30 nm in length.

Serological specificity: Comparison of Weybridge phage with phages M51, S708 and Tb in neutralization tests (Table 2) showed that all of these reacted with antiserum prepared against the Weybridge strain. The neutralization constant for Weybridge phage reacting with its homologous antiserum was much higher than the values obtained for the other phages with this serum. It was clear however that a serological relationship existed between all of the phages. The neutralization constants suggested a closer antigenic relationship of Weybridge phage to M51 and S708 than to Tb phage.

These results showed that the Weybridge phage shared many features in common with the previously described M51 and S708 phages (Morris et al. 1973). However, it was clear from the serological results that the Weybridge phage was distinct from the other phages and thus undoubtedly a new isolate. The resistance of Weybridge phage to lipid solvents
Table 2. Neutralization of brucella phages using absorbed antiserum to the Weybridge phage

<table>
<thead>
<tr>
<th>Phage</th>
<th>$k$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weybridge</td>
<td>47.5</td>
</tr>
<tr>
<td>M51</td>
<td>17.9</td>
</tr>
<tr>
<td>S708</td>
<td>15.3</td>
</tr>
<tr>
<td>Tb</td>
<td>4.4</td>
</tr>
</tbody>
</table>

and susceptibility to proteolytic enzymes and periodate oxidation were as previously observed for the other phages (Morris et al. 1973). These properties together with the phage adsorption studies, the morphology of the phage particle, the characteristics of plaque formation and the restriction of the lytic action of the phage to smooth Brucella cultures were similar to those reported previously for other brucella phages (McDuff, Jones & Wilson, 1962; Jones et al. 1968; Morris et al. 1973). This suggested that Weybridge phage belonged to the same phage family as the other hitherto described brucella phages (Morris et al. 1973).

The host range of Weybridge phage distinguished it from Tb phage. In its ability to lyse Brucella suis and Br. neotomae strains at low input multiplicities, Weybridge phage resembled M51 and S708. On the basis of this, together with the serological evidence, it is proposed that the known brucella phages which evidently belong to a single phage family (Morgan, 1963; Parnas, 1961; Morris et al. 1973), can be divided into two subgroups. The first subgroup would comprise those phages typified by the Tb reference phage, which are capable of effective replication only in Br. abortus. The second subgroup would comprise those phages typified by Weybridge phage, which are capable of replication in Br. abortus, Br. neotomae or Br. suis.

The one-step growth experiments showed that, in general, the characteristics of the infective cycle of Weybridge phage were similar to those reported for the Tb phage (Jones et al. 1968) but the mean burst size for Weybridge was only 25 when propagated on Brucella abortus compared with the figure of 121 reported for phages of the Tb group (McDuff et al. 1962).

Jones et al. (1968) reported no overall increase of Tb phage in Brucella neotomae 5K33 cultures. They considered that phage replication occurred in a small proportion of the bacterial population but that the remaining host cells were killed, the phage acting apparently as a bacteriocin. In the present study, Weybridge phage replicated readily in Br. neotomae, giving a high yield. The latent period was shorter than observed with Br. abortus but the time of maximum phage count and the burst size were similar to those observed with this strain.

The behaviour of the Weybridge phage in the one-step growth experiments was unexpected. If a proportion of the phage initiated non-productive infections this should be indicated by a reduction in p.f.u. immediately after adsorption. However, a reduction in the phage titre was not evident until 30 min after the end of the adsorption period. Usually the critical period during which a temperate phage initiates a productive or non-productive response occurs within a very short time of infection. In a non-productive response, if incorporation of the prophage into the bacterial genome were delayed, samples taken immediately after infection may yield a lower proportion of lysogenic bacteria than samples taken later in the infection period. The isolation history of the Weybridge phage is certainly consistent with lysogeny but preliminary attempts to demonstrate a lysogenic state in cells
Short communications

of *Brucella suis* surviving exposure to phage have been unsuccessful. Survivors showed no evidence of immunity to infection by Weybridge nor did they liberate phage after exposure to u.v. irradiation or mitomycin C. Nevertheless, the indisputable fact remains that phages such as M51, S708, and now Weybridge, are occasionally isolated from Brucella cultures, and, although lysogeny has yet to be demonstrated, these findings continue to be reported.

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**REFERENCES**


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