Lytic Activity of Vibrio Phages on Strains of *Vibrio fetus* Isolated from Man and Animals

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Five phages isolated from lysogenic strains of *Vibrio fetus* var. *venerealis* and two from *V. fetus* var. *intestinalis* were tested for lytic activity on 95 *V. fetus* strains from various animal and human hosts. In addition, virion and plaque morphology of the seven phages were compared. Electron micrographs showed that all were the kite-tailed variety with minor variations in head and tail dimensions. Plaques of V45 and V2 were small, clear and irregular; those of V3, V8, and V19 were large, clear and regular at the edge; the plaques of V16 and V20 were intermediate in size, clear, and very irregular at the edge with satellite plaques. The number of strains lysed by one or more phages were as follows: 29 of 30 from cattle; 7 of 11 from sheep; 1 of 5 from pigs; 1 of 1 from a monkey; and 33 of 42 from human hosts. Four natural groups of phages were derived by statistical measures of percentage of similarity in lytic activity. Group III lysed more strains (46 of 95) than any of the others. Twenty-five strains were lysed by group IV, 23 strains by group I, and 19 strains by group II. Results of this study indicate that phage typing should be a practical supplement to other differential tests for *V. fetus*.

*Vibrio fetus*, once known only as a cause of abortion in cattle and sheep, now is recognized as a pathogen that infects a wide variety of animals, birds, and man, producing diarrhea, septicemia, meningitis, arthritis, and numerous other conditions in addition to abortion (3, 4, 12). The recent isolation and characterization of bacteriophages for *V. fetus* supplements biochemical, serologic, and clinical criteria for identification and typing of *V. fetus* var. *venerealis* and *V. fetus* var. *intestinalis* (1, 5, 7).

The phages isolated to date have been derived from lysogenic cultures, and most have been obtained from the *venerealis* variety. However, the most useful propagating hosts for these phages have been strains of the *intestinalis* variety because of ease of cultivation and specificity of the phages (5). Phages utilized in the transmission of characteristics of streptomycin resistance and glycine tolerance by transduction also were derived from lysogenic *venerealis* strains with *intestinalis* strains serving as the propagating host (6).

This report presents the lytic spectra of seven phages isolated from *V. fetus* strains of bovine origin and compares the lytic activity of these phages for strains of *V. fetus* cultured from man and other animals.

**MATERIALS AND METHODS**

*Vibrio strains*. A total of 95 *Vibrio* strains that possessed most of the characteristics of *V. fetus* were employed in the present study. Thirty strains were from cattle, 11 from sheep, 5 each from pigs and birds, one each from a dog and a monkey, and 42 from humans. Soft agar lawns of the vibrio strains, as previously described (5), were used for testing the lytic activity of the phages.

**Phages**. Four phages (V2, V3, V8, and V45) were isolated from lysogenic *venerealis* strains of *V. fetus* by an enrichment method described earlier (5). Two phages [V16 (vfi-1) and V19 (vfi-6)] isolated from *intestinalis* strains were kindly supplied by B. D. Firehammer of Montana State University (7). A seventh phage (V20) was isolated by single plaque selection from a lawn of *V. fetus* strain 1138 infected with phage vfi-1. The propagating strain for *venerealis* phages was 1083-intestinalis; the propagating hosts for remaining 3 phages were: V16/1107-intestinalis, V19/1110-intestinalis, and V20/1138-intestinalis.

**Culture media**. Fluid thioglycolate medium (BBL,01-140) was used for maintenance of *V. fetus* stock cultures and for enrichment of phages in lyso-
genic strains as described (5). Broth for shaker cultures consisted of Albimi Brucella broth (Pfizer, 153A) with 0.1 M CaCl₂ added after autoclaving. The nutrient agar base and soft overlay agar used for phage tests were described earlier (5). Peptone broth for phage stock and dilution blanks contained, per liter of water: Na₂HPO₄, 1 g; KH₂PO₄, 2 g; NaCl, 3 g; MgSO₄, 0.5 g; CaCl₂, 0.5 g; and Albimi Peptone M, 10 g. The calcium chloride was added as a sterile 10% solution after autoclaving and cooling of the broth.

Selection of heat-resistant phages. Phage stocks were prepared in soft agar lawn cultures of the specific host. Appropriate dilutions of phage stock were used to obtain approximately 100 plaques per petri plate. A single clear plaque was picked and homogenized in 3 ml of peptone broth. Thereafter, the phages were heated in a water bath at 58°C for 1 h. The heated suspension was again plated as above, and the procedure was repeated four times, yielding phages that were resistant to heating at 60°C for 1 h.

Phage stock. The heat-resistant phages were mixed with propagating host cells in soft agar lawns at a concentration to produce almost complete lysis of the bacterial lawns. After incubation of cultures for 18 h at 37°C in microaerophilic atmosphere (5), the lysates were homogenized in 5 vol of peptone broth and centrifuged at 2,400 × g for 1 h, and the supernatant fluid was then filtered through a 0.45-μm membrane filter. The filtrates were preserved in 2-ml portions by freezing at −65°C and subsequently were used as phage stock in the following studies.

Titration of phage stock. A frozen sample of each phage stock was thawed, and a series of 10-fold dilutions was made in peptone broth. Stability of each phage stock was tested after dilution and storage at 5°C for 24 h and 1 week. Except for phage V2, the diluted stocks were stable for at least 1 week without loss of titer. In the case of V2, a fresh vial of stock was thawed, and used for only 1 day; the remaining six stocks were prepared fresh from the frozen state each week. The routine test dilution (RTD) was determined for each of the seven phages.

Phage tests on vibrio strains. In testing the lytic activity of phages on vibrio strains from various animal sources, three concentrations of phage were used; the RTD, 10× RTD, and 100× RTD. Each strain of vibrio was grown in an Albimi broth shaker culture. A 0.3-ml sample of the culture containing approximately 5 × 10⁷ log-phase vibrio cells was mixed with 3 ml of melted overlay agar at 49°C, poured onto Albimi agar base, and allowed to solidify for 15 min. One drop of each phage concentration was spotted onto the lawn surface by use of a capillary tube. The cultures were then incubated for 24 h at 37°C in desiccator jars in a microaerophilic atmosphere.

Phage grouping. A statistical analysis of the lytic spectra of the seven phages was performed to determine whether two or more of the phages were closely related to each other. The technique employed was a single-linkage algorithm as described by Sneath (10). The method is such that all members of a cluster possess a certain level of similarity with other members of the group.

The measure of similarity chosen for use here was the amount of agreement between any two phages with respect to their ability to lyse the 30 strains of bovine vibrios. The degree of lysis was given values from 0 to 4, where the maximum ability to lyse a given strain is 4. This factor is a natural extension of the matching coefficient proposed by Sokol and Michener (11), but incorporates data coded 0 to 4 rather than positive and negative, and the values are expressed as percentages (see Table 3).

RESULTS AND DISCUSSION

Phage characteristics. Considerable variation was noted in plaque morphology among the phages. Plaques of V45 and V2 were small (1–1.5 mm), clear, and irregular; the plaques of V3, V8, and V19 were large (3–4 mm), clear, and more uniformly regular at the edge; and plaques of V16 and V20 were intermediate in size (2–3 mm), clear, and very irregular at the edge, with satellite plaques (Fig. 1).

In electron micrographs, virions appeared similar to those described earlier (5, 7). All were of the kite-tailed variety with average measurements of the following: head diameter, 45 nm; tail diameter, 8 nm; and tail length, 197 nm (Fig. 2).

Although each of the seven phages was stable at 60°C for 1 h and the RTD titers did not diminish over periods of several weeks at 4°C, a large number of phage ghosts was noted in electron micrographs. This was probably due to slow release of phage from infected vibrios with concurrent adsorption of phage to cell fragments and expulsion of nucleic acid.

The lytic activity of each phage on its propagating host is shown within the blocked area of Table 1. The lytic spectra of phages on other strains used for propagation are also given. Phages V45 and V2 lysed only 1083; V3, V8, and V19 lysed three strains, 1083, 1107, and 1110; V16 lysed a different group of three strains, 1107, 1110, and 1138; whereas V20 lysed only strain 1138. Phage V3 was the most active and V2 was the least active in these tests. In most instances the RTD was a 10⁻³ dilution of thawed phage stock after deep-freeze preservation. An example of the lysis of a host lawn is V3 on host 1083 (Fig. 3).

Phage lysis of test strains. V. fetus strains lysed by one or more phages are presented in Table 2. The number lysed per species of origin was greater for cattle, i.e., 29 or 30 (96.7%), which was expected because the phages used in the study were derived from lysogenic V. fetus strains isolated from cattle. However, V. fetus strains cultured from humans were lysed at the high rate of 33 of 42 (78.6%), indicating a close
relationship between these strains and those from cattle. The ratio was only a little lower for *V. fetus* strains from sheep, i.e., 7 of 11 (63.7%). The number of strains tested from other animals was too small to be considered to be of significance. However, one strain from a pig and the strain from the monkey were lysed by several phages.

**Phage groups.** The seven phages appeared to fall into four natural groups based on their lytic activity on *V. fetus* strains from cattle, using a single linkage algorithm analysis. Statistical measures of similarity of the phages with numerical values expressed as a percentage of possible agreement are given in Table 3. A further analysis of phage lytic activity on 11

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**FIG. 1.** Morphology of phage plaques in soft agar lawns of *V. fetus*. *a*, Plaques of phage V3 in lawn of host strain 1083. *b*, Typical plaque of phage V16 in host strain 1107 showing satellite microplaques.

**FIG. 2.** Morphology of *V. fetus* phage virions. *a*, Two virions of phage V3 attached to a cell fragment; ×280,000. *b*, A virion of phage V16 with full head and receptor substance associated with tip of tail; ×360,000.
Table 1. Lytic spectra of vibrio phages on propagating strains

| Propagating strains | Phage dilution | V45 | V2 | V3 | V8 | V16 | V19 | V20 |
|---------------------|----------------|-----|----|----|----|-----|-----|-----|
| 1083                | $10^{-0}$      | CL  | CL | CL | CL | -   | CL  | -   |
|                     | $10^{-1}$      | CL  | CL | CL | CL | -   | CL  | -   |
|                     | $10^{-2}$      | ++  | CL | CL | CL | -   | CL  | -   |
|                     | $10^{-3}$      | +++ | +  | CL | +++| -   | +++ | -   |
|                     | $10^{-4}$      | ++  | -  | +++| ++ | -   | ++  | -   |
| 1107                | $10^{-0}$      | -   | -  | CL | CL | CL  | CL  | -   |
|                     | $10^{-1}$      | -   | -  | CL | CL | CL  | CL  | -   |
|                     | $10^{-2}$      | -   | -  | CL | +++| CL  | CL  | -   |
|                     | $10^{-3}$      | -   | -  | CL | ++ | +++ | +++ | -   |
|                     | $10^{-4}$      | -   | -  | +++| +  | +   | +++ | -   |
| 1110                | $10^{-0}$      | -   | -  | CL | CL | CL  | CL  | -   |
|                     | $10^{-1}$      | -   | -  | CL | CL | CL  | CL  | -   |
|                     | $10^{-2}$      | -   | -  | CL | +++| +++ | CL  | -   |
|                     | $10^{-3}$      | -   | -  | CL | ++ | ++  | +++ | -   |
|                     | $10^{-4}$      | -   | -  | +++| +  | +   | +++ | -   |
| 1138                | $10^{-0}$      | -   | -  | -  | -  | CL  | -   | CL  |
|                     | $10^{-1}$      | -   | -  | -  | -  | +++ | -   | CL  |
|                     | $10^{-2}$      | -   | -  | -  | -  | +++ | -   | CL  |
|                     | $10^{-3}$      | -   | -  | -  | -  | +   | -   | +++ |
|                     | $10^{-4}$      | -   | -  | -  | -  | -   | -   | -   |

*The values enclosed in blocks represent the lytic activity of the phage on its specific propagating host.

*CL, Confluent lysis; +++ = lysis with secondary growth; ++ = more than 50 plaques; + = 1 to 49 plaques; - = no lysis.

Fig. 3. Lysis of V. fetus host strain 1083 in soft agar lawn after spotting drops of phage suspension in 10-fold dilutions. The $10^{-3}$ dilution was taken as the RTD for this phage.
strains from sheep and 42 strains from humans was not significantly different. Phages V2 and V45 were designated group I; V3, V8, and V19 were group II; phage V16 represented group III; and V20, group IV.

Phage group III lysed a greater number of V. fetus strains than any other group, with a total of 46. Group IV lysed 25 strains and group I lysed 23 strains. Although group II phages were the most active in lytic ability on susceptible strains, they lysed only 19 of 95 strains tested. In all, 71 of 95 strains were lysed by one or more phages.

There appeared to be greater similarity between groups III and IV than among other groups when lytic activity and plaque morphology were considered. The difference in the lytic activity on vibrio strains could be due to "host-mediated variation" described by Bertani and Weigle (2) and Luria and Human (8). In all cases where V20 lysed a bovine strain, V16 also lysed this strain to the same or greater degree. Therefore, V16 would have selected V. fetus strains of this phage type. However, several vibrio strains of human origin were lysed by V20 but not by V16, which might justify using both phages in typing trials.

Phage lysis of serotypes. Table 4 gives the lytic spectra of the four phage groups on 18 strains representative of V. fetus serotypes A, B, and C, using the revised classification of Berg et al. (1). The serotype A strains were not lysed by groups I or II phages, but were all lysed by group III. Most of the serotype B strains were lysed by group II, although some were also lysed by the other groups.

The serotype C strains from sheep were not lysed by any of the phages tested, indicating that they were distinctly different than the other V. fetus strains. These strains are commonly isolated from intestines and gallbladder of animals and birds (5), whereas serotype A

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**Table 2. Number of V. fetus strains from various animal hosts lysed by Vibrio phages**

| Animal host | Strains tested | V45 | V2 | V3 | V8 | V16 | V19 | V20 | Total* |
|-------------|----------------|-----|----|----|----|-----|-----|-----|-------|
| Cattle      | 30             | 6   | 7  | 7  | 6  | 22  | 8   | 11  | 29/30 |
| Sheep       | 11             | 1   | 2  | 1  | 4  | 2   | 0   | 1/5 |
| Pigs        | 5              | 0   | 0  | 0  | 1  | 0   | 0   | 0/5 |
| Birds       | 5              | 0   | 0  | 0  | 0  | 0   | 0   | 0/5 |
| Dog         | 1              | 0   | 0  | 0  | 0  | 0   | 0   | 0/5 |
| Monkey      | 1              | 1   | 1  | 0  | 1  | 1   | 0   | 1/1 |
| Human       | 42             | 6   | 9  | 4  | 2  | 18  | 4   | 12  | 33/42 |
| Total       | 95             | 15  | 19 | 12 | 9  | 46  | 14  | 25  |

* Number of strains lysed/strains tested.
* Number of strains lysed by 10 x RTD phage concentration.

**Table 3. Similarity measures of the lytic activity of seven vibrio phages on V. fetus strains of bovine origin**

| Phages | V45 | V2  | V3  | V19 | V8  | V16 | V20 |
|--------|-----|-----|-----|-----|-----|-----|-----|
| V45    | 100 | 90  | 73  | 68  | 73  | 44  | 65  |
| V2     | 90  | 100 | 65  | 60  | 62  | 50  | 70  |
| V3     | 73  | 65  | 100 | 95  | 93  | 35  | 44  |
| V19    | 68  | 60  | 95  | 100 | 89  | 31  | 40  |
| V8     | 73  | 62  | 93  | 89  | 100 | 42  | 51  |
| V16    | 44  | 50  | 35  | 31  | 42  | 100 | 73  |
| V20    | 65  | 70  | 44  | 40  | 51  | 73  | 100 |

| Similarity groups | I | II | III | IV |
|-------------------|---|----|-----|----|

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strains from the fetus were lised only.
strains are found mostly in cattle, and serotype B strains infect cattle, sheep, and man. Phages capable of lysing serotype C strains should be included in the phage-typing scheme for *V. fetus*. Ogg and Chang (9) reported isolating a phage from a lysogenic strain of serotype C *V. fetus* that lysed other type C strains. With the rapid increase in the number of reported cases of human vibriosis, there is a need for additional phages to facilitate taxonomic and epidemiologic characterization of new vibrio isolates and to elucidate the importance of natural transmission of these organisms between animal species.

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### Table 4. Lysis of serotype A, B, and C strains of *V. fetus* by four groups of phages

| Strains | Lysis by phage groups |
|---------|----------------------|
|         | I | II | III | IV |
| Serotype A* |   |   |   |   |
| 661     |   |   | +  | +  |
| 779     |   |   | +  | -  |
| 993     |   |   | +  | -  |
| 998     |   |   | +  | -  |
| 1104    |   |   | +  | -  |
| 1105    |   |   | +  | -  |
| 1106    |   |   | +  | -  |
| Serotype B* |   |   |   |   |
| 436     | + | - | +  | +  |
| 1083    | + | + | -  | -  |
| 1107    | - | + | -  | -  |
| 1108    | - | + | -  | -  |
| 1109    | - | + | -  | -  |
| 1110    | - | + | -  | -  |
| 1121    | + | + | -  | -  |
| Serotype C* |   |   |   |   |
| 495     |   | - | -  | -  |
| 652     | - | - | -  | -  |
| 917     | - | - | -  | -  |
| 1114    | - | - | -  | -  |

* Classed as biotype 1, *venerealis*, from cattle (1).
* Classed as biotype 2, *intestinalis*, from cattle.
* Classed as biotype 2, *intestinalis*, from sheep.