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NOTES

Isolation of *Brucella melitensis* Phage of Broad Biotype and Species Specificity

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A *Brucella melitensis* phage was isolated. Stocks of phage were established which produced early large plaques. The phage host range included all smooth *Brucella* species.

Information on the sensitivity of *Brucella melitensis* to brucellaphages is contradictory and often applicable to a single strain, the identification of which may itself be dubious (4, 7, 14-16). Oxidative metabolism studies of susceptible strains established that the initial brucellaphages were restricted in host range to *B. abortus* and *B. suis* (7, 9). Although occasional reports of bacteriophage activity on *B. melitensis* exist, the host range has been limited to an individual strain of *B. melitensis* or to strains with varying degrees of susceptibility within one biotype (3, 10, 13). We report here the existence and availability of a *Brucella* bacteriophage with a host range that includes all three biotypes of *B. melitensis*, as well as other species of *Brucella*. In keeping with the tradition of naming *Brucella* phages after the location of their discovery, we have designated our isolate the "Berkeley phage" (4, 12).

The Berkeley phage was isolated from very small plaques that appeared on a soft-agar lawn containing a mixture of *B. melitensis*, strain "Isfahan," and the Weybridge phage (12). As with this initial isolation, all further propagations and tests for host range were performed on soft agar either by the mixture technique or by placing drops of phage suspension on a lawn (1, 2, 5). After two successive propagations on strain Isfahan, the isolate was then tested on three biotypes of *B. melitensis* represented by strains Isfahan, 63/10, and Ether. Small plaques were seen on each strain. These isolates were passaged on their respective hosts. By the sixth parallel passage, a mixture of large and small plaque sizes was seen on each strain. As the number of passages increased, the percentage of large plaques increased. Also, with passage the emergence of early- and late-plaque types was noted. In keeping with these characteristics, phage causing large, early appearing plaques were isolated from each strain (cf. reference 8). The isolates were then reselected and propagated for three consecutive cycles on their respective hosts. This purification technique resulted in biotype phage stocks that produced early, large, uniform plaques (see Fig. 1b).

The phage stocks were then plated on different biotypes of *B. melitensis* to compare their host range, efficiency of plating, and homogeneity. All three phage stocks produced similar plaque types on each biotype. It was observed that the host strain controlled plaque morphology in terms of the degree of clarity. On some strains uniformly clear plaques were seen, whereas on others turbid plaques were observed (cf. 6). This property remained constant when different biotype phage stocks were reacted with each *Brucella* strain.

The efficiency of plating of the three phage stocks was determined from the routine test dilution (RTD). The RTD was established for each phage preparation on its host strain. It was found that each phage stock plated with equal efficiency at the RTD on all three biotypes of *B. melitensis*.

The phage stock that had been passed only on strain Isfahan was designated the Berkeley phage. RTD of this stock was then tested against other strains of *B. melitensis* as well as other species of *Brucella*. To make a more meaningful comparison, the Tbilisi phage was used as a control. Table 1 shows that all strains tested were susceptible to the RTD of the Berkeley phage. Only those strains designated *B. abortus* or *B. suis* were sensitive to the Tbilisi phage.

Since it has been established that the Weybridge phage replicated on strain Isfahan, the remaining *B. melitensis* strains listed in Table 1 were examined for their susceptibility to the

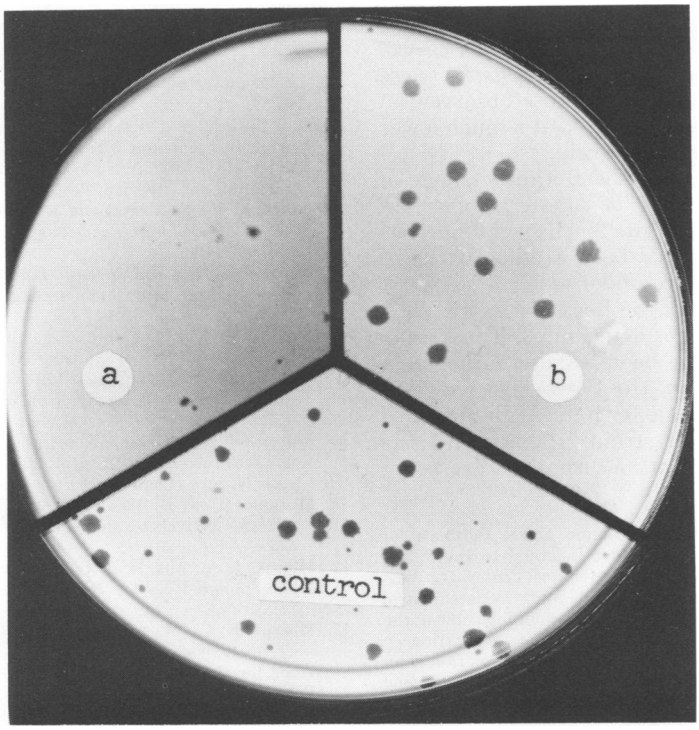

FIG. 1. *Composite photograph presents the comparison of plaque morphology of the Berkeley and Weybridge phages. (a) Weybridge phage on B. melitensis strain Isfahan; (b) Berkeley phage on B. melitensis strain Isfahan; (control) Weybridge phage on B. suis 1330, the propagation host for Weybridge phage. The photographs were taken of soft-agar plates after 48 h of incubation.*

TABLE 1. *Host range of the Berkeley and Tbilisi bacteriophages at their RTDs*[a]

Organism	Origin	Berkeley phage		Tbilisi phage	
		RTD[b]	RTD × 10^{-2}	RTD[c]	RTD × 10^{4}
Brucella melitensis					
Biotype 1					
16M (23456)[d]	U.S.A.	+[e]	±	−	−
Isfahan	Iran	+	±	−	−
65/87	−[f]	+	±	−	−
6840-1-75	U.S.A.	+	±	−	−
6015	Mexico	+	±	−	−
Biotype 2					
63/10	−[f]	+	±	−	−
Biotype 3					
Ether (23458)[d]	−[f]	+	±	−	−
65/155	Mongolia	+	±	−	−
65/59	India	+	±	−	−
B. abortus					
R-19	U.S.S.R.	+	±	+	+
B. suis					
1330 (23444)[d]	U.S.A.	+	±	−	+[g]

[a] RTD consists of a drop of phage suspension on a lawn of bacteria that contains the highest dilution of phage that results in confluent plaques.
[b] RTD established on strain Isfahan.
[c] RTD established on strain R-19.
[d] World Health Organization reference strains (ATCC no.).
[e] +, Confluent plaques; ±, individual plaques; −, no plaques.
[f] Received from World Health Organization Reference Laboratory, Weybridge, England; origin not noted.
[g] Inhibition, no plaques.

Weybridge phage. A few small plaques were seen on strains 63/10 and Ether; none was seen on the other strains of *B. melitensis*. These plaques were smaller than those observed on Isfahan (Fig. 1a) and appeared at a much lower frequency and at a slower rate.

The susceptibility to the Berkeley phage of strains from a variety of countries of origin (Table 1) may compensate for the small number of strains which have been studied to date. Additional strains are under test.

The relationship of the Berkeley to the Weybridge phage was not determined. The Berkeley phage may have been isolated either as a host range mutant of the Weybridge phage or as the product of some form of rescue initiated by superinfection of strain Isfahan with the Weybridge phage. Both alternatives are being investigated.

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