APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1977, p. 506-511
Vol. 34, No. 5

Printed in U.S.A.

Bdellovibrio and the Intestinal Flora of Vertebrates

J. M. WESTERGAARD* AND T. T. KRAMER

Department of Microbiology, School of Veterinary Medicine, Auburn University, Auburn, Alabama 36830

Received for publication 29 April 1977

Bdellovibrio strain MS7 force-fed to fish and frogs via an intragastric tube did not become an integral component of the intestinal microflora. Strain MS7 fed to mice in drinking water was not recovered from the intestinal tract of mice. However, in vitro, the organism multiplied in intestinal contents of frogs and mice. *Bdellovibrio* inoculated into rabbit ileal loops was greatly reduced in number within 24 h. It was concluded that strain MS7 could be considered nonpathogenic to animals, at least when introduced into the digestive tract, and that it is not feasible at the present time to lyse pathogenic, gram-negative bacteria in the alimentary tract with *Bdellovibrio*.

The intestinal tract of most vertebrates contains high concentrations of gram-negative bacteria (4, 5, 7, 14), many of which are susceptible hosts for *Bdellovibrio* (16, 18). However, information on a possible association in the intestinal tract of animals between *Bdellovibrio* and susceptible host cells is scarce. Nakamura (10) indicated that *B. bacteriovorus* could reduce the population of *Shigella* in the intestine of rabbits when *S. flexneri* and *B. bacteriovorus* were inoculated simultaneously into ileal loops. In some of the loops, the number of *Shigella* was reduced substantially during a 24-h period, whereas other loops did not yield any *Shigella*. The purpose of this study was to examine the fate of *Bdellovibrio* cells administered per os to fish, frogs, and mice and of *Bdellovibrio* cells inoculated into rabbit ileal loops.

MATERIALS AND METHODS

Bacterial cultures. *Bdellovibrio* strain MS7, originally isolated from wastewater by the procedure of Stolp and Starr (16), was used throughout this investigation. It possesses morphological and physiological characteristics similar to those of *B. bacteriovorus* (J. M. Westergaard, Ph.D. dissertation, Auburn University, Auburn, Alabama, 1977). Its host range includes species of the genera: *Citrobacter, Edwardsiella, Enterobacter, Erwinia, Escherichia, Klebsiella, Proteus, Salmonella, Serratia, Shigella, Branhamella, Acinetobacter, Pseudomonas,* and *Aeromonas. E. coli* O15 suspended in a mineral salt solution (5) was routinely used for *Bdellovibrio* propagation and in lawns for enumerating *Bdellovibrio* by the double-layer agar technique (16). Human enteropathogenic *E. coli* cultures, strains 1298 and 1484, obtained from H. W. Moon (National Animal Disease Center, Ames, Iowa), were used to study *Bdellovibrio*-host interactions in rabbit ileal loops. *Aeromonas hydrophila,* provided by J. A. Plumb (Department of Fisheries and Allied Aquacultures, Auburn University), was used for in vitro studies on *Bdellovibrio* growth in intestinal contents of frogs.

Media. Peptone yeast extract, consisting of 1% peptone (Difco Laboratories, Detroit, Mich.) and 0.3% yeast extract (Difco), was used for propagation of *E. coli* O15. Trypticase soy broth (BBL, Cockeysville, Md.) was employed to propagate human enteropathogenic *E. coli* cultures. Plate count agar and M-endo broth, employed for enumeration of total bacterial counts and total coliform counts, respectively, were prepared as outlined in *Standard Methods* (17). Dilute-nutrient-broth agar described by Seidler and Starr (12) was applied in the double-layer technique for *Bdellovibrio* cultivation.

***Bdellovibrio*-intestinal flora interaction in vivo.** *Bdellovibrio*-intestinal flora interaction in channel catfish (*Ictalurus punctatus*) was studied in two experiments. Fish were 10 to 15 cm long and were kept in a 15-gallon (ca. 56.9-liter) aquarium. Water was aerated and kept within 15 to 24°C. A *Bdellovibrio* suspension was administered into the esophagus and/or stomach of 12 fish by a thin plastic tube. Each fish received about 1 ml of *Bdellovibrio* suspension, which in the first experiment contained 2.5×10^8 cells per ml and in the second experiment contained 8.8×10^8 cells per ml. The bacterial concentration was determined by dark-field microscopy, using a Petroff-Hausser counting chamber. *Bdellovibrio* were not administered to a contact control group of eight fish. The control group was kept in the same aquarium as the fish force-fed with *Bdellovibrio*.

Frogs (*Rana pipiens*) were kept in stainless-steel trays constructed to provide a dry area and an area with a constant supply of fresh tap water. Five trays with six frogs each were used. The frogs were kept at room temperature (20 to 24°C). After 24 h in the trays, each of 27 frogs received an intragastric inoculation of a 1-ml *Bdellovibrio* suspension (2.2×10^8 cells per ml) via a thin stomach tube. Three frogs were not force-fed with *Bdellovibrio*.

The *Bdellovibrio*-intestinal flora interaction in mice was studied in two identical experiments by examining intestinal contents from mice provided with *Bdello-*

vibrio-inoculated drinking water. Sixteen mice, which were of either sex and 4 to 6 weeks old, were used in each study. All mice, apart from four control mice, were provided with drinking water containing *Bdellovibrio* during a 3-day period. Two mice were sacrificed daily during these 3 days and on each of the following 3 days. The water supply was renewed daily, and the amount of water removed from the bottles was recorded each day. The *Bdellovibrio* concentration in the drinking water was determined immediately before water was offered to the mice and when it was removed.

The intestinal contents of fish, frogs, and mice fed *Bdellovibrio* were examined quantitatively for the organisms at selected time intervals. The intestinal tract was removed in toto from sacrificed animals, and the contents were squeezed into 10 ml of mineral salt solution. The suspended material was stirred with a magnetic stirrer until the contents were evenly dispersed. Three milliliters of the suspension was filtered through a 1.2-μm-pore-size filter, and another 3 ml was filtered through a 0.45-μm-pore-size filter. Filtrates were examined for *Bdellovibrio* by the double-layer technique.

Water samples from the aquarium with channel catfish were examined for total bacterial counts, total coliform counts, and *Bdellovibrio* counts. Samples were collected (i) before fish were placed in the aquarium, (ii) after fish were placed in the aquarium but before being given *Bdellovibrio*, and (iii) after *Bdellovibrio* were given to fish. Total bacterial counts were determined by a micro technique. Six replicate 0.025-ml portions of each sample were placed on a plate containing plate count agar with an Eppendorf pipette. Cultures were incubated inverted at 35°C for 20 ± 2 h. Counts were made on plates that contained 10 to 60 colonies within each area. Coliform counts were determined by the membrane filter technique and with M-endo broth as described in *Standard Methods* (17).

Ileal loops were prepared in six New Zealand albino rabbits as described by Moon and Whipp (9). Replicate loops in each rabbit were inoculated separately with (i) an axenic culture of *Bdellovibrio*, (ii) an axenic culture of *E. coli* 1298 or *E. coli* 1484, (iii) a 24-h *Bdellovibrio-E. coli* culture, and (iv) a 10- to 20-min *Bdellovibrio-E. coli* culture. The volumes of the cultures injected into loops in two rabbits were 0.3 ml, and those in four rabbits were 1 ml. The cell concentrations of *Bdellovibrio* cultures were adjusted to 10^7/ml. The *E. coli* cultures had cell concentrations of 10^9/ml. The *Bdellovibrio-E. coli* cultures consisted of 50% (volume) *Bdellovibrio* culture and 50% *E. coli* culture.

After surgery, rabbits were kept in separate cages at room temperature. The abdominal cavity was reopened immediately upon death of the rabbits, the fluid accumulated in distended loops was aspirated with a syringe, and the volume was recorded. Contents of loops inoculated with *Bdellovibrio* cultures were collected and examined for *Bdellovibrio* survival by the double-layer technique, using *E. coli* as host cells.

***Bdellovibrio*-intestinal flora interaction in vitro.** Intestinal contents were used from frogs and mice. The interaction of the intestinal contents of bullfrogs (*R. catesbeiana*) and *Bdellovibrio* was investigated in two identical studies, with pooled intestinal contents from three frogs in each study. Intestinal contents of frogs were added to 15 ml of mineral salt solution and stirred with a magnetic stirrer until the contents were evenly dispersed. The suspension was filtered through a 5.0-μm-pore-size filter and tested for its ability to support *Bdellovibrio* growth by the double-layer technique. The filtrate was used in top layers as a replacement for (i) an axenic host cell culture and (ii) a diluent for the *Bdellovibrio* culture inoculated into top layers. *Aeromonas hydrophila* cells were used in host lawns for *Bdellovibrio* in control top layers.

Growth of *Bdellovibrio* in the intestinal contents of mice and in extracts of homogenized intestinal tissue mixed with the intestinal contents was examined by comparing the number of *Bdellovibrio* plaques in six different top layers, A through F, composed as shown in Table 1. Intestinal contents included in top layers A, B, and D were pooled from two mice, thoroughly mixed with 10 ml of mineral salt solution, and filtered through a 5.0-μm-pore-size filter before used. The intestinal components included in top layers E and F originated from the intestinal tracts of two mice. The intestinal tracts were homogenized by a macro homogenizer, Virtis model 45 (Virtis Research Equipment, Gardiner, N.Y.), and mixed with 20 ml of mineral salt solution, before the suspension was filtered through a

TABLE 1. *Composition of top layers used to study the Bdellovibrio-intestinal flora interaction in cultures, which include 5.0-μm-membrane filtrates from suspended intestinal contents from mice*[a]

Top layers	Composition of top layers
A	Host cells: *E. coli* suspension (1 ml) *Bdv.* source: Intestinal filtrate (1 ml)
B	Host cells: Intestinal filtrate (1 ml) *Bdv.* source: *Bdv.* strain MS7 (1 ml) Diluent for *Bdv.* source: MSS
C	Host cells: *E. coli* suspension (1 ml) *Bdv.* source: *Bdv.* strain MS7 (1 ml) Diluent for *Bdv.* source: MSS
D	Host cells: *E. coli* suspension (1 ml) *Bdv.* source: *Bdv.* strain MS7 (1 ml) Diluent for *Bdv.* source: Intestinal filtrate
E	Host cells: Filtrate, homogenized intestinal tracts (1 ml) *Bdv.* source: *Bdv.* strain MS7 (1 ml) Diluent for *Bdv.* source: MSS
F	Host cells: *E. coli* suspension (1 ml) *Bdv.* source: *Bdv.* strain MS7 (1 ml) Diluent for *Bdv.* source: Filtrate, homogenized intestinal tracts

[a] All top layers contained 4 ml of dilute-nutrient-broth agar. *Bdv.*, *Bdellovibrio*; MSS, mineral salt solution.

5.0-μm-pore-size filter. The filtrate was included in top layers as given in Table 1.

RESULTS

Bdellovibrio were undetectable in the intestinal contents of fish after 24 h in the first experiment (Fig. 1) but were isolated in small numbers from fish on day 6 in the second experiment (Fig. 2). The numbers of *Bdellovibrio* isolated from fish sacrificed 10 to 15 min after the fish had been given *Bdellovibrio* are given in Table 2. The data indicate that the loss of *Bdellovibrio* by (i) the force-feeding technique, (ii) the 10- to 15-min stay of *Bdellovibrio* in the digestive tract of fish, and (iii) the isolation procedure for *Bdellovibrio* amounted to about 99% in both experiments. *Bdellovibrio* counts given in Table 2 originated from 1.2-μm-membrane filtrate. The recovery of *Bdellovibrio* from

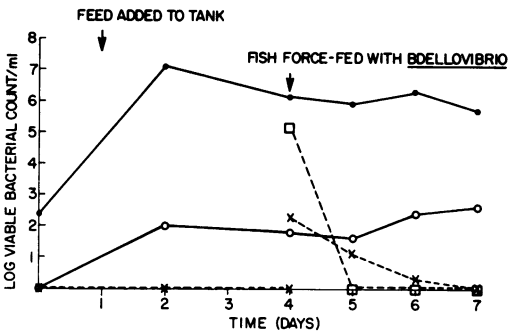

FIG. 1. *Bdellovibrio counts from channel catfish force-fed with Bdellovibrio suspensions, and total bacterial counts, total coliform counts, and Bdellovibrio counts of sampled tank water. Experiment 1, Total bacterial counts (●), total coliform counts (○), Bdellovibrio counts of water (×), Bdellovibrio recovered from fish force-fed with Bdellovibrio (□).*

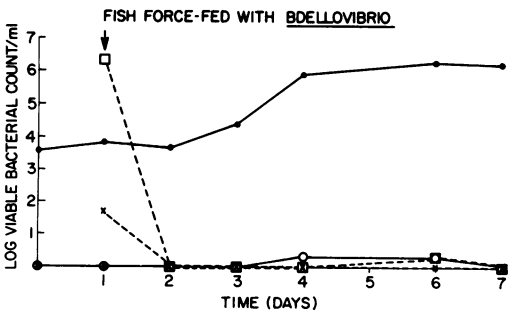

FIG. 2. *Bdellovibrio counts from channel catfish force-fed with Bdellovibrio suspensions, and total bacterial counts, total coliform counts, and Bdellovibrio counts of sampled tank water. Experiment 2, Total bacterial counts (●); total coliform counts (○); Bdellovibrio counts of water (×), Bdellovibrio recovered from fish force-fed with Bdellovibrio (□).*

TABLE 2. *Recovery of Bdellovibrio from channel catfish 10 to 15 min after the fish had been force-fed with Bdellovibrio*

Expt	*Bdellovibrio* inoculum (total cells/fish, cells/ml)	*Bdellovibrio* isolated from force-fed fish		
		Fish no.	Cells/ml of intestinal contents	Mean counts (cells/ml)
I	2.5×10^8	1	3.2×10^5	1.6×10^5
		2	7.5×10^2	
II	8.8×10^8	1	3.8×10^6	2.3×10^6
		2	8.7×10^5	

the 0.45-μm-membrane filtrate was slightly lower. *Bdellovibrio* were not isolated from contact control fish in which the principals were given 2.5×10^8 *Bdellovibrio*. However, two contact fish examined 6 days after the administration of 8.8×10^8 *Bdellovibrio* to principals yielded one and two *Bdellovibrio* plaques on *E. coli* lawns per fish. *Bdellovibrio* were unable to multiply in the aquarium water, and the organism was undetectable in the water a few days after *Bdellovibrio* had been administered to the fish (Fig. 1 and 2). The number of *Bdellovibrio* isolated from the aquarium water represented, at any time of the study, less than 1% of the *Bdellovibrio* fed to fish.

Recovery of *Bdellovibrio* from frogs force-fed with *Bdellovibrio* is shown in Fig. 3. *Bdellovibrio* were isolated 10 to 15 min after *Bdellovibrio* were administered. Mean counts of *Bdellovibrio* isolated from the 1.2- and the 0.45-μm-pore-size filtrates of the intestinal contents (Fig. 3) showed a very low survival of *Bdellovibrio* within the first 48 h of the study. The data presented in Fig. 3 show, furthermore, that a higher number of *Bdellovibrio* could be recovered from the 1.2-μm-membrane filtrate than from the 0.45-μm-membrane filtrate. *Bdellovibrio* were not isolated from frogs in the control group.

Bdellovibrio were not isolated from any of the mice offered drinking water containing *Bdellovibrio* nor from the four mice in the control group. The concentrations of *Bdellovibrio* in the drinking water provided for the mice varied from 2.0×10^6 to 1.6×10^7 cells per ml, and the concentrations were reduced by 1 log in the water during the initial 24 h.

Very few of the *Bdellovibrio* inoculated into rabbit ileal loops survived (Table 3). They were recovered from 5 out of the 12 loops inoculated with a culture consisting of *Bdellovibrio* and enteropathogenic *E. coli* cells. The axenic cultures of *Bdellovibrio* inoculated into the loops contained 10^7 cells per ml, but *Bdellovibrio*

could only be recovered from the 22- to 28-h-old loops at concentrations below 10^3 cells per ml.

The in vitro study of *Bdellovibrio* growth in intestinal contents from frogs showed (Table 4) that a 5.0-μm-membrane filtrate of the contents contained a sufficient number of host cells to support growth. Top layer (A), including the filtrate in place of an axenic culture of host cells, however, produced a lower number of plaques than the control culture (B). Top-layer (C), which included *A. hydrophila* as the host cells and the 5.0-μm-membrane filtrate as a diluent for the *Bdellovibrio* culture, produced a slightly higher number of plaques than top layer (A) but a slightly lower number of plaques than the control top layer (B).

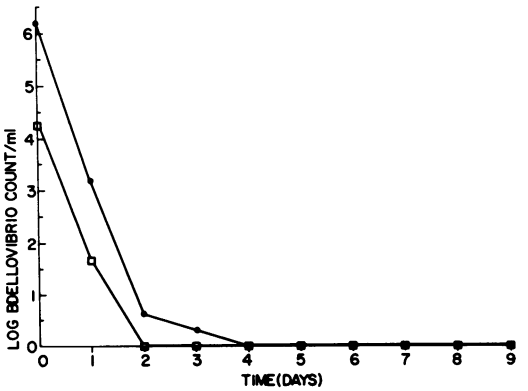

FIG. 3. *Bdellovibrio isolated from suspended intestinal contents collected from leopard frogs force-fed with Bdellovibrio suspensions. Symbols: ●, Bdellovibrio isolated from 1.2-μm-membrane filtrate, □, Bdellovibrio isolated from 0.45-μm-membrane filtrate.*

The number of plaques formed by *Bdellovibrio* in top layers containing filtrates of intestinal contents or filtrates of homogenized intestinal tracts from mice are shown in Table 5. The data indicate that a replacement of an axenic culture of host cells with any of the two filtrates resulted in a 2- to 4-log reduction in the numbers of plaques. The use of the filtrates in place of mineral salt solution as the diluent for *Bdellovibrio* cultures resulted in a 1-log or more reduction of plaques formed in three of the six top layers.

DISCUSSION

It is well known that certain bacteria may only appear as transient residents of the intestinal microflora of animals, although the intestinal tract supposedly provides adequate conditions for growth and multiplication of these organisms. Sears et al. (11) studied the fate of *E. coli* strains deliberately swallowed in large numbers by human subjects. It was found that the ingested *E. coli* strains were sometimes undetectable in the feces and that, when they were isolated, it was on an intermittent basis. None of the ingested *E. coli* strains became residents in the intestinal tract, although the environment was suitable for growth of *E. coli* and contained resident *E. coli* strains.

The reason(s) for the inability of *Bdellovibrio* to become a normal component of the microflora of fish is unknown. The short survival period in

TABLE 3. *Concentrations of Bdellovibrio suspensions inoculated into loops and the numbers recovered from the same loops at the time of death of rabbits*[a]

Rabbit no.	Time (h) difference between inoculation and recovery of *Bdellovibrio*	*Bdv.* inoculated into and recovered from loops			
		Bdv.	Axenic *Bdv.* culture	10-min *Bdv.-E. coli* culture	24-h *Bdv.-E. coli* culture
1	28	I	2.2×10^7	1.9×10^6	1.1×10^8
		R	9.0×10^0	None	None
2	26	I	2.2×10^7	1.9×10^6	1.1×10^8
		R	8.1×10^1	None	2.0×10^0
3	1	I	4.1×10^7	2.9×10^6	8.6×10^8
		R	1.4×10^3	9.1×10^2	1.2×10^3
4	22	I	4.1×10^7	1.4×10^6	1.0×10^8
		R	2.8×10^2	2.1×10^2	None
5	25	I	1.5×10^7	2.1×10^6	8.0×10^7
		R	5.7×10^2	None	None
6	25	I	1.5×10^7	1.2×10^6	3.4×10^7
		R	8.0×10^2	None	1.4×10^1

[a] *Bdv., Bdellovibrio*; I, inoculated; R, recovered.

TABLE 4. *Growth of Bdellovibrio in top layers with and without 5.0-μm-pore-size filtrates of intestinal contents from frogs*[a]

Top layer	Composition of top layer	*Bdv.* counts/ml		
		Expt 1	Expt 2	Mean count ± 1 SD
A	Host cells: intestinal filtrate (1 ml) *Bdv.* source: *Bdv.* strain MS7 (1 ml) Dil. for *Bdv.*: MSS	6.7×10^4	5.7×10^7	$2.9 \times 10^7 \pm 4.0 \times 10^7$
B (control)	Host cells: *A. hydrophila* (1 ml) *Bdv.* source: *Bdv.* strain MS7 (1 ml) Dil. for *Bdv.*: MSS	2.3×10^8	5.0×10^8	$3.7 \times 10^8 \pm 1.9 \times 10^8$
C	Host cells: *A. hydrophila* (1 ml) *Bdv.* source: *Bdv.* strain MS7 (1 ml) Dil. for *Bdv.*: intestinal filtrate	6.9×10^6	8.1×10^7	$4.4 \times 10^7 \pm 5.2 \times 10^7$

[a] All top layers contained 4 ml of dilute-nutrient-broth agar. *Bdv.*, *Bdellovibrio*; MSS, mineral salt solution; Dil., diluent; SD, standard deviation.

TABLE 5. *Bdellovibrio counts in top layers with and without components from intestinal tracts from mice*[a]

Top layer	Mouse component in top layer	*Bdv.* counts		
		Expt 1	Expt 2	Expt 3
A	Filtrate of intestinal contents included as *Bdv.* source	0	0	0
B	Filtrate of intestinal contents included to replace host cells	7.0×10^4	1.0×10^4	1.1×10^1
C (control)	None	5.7×10^8	1.3×10^7	1.2×10^4
D	Filtrate of intestinal contents used as diluent for *Bdv.* culture	3.4×10^8	3.5×10^6	6.7×10^3
E	Filtrate of homogenized intestinal tract included to replace host cells	1.6×10^5	8.0×10^5	2.1×10^1
F	Filtrate of homogenized intestinal tract used as diluent for *Bdv.* culture	3.7×10^8	1.4×10^7	5.0×10^2

[a] *Bdv.*, *Bdellovibrio*.

the intestinal tract of *Bdellovibrio* force-fed to fish corresponds to observations made by Glantz and Krantz (6), who reported that brown trout receiving *E. coli*-dosed food and water retained the organism in the gut for 1 to 14 days. Geldreich and Clarke (5) reported that tracer coliforms fed to bluegills and carp could only be recovered consistently from intestinal contents when the water environment of the fish contained high levels of the tracer organism.

Bdellovibrio were unable to establish themselves as a component of the intestinal microflora of frogs and mice, although suspended intestinal contents from both animals (Tables 4 and 5) supported *Bdellovibrio* growth when contents were included in top layers. The factors that prevented *Bdellovibrio* from multiplying in intestinal contents in vivo, a system far more complex than the in vitro system, are unknown at the present time. The demand for "a good supply of oxygen" for *Bdellovibrio* growth has been reported by Stolp and Starr (16). Burger et al. (1) observed in their work on the oxygen requirements of *B. bacteriovorus* W that the life cycle of the organism could be completed under aerobic or semiaerobic conditions. Their observations showed the required minimum oxygen partial pressure for growth of strain W to be 3 to 5 mm of Hg, a pressure under which many anaerobic species grow (3). Failure of in vivo growth of *Bdellovibrio* may be due to the lack of oxygen, since gas present in the intestinal tracts of animals is unevenly distributed, and the oxygen content is relatively low (2). The reduction in plaque number recorded for top layers when the intestinal contents replaced axenic *A. hydrophila* or axenic *E. coli* cultures may be a result of a low concentration of suscep-

tible host bacteria.

The recovery of small numbers or no *Bdellovibrio* from rabbit ileal loops inoculated with *Bdellovibrio* cultures suggests that *Bdellovibrio* did not multiply within the loops. The small recovery (Table 3) seems to be in agreement with results obtained from the force-feeding experiments of fish, frogs, and mice. Nakamura (10), working with *B. bacteriovorus* and *S. flexneri*, obtained somewhat different results. He found that the injection of *S. flexneri* into 16 rabbit ileal loops resulted in accumulation of fluid in all 16; simultaneous injection of *S. flexneri* and *B. bacteriovorus* into 16 loops, however, caused accumulation of fluid in only 1 loop. The latter result was assumed to be caused by a reduction of *Shigella* cells due to destruction by *Bdellovibrio*.

Results obtained from force-feeding experiments and from ileal loops inoculated with *Bdellovibrio* suggest that *Bdellovibrio* are probably not pathogenic to animals, at least when introduced into the digestive tract. The results also suggest that, since *Bdellovibrio* failed to multiply in host cells in the digestive tract, they cannot inhibit the growth of pathogenic, gram-negative enteric bacteria in the intestinal tract. This contrasts with the observations of Nakamura (10). The apparent lack of pathogenicity observed for *Bdellovibrio* concurs with observations made by Simpson (13), as cited by Starr and Huang (15) and by Verklova (19). Simpson (13) reported on the inability of *Bdellovibrio* to infect a variety of tissue cultures of mammalian cells, and Verklova (19) stated that *Bdellovibrio* showed no signs of pathogenicity when injected into mice, rabbits, and guinea pigs.

ACKNOWLEDGMENTS

This work was supported by a grant from the U.S. Department of Interior, Office of Water Research and Technology through the Water Resources Research Institute (B-061-ALA).

LITERATURE CITED

1. **Burger, A., G. Drews, and R. Ladwig.** 1968. Wirkskreis and Infektioncyklus eines neu isolierten *Bdellovibrio bacteriovorus* stammes. Arch. Microbiol. **61**:261-279.
2. **Code, D. F., and W. Heidel (ed.).** 1968. Handbook of physiology, vol. 5, section G: alimentary canal. American Physiological Society, Washington, D.C.
3. **Finegold, S. M.** 1977. Anaerobic bacteria in human disease. Academic Press Inc., New York.
4. **Geldreich, E. E.** 1966. Sanitary significance of fecal coliforms in the environment. U.S. Department of the Interior, Federal Water Pollution Control Administration, Cincinnati.
5. **Geldreich, E. E., and N. A. Clarke.** 1966. Bacterial pollution indicators in the intestinal tract of freshwater fish. Appl. Microbiol. **14**:429-437.
6. **Glantz, P. J., and G. E. Krantz.** 1965. *Escherichia coli* serotypes isolated from fish and their environment. Health Lab. Sci. **2**:54-63.
7. **Kaura, Y. K., V. K. Sharma, I. P. Singy, R. Sakazaki, and R. Rohde.** 1972. Snakes as reservoirs of *Arizona* and *Salmonella*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **219**:506-513.
8. **Matin, A., and S. C. Rittenberg.** 1972. Kinetics of deoxyribonucleic acid destruction and synthesis during growth of *Bdellovibrio bacteriovorus* strain 109D on *Pseudomonas putida* and *Escherichia coli*. J. Bacteriol. **111**:664-673.
9. **Moon, H. W., and S. C. Whipp.** 1971. Systems for testing the enteropathogenicity of *Escherichia coli*. Ann. N.Y. Acad. Sci. **176**:197-211.
10. **Nakamura, M.** 1972. Alteration of *Shigella* pathogenicity by other bacteria. Am. J. Clin. Nutr. **25**:1441-1451.
11. **Sears, H. J., I. Brownlee, and J. K. Uchiyama.** 1950. Persistence of individual strains of *Escherichia coli* in the intestinal tract of man. J. Bacteriol. **59**:293-301.
12. **Seidler, R. J., and M. P. Starr.** 1969. Factors affecting the intracellular parasitic growth of *Bdellovibrio bacteriovorus* developing within *Escherichia coli*. J. Bacteriol. **97**:912-23.
13. **Simpson, F. J.** 1972. Cited by M. P. Starr, and J. C. C. Huang. 1972. Physiology of the bdellovibrios. Adv. Microbiol. Physiol. **8**:215-261.
14. **Smith, H. W., and W. E. Crabb.** 1961. The faecal bacterial flora of animals and man: its development in the young. J. Pathol. Bacteriol. **82**:53-66.
15. **Starr, M. P., and J. C. C. Huang.** 1972. Physiology of the bdellovibrios. Adv. Microb. Physiol. **8**:215-261.
16. **Stolp, H., and M. P. Starr.** 1963. *Bdellovibrio bacteriovorus* gen. et sp. n., a predatory, ectoparasitic, and bacteriolytic microorganism. Antonie van Leeuwenhoek J. Microbiol. Serol. **29**:217-248.
17. **Taras, M. J., A. E. Greenberg, R. D. Hoak, and M. C. Rand (ed.).** 1971. Standard methods for the examination of water and wastewater, 13th ed. American Public Health Association, Washington, D.C.
18. **Uematsu, T., and S. Wakimoto.** 1970. Biological and ecological studies on *Bdellovibrio*. 1. Isolation, morphology, and parasitism of *Bdellovibrio*. Ann. Phytopathol. Soc. Jpn. **36**:48-55.
19. **Verklova, Z. S.** 1973. A Study of virulence, toxicity and immunogenicity of various strains of *Bdellovibrio bacteriovorus*. (In Russian.) Gig. Sanit. **38**:10-13.