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Characterization of Bdellocysts of *Bdellovibrio* sp.

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Bdellovibrio sp. strain W will infect and produce resting cells, termed bdellocysts, in a variety of gram-negative bacteria. Bdellocysts appeared to be produced only within susceptible prey and never in their absence. Optimum conditions for encystment included infection of stationary-phase prey cells in 0.05 M potassium phosphate buffer (pH 7.5) at concentrations of prey and bdellovibrios of 2×10^9 cells per ml with a multiplicity of infection of unity. Bdellocysts contained more deoxyribonucleic acid, ribonucleic acid, protein, and carbohydrate per cell than did vegetative cells. Poly-β-hydroxybutyrate and dipicolinic acid were not detected. Bdellocysts were more resistant than vegetative cells to effects of elevated temperatures, sonic treatment, and desiccation. Bdellocysts remained viable for extended periods when incubated in the absence of prey, whereas vegetative cells lost viability rapidly under the same conditions. Their survival under starvation conditions may be due to the low rate of endogenous respiration by the bdellocysts. Bdellocysts are capable of germination in the presence or absence of prey cells in rich medium such as peptone-yeast extract.

The genus *Bdellovibrio* is comprised of unique bacteria that are capable of infection and growth in the periplasmic space of a variety of gram-negative bacteria. The life cycle of predacious bdellovibrios has been studied extensively (18, 20, 21). One strain of *Bdellovibrio*, isolated and described by Burger et al. (4) and designated strain W, differed dramatically from other known *Bdellovibrio* strains by the occasional production of resting stages exclusively in the prey bacterium *Rhodospirillum rubrum*. Ultrastructural investigations of resting stages of *Bdellovibrio* sp. strain W (4, 7) revealed that they were structurally more simple than the resting forms of other bacteria. The resting cell was approximately twice as wide as the vegetative cell and was surrounded by an outer layer of amorphous electron-dense material.

These resting forms have been referred to as "encysted resting stages" (4) and "resting bodies" (7). The production of resting stages by *Bdellovibrio* is unique since strain W is an obligate predator and the encysted forms must develop within their prey. We have therefore designated these unique resting bodies "bdellocysts," based on their cystlike morphology, resistance properties, and reduced respiration. The biological properties and germination of *Bdellovibrio* sp. strain W bdellocysts have not been reported. The work described in the present paper was undertaken to characterize bdellocysts in an effort to help elucidate their role in the life cycle of the bdellovibrios.

MATERIALS AND METHODS

Organisms and culture conditions. *Bdellovibrio* sp. strain W was obtained from G. Drews, University of Freiburg, Freiburg, Germany. Prey organisms routinely used throughout this study were *Escherichia coli* B (obtained from D. Abram, University of Pittsburgh, Pittsburgh, Pa., as ATCC 15144) and *R. rubrum* Hughes (obtained from G. Sojka, Indiana University, Bloomington, Ind.). Other bacteria used in the prey range study were: *Pseudomonas fluorescens* ATCC 13525, *Erwinia amylovora* ATCC 15580, *Spirillum serpens* ATCC 12638, *Lactobacillus plantarum* ATCC 14917, and *Streptococcus faecalis* ATCC 19433 obtained from the American Type Culture Collection, Bethesda, Md.; *E. coli* K-12 P678-54 obtained from H. I. Adler, Oak Ridge, Tenn.; and laboratory strains of *Alcaligenes faecalis*, *Enterobacter aerogenes*, and *Serratia marcescens*.

R. rubrum was cultured in tryptic soy broth (Difco), *S. faecalis* was cultured in brain heart infusion broth (Difco), and *L. plantarum* was cultured in Lactobacillus MRS broth (Difco). All other prey were grown in peptone-yeast extract (PYE) broth (1). Stock cultures were maintained on agar plates, and transfers to liquid media were from single colonies.

To obtain vegetative cells of *Bdellovibrio* sp. strain W, prey cells were harvested by centrifugation in the late exponential phase of growth, washed once, and suspended in dilute nutrient broth (DNB) supplemented with 0.002 M $CaCl_2$ and 0.003 M $MgCl_2$ (17). This prey suspension was then infected with a 10% (vol/vol) inoculum of a 12-h bdellovibrio lysate and incubated at 30°C in a shaker incubator for 12 to 18 h; lysis of the prey cells was complete at this time. Stock cultures of bdellovibrios were maintained as lysates on *E. coli* B and *R. rubrum* at 4°C.

Prey cells used for the production of bdellocysts were grown to stationary phase before harvesting. Optimal conditions for encystment were determined by varying the cultural conditions to obtain maximum production of bdellocysts. The media in which bdellocyst production were tested included a modified Drews R8AH broth (7), yeast nitrogen base (Difco), PYE, DNB, Burk nitrogen-free buffer (23), 5 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (Nutritional Biochemicals Corp.), 0.05 M potassium phosphate buffer, and 5 mM *N*-tris(hydroxymethyl)-methyl glycine (Tricine) buffer (Sigma). All media were adjusted to pH 7.5 for these experiments. Direct microscopic counts of prey cells, bdellovibrios, and bdellocysts were made with a Petroff-Hauser bacterial counting chamber mounted in a Zeiss Universal microscope equipped with phase-contrast optics.

Purification of bdellocysts. Cultures containing bdellocysts were practically devoid of free prey cells; prey had either been lysed or contained bdellocysts. However, there were many vegetative bdellovibrios present, which were lysed by adding Triton X-100 (Fisher Scientific Co.) to a final concentration of 0.02% (wt/vol) to the culture and incubating for 30 min at room temperature (22 to 25°C). There was no apparent effect of the Triton X-100 on the bdellocysts. The bdellocysts were harvested by centrifugation at 5,000 × *g* for 15 min, washed five times, and suspended in sterile distilled water. The purified bdellocysts were stored at 4°C until needed. Bdellocysts used in these experiments were not stored for longer than 7 days.

Prey range. To determine if prey bacteria would support vegetative growth of *Bdellovibrio* sp. strain W, liquid cultures were prepared in DNB as described above and examined microscopically for lysis of the prey and growth of the bdellovibrios. Vegetative growth was also determined by plaque formation on lawns of prey cells in PYE agar prepared by the double-layer agar overlay method (22). Bdellocyst production in the various prey was determined by direct microscopic examination of liquid cultures.

Chemical analyses. *Bdellovibrio* vegetative cells that were used for chemical analyses were grown for 12 to 18 h on *E. coli* B in DNB, harvested by centrifugation at 16,000 × *g*, and washed three times in distilled water. Bdellocysts were purified and washed as described above.

Samples containing 5 × 10^{10} vegetative cells or bdellocysts were extracted in 5 ml of cold 10% (wt/vol) trichloroacetic acid for 30 min. The suspensions were centrifuged at 5,000 × *g* for 10 min, and the pellets were suspended in 5 ml of hot 5% trichloroacetic acid and hydrolyzed at 100°C for 30 min. The hydrolysate was centrifuged at 5,000 × *g* for 10 min, and the supernatant fluid was used for determination of deoxyribonucleic acid (DNA) by the Burton modification of the diphenylamine reaction (5). A 1:10 dilution of the supernatant fraction was used to determine ribonucleic acid (RNA) by the orcinol reaction (2). The residue was digested in 2 ml of 1 N NaOH for 30 min, and the protein was determined on a 1:10 dilution by the Lowry method (12). Calf thymus DNA (Sigma type V), yeast RNA (Sigma type XI), and crystalline bovine serum albumin (Sigma) were used as standards for DNA, RNA, and protein, respectively.

Poly-β-hydroxybutyrate was determined by the method of Law and Slepecky (10), dipicolinic acid was determined by the method of Jannsen et al. (8), and total carbohydrate was determined by the phenol-sulfuric acid method (3) with glucose as the standard; these analyses were conducted on samples containing 1 × 10^{11} vegetative cells or bdellocysts.

Oxygen uptake. *Bdellovibrio* vegetative cells used for oxygen uptake experiments were grown on *E. coli* B in DNB for 12 to 18 h, harvested and washed twice by centrifugation at 12,000 × *g*, and suspended in 0.05 M potassium phosphate buffer, pH 7.5. Purified bdellocysts were also suspended in 0.05 M phosphate buffer. Oxygen uptake was determined on 3-ml samples containing 5 × 10^9 cells per ml with a YSI model 53 oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio). After the endogenous rate was determined, sodium succinate or sodium glutamate was added to a final concentration of 5 mM, and the rate of oxygen uptake was again determined.

Germination. Bdellocysts were germinated by incubation in PYE broth or on PYE agar at 30°C in either the presence or absence of prey.

Microculture and photomicrography. Microcultures were prepared by adding a drop of a mildly sonically treated bdellocyst suspension to a 0.5-mm-thick square of PYE agar on a microscope slide. A bdellocyst suspension containing 1 × 10^9 cells per ml was sonically treated with a Branson model S125 Sonifier (Branson Instruments, Danburg, Conn.) at a setting of 4 for 5 s. The mild sonic treatment of the bdellocysts was employed to partially remove prey ghosts to permit observation of germination. The inoculum was covered with a cover glass and sealed, leaving two 1-mm openings for aeration. The microculture was observed through a Zeiss Universal microscope equipped with phase-contrast optics. Photomicrographs were taken on Kodak Plus-X pan film with a Nikon model AFM Automatic camera attachment.

Resistance studies. In all determinations of sensitivity of bdellocysts and vegetative cells to stresses, viable counts were determined by plating onto PYE agar by the agar overlay method with *R. rubrum* as prey.

To determine heat resistance, 0.1-ml samples of vegetative cells and bdellocysts containing 1 × 10^{10} cells were introduced into screw-capped tubes containing 9.9 ml of 10^{-3} M Tricine buffer, pH 7.2, preheated to the desired temperature in a constant-temperature water bath. After 15 min, the tubes were removed and rapidly cooled to 25°C, and viability was determined.

Sensitivity to ultraviolet irradiation was determined by placing 5 ml of either vegetative cells or bdellocysts (2 × 10^9 cells per ml in 10^{-3} M Tricine buffer, pH 7.2) in a petri dish and irradiating with a Spectroline R-51 high-intensity short-wave (wavelength, 260 nm) ultraviolet lamp (Spectronics Corp., Westbury, N.Y.) at a distance of 14 cm. Samples were removed at intervals, and viability was determined by plating.

The effect of sonic treatment on viability was

determined by sonically treating 10-ml samples of vegetative cells and bdellocysts containing 2×10^{10} cells per ml with a Branson model S125 Sonifier set at maximum power output. During sonic treatment, samples were cooled in ice to keep the sample temperature below 10°C. Samples were sonically treated for 5 to 15 s, with a 1-min cooling time between bursts. Samples were removed at intervals and plated for viable counts.

To determine the effects of desiccation on viability, 0.1-ml samples of vegetative cells and bdellocysts were placed in tubes and dried over desiccant (silica gel) under slight vacuum. Tubes were removed at selected intervals, and the cells were resuspended in 10 ml of 10^{-3} M Tricine buffer (pH 7.2) with vigorous agitation. Viability was determined by plating.

RESULTS

Prey range. Burger et al. (4) reported that *Bdellovibrio* sp. strain W exhibited a wide prey range. However, these workers and Hoeniger et al. (7) indicated that bdellocysts were produced only in *R. rubrum* as the prey cell. We examined the interaction of strain W with a variety of other prey. Table 1 shows the results of these experiments. Contrary to the earlier reports, all of the gram-negative bacteria tested served as prey for both vegetative growth and bdellocyst production. The two gram-positive bacteria reported by Burger et al. (4) to be lysed by *Bdellovibrio* sp. strain W failed to support growth of the bdellovibrios in these experiments. In addition, bdellocysts were produced on appropriate prey on agar medium in contrast to the report by Hoeniger et al. (7) that bdellocysts were formed only in liquid medium. Bdellocysts produced in different prey organisms were morphologically indistinguishable from one another when observed by phase-contrast microscopy. Figure 1 shows phase-contrast micrographs of bdellocysts produced in *R. rubrum* (a) and *E. coli* B (b). The bdellocysts developed inside their prey, and the ghosts of the prey remained around the bdellocysts (arrows). Strain W was not observed to encyst outside the prey cells or in the absence of prey.

Encystment. The encystment of *Bdellovibrio* sp. strain W was tested in a variety of media with both *R. rubrum* and *E. coli* B as prey. Table 2 shows the effects that some media have on bdellocyst production. Results are expressed as the percentage of the bdellovibrio inoculum that developed into bdellocysts as determined by Petroff-Hauser microscopic counts. In media that will sustain at least limited growth of the prey (experiments 1 through 4), the bdellovibrios grew vegetatively with little production of bdellocysts. When bdellovibrios and prey were incubated in buffers (experiments 5 through 8), bdellocyst production was greater than that in the richer media, with the greatest production of bdellocysts consistently occurring in 0.05 M potassium phosphate buffer. Higher ionic strengths of phosphate buffer inhibited both encystment and vegetative growth. Under similar procedures, the optimum pH range for encystment was found to be between 7 and 8; this pH range was also optimum for vegetative growth.

The concentrations of predator and prey cells and multiplicity of infection (MOI) were found to be important for maximum encystment (Table 3). The medium employed in these experiments was 0.05 M potassium phosphate buffer, pH 7.5. At concentrations below 2×10^9 cells per ml for either *Bdellovibrio* or its prey (experiments 1 and 2), bdellocyst production was low. At 2×10^9 cells per ml or above for both predator and prey, an MOI of 1 (experiments 3, 9, and 10) proved to be near optimal for encystment. At a prey concentration of 2×10^9 cells per ml, MOIs above 1.5 (experiments 6 and 7) or below 1 (experiment 5) yielded low numbers of bdellocysts. When the bdellovibrio concentration was 2×10^9 cells per ml, the prey concentration could be raised as high as 4×10^9 cells per ml (experiment 8) with near optimal bdellocyst production. The maximal bdellocyst production of 32% was consistently achieved by using an MOI of 1 at a concentration of 2×10^9 cells per ml for both bdellovibrios and prey.

Compounds such as β-D-hydroxybutyrate and *n*-butanol, which induce encystment of *Azotobacter vinelandii* (11, 19), had no effect on encystment of *Bdellovibrio* at concentrations

TABLE 1. *Prey range of Bdellovibrio sp. strain W in dilute nutrient broth*

Organism	Lysis[a]	Bdellocyst production[b]
R. rubrum Hughes	+	+
E. coli B ATCC 15144	+	+
A. faecalis	+	+
E. aerogenes	+	+
S. marcescens	+	+
P. fluorescens ATCC 13525	+	+
E. amylovora ATCC 15580	+	+
S. serpens VHL ATCC 12638	+	+
E. coli K-12 P678-54	+	+
L. plantarum ATCC 14917	−	−
S. faecalis ATCC 19433	−	−

[a] Lysis of prey was determined by both microscopic examination of broth cultures and observation of plaque formation on double-layer agar plates.

[b] Production of bdellocysts was determined by microscopic examination of broth lysates.

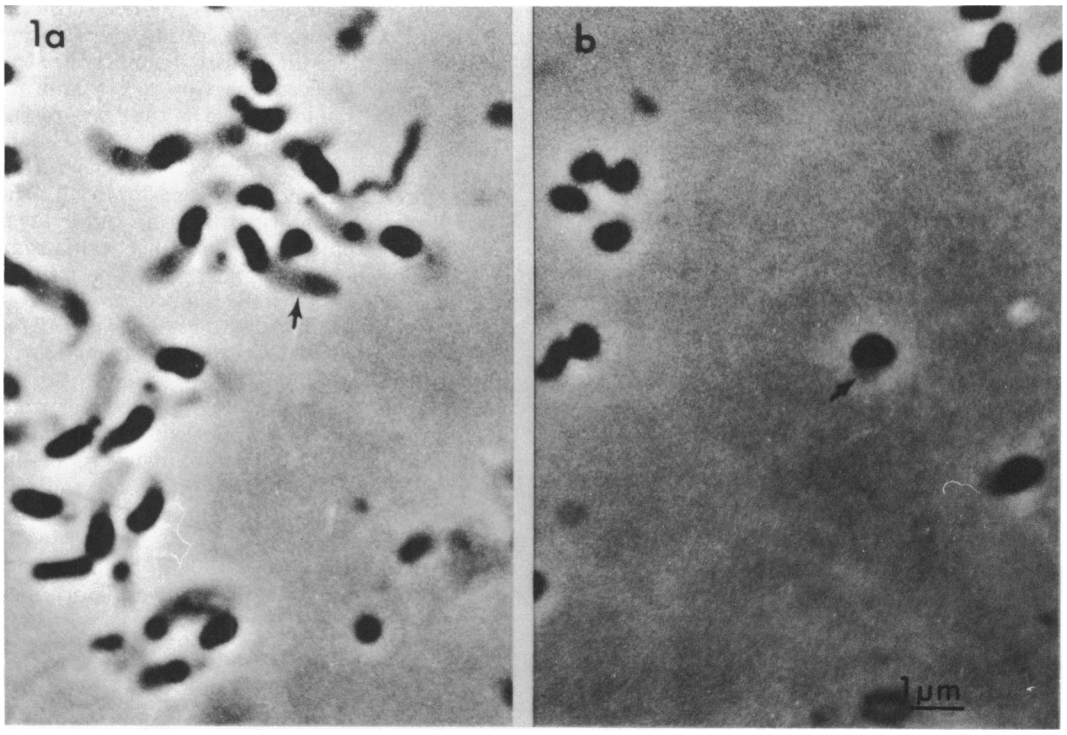

FIG. 1. *Phase-contrast micrographs of bdellocysts produced in R. rubrum (a) and E. coli B (b). The ghosts of the prey remain around the bdellocysts (arrows). Samples were from cultures 21 h postinfection.*

TABLE 2. *Effect of medium on production of bdellocysts*

Expt no.	Medium[a]	Encystment and range (%)[b]
1	Drews R8AH	<5
2	Yeast nitrogen base	<5
3	PYE	<5
4	DNB	8 ± 4
5	Burk nitrogen-free buffer	23 ± 8
6	5 mM HEPES buffer	15 ± 4
7	0.05 M potassium phosphate buffer	31 ± 4
8	5 mM Tricine buffer	12 ± 3

[a] pH 7.5. For references to compositions of media, see text.

[b] Results are expressed as the percentage of the initial bdellovibrio inoculum present as bdellocysts as determined by Petroff-Hauser microscopic counts. The concentration of both *Bdellovibrio* sp. strain W and *E. coli* B initially was 2×10^9 cells per ml. Data given are averages of results from five separate experiments.

TABLE 3. *Effect of MOI and cellular concentration on production of bdellocysts*

Expt no.	Cell concn ($\times 10^9$/ml)		MOI	Encystment and range[a] (%)
	Bdellovibrio	Prey		
1	0.2	0.2	1	<5
2	0.2	2	0.1	<5
3	2	2	1	32 ± 4
4	3	2	1.5	25 ± 5
5	1	2	0.5	10 ± 5
6	4	2	2	6 ± 2
7	5	2	2.5	<5
8	2	4	0.5	31 ± 5
9	4	4	1	26 ± 4
10	5	5	1	24 ± 5

[a] Results are expressed as the percentage of the initial bdellovibrio inoculum present as bdellocysts as determined by Petroff-Hauser microscopic counts. Data given are averages of results from three experiments.

below 0.1% and inhibited encystment somewhat at higher concentrations.

Chemical analyses. Table 4 lists various chemical analyses for both vegetative cells and bdellocysts of *Bdellovibrio* sp. strain W. Since bdellocysts are significantly larger than vegetative cells (approximately 0.5 by 1 μm for bdellocysts compared with 0.25 by 1 μm for vegetative cells), one would expect greater amounts of RNA, protein, and carbohydrate, which was the case. The ratio of RNA to protein for bdellocysts was similar to that for vegetative cells,

TABLE 4. *Chemical analysis of Bdellovibrio sp. strain W vegetative cells and bdellocysts*[a]

Determination	Vegetative cells (μg)	Bdellocysts (μg)
DNA		
Average	2.2	3.6
Range	1.8–2.4	3.3–3.8
RNA		
Average	11.1	19.8
Range	10.6–12.7	18.8–20.4
Protein		
Average	41.0	69.2
Range	37.2–42.4	68.0–72.2
Carbohydrate		
Average	20.3	60.5
Range	18.4–22.1	57.2–62.7
Poly-β-hydroxybutyrate	ND[b]	ND
Dipicolinic acid	ND	ND

[a] Results are expressed as micrograms per 10^9 cells and represent triplicate experiments.

[b] ND, Less than the lower limits of detectability of the methods employed.

and the amount of protein in the vegetative cells was within the range found for other *Bdellovibrio* strains (15). The relatively large amount of total carbohydrate present in the bdellocysts may be attributed, in part, to the additional wall structure surrounding the bdellocysts. The concentration of DNA in the bdellocysts was about 50% greater than that in the vegetative cells, perhaps indicating additional DNA replication prior to development of the bdellocysts. The quantity of DNA present in the vegetative cells was comparable to that found for other bdellovibrios (13, 17). Analyses for dipicolinic acid, a compound present in bacterial endospores (14), and for poly-β-hydroxybutyrate were less than the lower limits of detectability of the methods employed.

Oxygen uptake. The rate of endogenous respiration of bdellocysts was less than 20% of that of the vegetative cells (Table 5). When glutamate or succinate was added, the rate of oxygen uptake was not appreciably altered for either vegetative cells or bdellocysts. It is conceivable that at least a portion of the oxygen consumed by the bdellocyst suspension was due to low numbers of contaminating prey cells.

Germination. Bdellocysts were capable of germination in a complex medium such as PYE in either the presence or absence of prey. When plated onto PYE agar containing *R. rubrum*, from 70 to 95% of the bdellocysts germinated and produced plaques. In PYE broth, more than 95% of the bdellocysts germinated to form highly motile vegetative cells.

Time-lapse, phase-contrast micrographs of bdellocysts germinating on PYE agar are shown in Fig. 2. Bdellocysts are not refractile like endospores; therefore, initial germination events were more difficult to demonstrate. Initially, the bdellocyst was very dense and appeared to become less dense as time progressed (2b and c). The germinating bdellocyst elongated somewhat, becoming "S"-shaped rather than vibrioid (2d and e). After 90 min (2f), the germinated cell emerged from an outer layer (arrow). This outer layer probably was a remnant of the prey cell ghost, which remained closely associated with the bdellocyst even after mild sonic treatment. After emergence from this outer layer, the germinant became motile and continued to elongate (2g, h, and j). As the germinant elongated, it became thinner until eventually its thickness approximated that of a vegetative cell. The germinants were predacious upon gaining motility and infected and lysed its prey in the same manner as vegetative cells.

Resistance studies. The heat resistance of vegetative cells and bdellocysts was determined by exposing both to temperatures of from 40 to 60°C for 15 min (Fig. 3). Although bdellocysts did not exhibit heat resistance comparable to that of endospores, they were more resistant than were vegetative cells. More than 99% of the vegetative cells were killed at 50°C, whereas it required temperatures of nearly 60°C to inactivate 99% of the bdellocysts. Bdellocysts retained 100% viability after 30 min of exposure to either 45 or 50°C, but they lost viability rapidly at 55°C. This thermal tolerance of bdellocysts is comparable to that reported for *Azotobacter* cysts (19).

Bdellocysts were considerably more resistant to sonic disruption than were vegetative cells (Fig. 4). Vegetative cells lost viability rapidly, with only 2% surviving after sonic treatment for 10 s; concomitantly, the turbidity at 600 nm decreased by 90% due to disruption of the cells. On the other hand, after 30 s of sonic treatment, the bdellocysts retained 35% viability and showed a 40% drop in turbidity.

The effect of ultraviolet irradiation on the

TABLE 5. *Oxygen uptake by Bdellovibrio sp. strain W vegetative cells and bdellocysts*

Specimen	Oxygen uptake[a]		
	Endogenous	5 mM succinate	5 mM glutamate
Vegetative cells	1.83	1.83	1.83
Bdellocysts	0.34	0.40	0.40

[a] Determined on 3-ml samples by a YSI model 53 oxygen monitor. Values are nanomoles per minute per 10^9 cells.

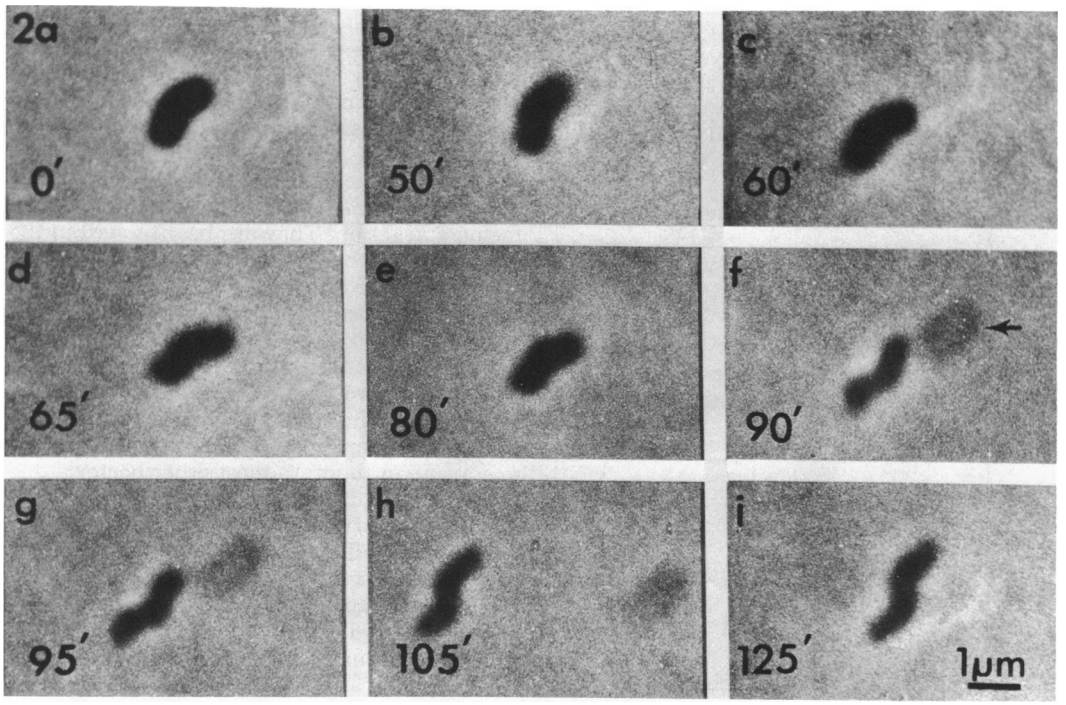

FIG. 2. *Time-lapse phase-contrast photomicrographs of a germinating bdellocyst. Bdellocysts were produced as described in the text, and slide cultures were prepared on peptone-yeast extract agar. Arrow points to outer layer from which the germinant has emerged.*

viability of vegetative cells and bdellocysts is shown in Fig. 5. The curves are multitarget in character and indicate that bdellocysts and vegetative cells were inactivated at nearly the same rate.

It has been shown that in the absence of prey organisms and of suitable sources of carbon and energy, several strains of *Bdellovibrio* rapidly lose viability due to their high rate of endogenous respiration (4, 6). When bdellocysts were incubated at 30°C in 10^{-3} M Tricine buffer (pH 7.2), they retained greater than 95% viability for up to 15 days, whereas vegetative cells lost more than 80% viability after only 48 h under the same conditions.

Bdellovibrio vegetative cells lost viability immediately upon desiccation and could not be recovered. Bdellocysts, however, retained from 45 to 80% viability after 6 days of desiccation. The results of these experiments were variable, ranging from 45 to 80% survival; perhaps this was due to adherence of the bdellocysts to the walls of the tubes upon drying, resulting in a variation in recovery.

When treated with the nonionic detergent Triton X-100 (0.02%, wt/vol), bdellovibrio vegetative cells were lysed rapidly, but bdellocysts were unaffected, retaining 100% viability after 2 h of exposure to concentrations as great as 1%. However, ionic detergents such as sodium dodecyl sulfate and desoxycholate (0.02%, wt/vol) reduced bdellocyst viability by as much as 45% after exposure for 15 min.

DISCUSSION

That *Bdellovibrio* sp. strain W bdellocysts differ morphologically from vegetative cells was shown previously (4, 7). Our results showed clearly that there are other differences. Bdellocysts appear to differ from vegetative cells in quantity of major macromolecules. The greater amounts of RNA and protein may be attributed simply to the larger size of the bdellocysts. The additional carbohydrate in the bdellocysts may be due as well to the presence of additional wall material. The specific chemistry of this wall material and of the inclusion material present in the bdellocysts is not known at this time.

Bdellocysts were produced in all media that were tested, but the greatest observed production occurred in dilute phosphate buffer. Such conditions may be considered to be "starvation" conditions for the prey and also for the free bdellovibrios, and they appear to play a role in

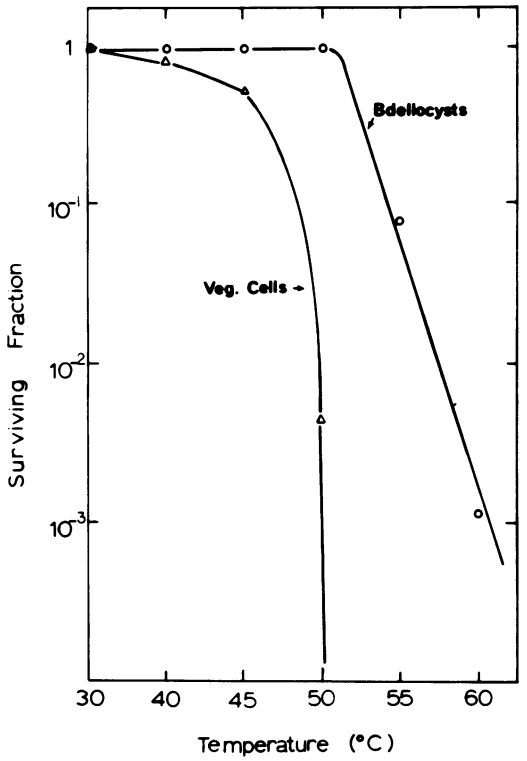

FIG. 3. *Resistance of Bdellovibrio sp. strain W vegetative cells and bdellocysts to heat. Samples were heated to temperatures from 40 to 60°C for 15 min and then plated for viability.*

initiating bdellocyst production. MOI and cellular concentration are also important in determining whether the bdellovibrios grow vegetatively or encyst. At cellular concentrations below 2×10^9 cells per ml and at MOIs below unity when cellular concentration equals 2×10^9 cells per ml, the bdellovibrios grew vegetatively with little production of bdellocysts. Likewise, at MOIs above 1.5, bdellocyst production was low. Conversely, when the MOI was 1 and the cellular concentration was at 2×10^9 cells per ml or above, bdellocyst production was enhanced. Under such conditions, over 30% of the infecting bdellovibrios developed into bdellocysts, with production of bdellocysts reaching a maximum at 12 to 16 h postinfection.

The bdellocysts were produced directly from infecting bdellovibrios prior to any intracellular multiplication. This fact has raised an interesting question (20); of what selective advantage is the immediate formation of a bdellocyst after the bdellovibrio enters its prey and before propagation of its progeny? Upon entry into a prey cell, the bdellovibrio is presumably not under starvation conditions and is in an environment capable of supporting production of at least 5 to 10 progeny bdellovibrios (21). A speculation as to why bdellocysts are produced under such circumstances can perhaps be formed by drawing an analogy with endospore formation. Knaysi stated that "spores are formed by healthy cells facing starvation" (9). Bdellocysts may well be produced by healthy bdellovibrios facing starvation. They are healthy within the prey cell, which contains the necessary nutrients for growth. However, they face starvation from two fronts: (i) the bdellovibrios find themselves inside a prey that is itself in a lean environment, and (ii) at high MOIs those prey cells present would soon be lysed, resulting in starvation conditions for the bdellovibrios. Bdellovibrios are much more acutely sensitive to starvation than are most other bacteria due to their high rate of endogenous respiration and the absence of any known storage material; vegetative bdellovibrios lose viability very rapidly under starvation conditions (4, 6). Bdellocysts have a much reduced level of endogenous respiration and are able to survive starvation conditions for extended periods. When cultures are prepared at low MOIs, the bdellovibrios

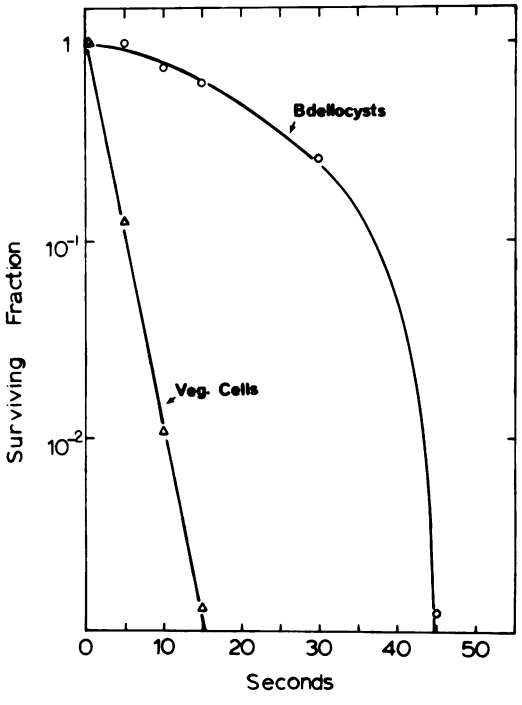

FIG. 4. *Susceptibility of Bdellovibrio sp. strain W vegetative cells and bdellocysts to sonic disruption. Samples were sonically treated as described in the text. At the time intervals indicated, samples were removed and plated for viability.*

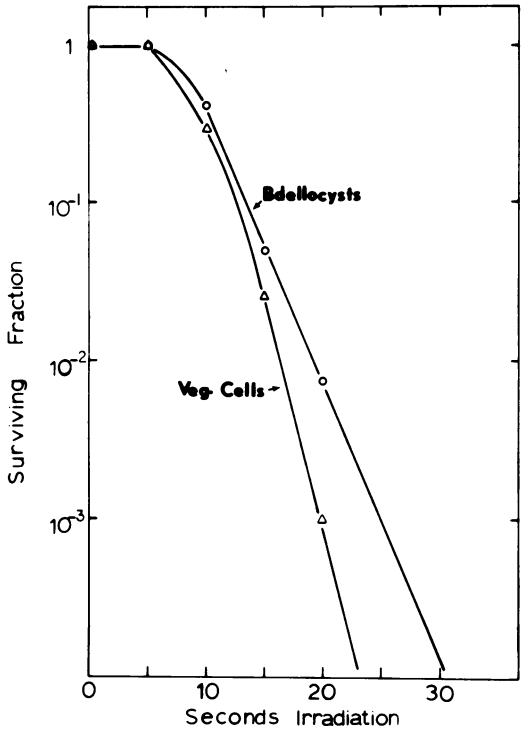

FIG. 5. *Effect of ultraviolet irradiation on the viability of Bdellovibrio sp. strain W vegetative cells and bdellocysts. Samples of 5 ml were irradiated as described in the text.*

grow vegetatively for a time; a few (2 to 8%) will encyst when prey cells have become limiting and the bdellovibrios are facing starvation.

Bdellocysts are capable of germination in rich media such as PYE in either the presence or absence of their prey. This is the first report that bdellocysts are capable of germination. Once germinated, the motile germinants can attack, penetrate, and grow in the periplasmic space of susceptible bacteria. The germination of bdellocysts in the absence of prey suggests that in nature the encysted cells will germinate when conditions are sufficient to support the growth of prey organisms. The sequence of events in germination as revealed by phase-contrast microscopy is that the bdellocysts elongate, become "S" shaped, emerge from an outer layer, and eventually become motile. This outer layer is probably a remnant of the prey ghost as revealed by electron microscopy (manuscript in preparation). A detailed study of the ultrastructural events accompanying germination is currently underway.

Bdellovibrio sp. strain W bdellocysts are more resistant than are vegetative cells to heat, sonic disruption, desiccation, and detergents. The lysis of vegetative cells by nonionic detergents has been reported for other bdellovibrios (D. Abram and J. Castro e Melo, Abstr. Annu. Meet. Am. Soc. Microbiol., 1975, J6, p. 144). Bdellocysts are resistant to the effects of the nonionic detergent Triton X-100 and somewhat resistant to ionic detergents. This resistance may be due to the inacessability of the cell wall due to the presence of the outer wall surrounding the cysts.

The fact that bdellocysts can withstand drying conditions could be a selective advantage in allowing the bdellovibrios to survive limited desiccation encountered under natural conditions in the soil. Even though bdellocysts do not appear to be as resistant to drying as other dormant forms of bacteria (e.g., endospores), their resistance to desiccation combined with their low respiration rate could allow bdellocysts to survive in areas of low prey density and fluctuating moisture content that otherwise would be hostile to the existence of bdellovibrios.

Bdellocysts are only slightly more resistant to heat than are vegetative cells. This slight heat resistance probably does not offer any selective advantage for survival. This also appears to be the case for resistance to ultraviolet irradiation.

If the production of bdellocysts were confined to a single prey-predator system as previously indicated (4, 7), or to a single medium, the influence of such prey or medium on initiating bdellocyst production could not be ruled out. However, as our results show, prey that were capable of supporting vegetative growth of the bdellovibrios also supported the development of bdellocysts. The fact that bdellocysts are produced in a variety of prey makes their production a more generalized phenomenon than originally thought. The production of bdellocysts on solid medium and in prey other than *R. rubrum*, in contrast with other reports (4, 7), could be due to differences in media or other conditions employed during the experiments. However, it should be emphasized that although certain conditions (e.g., media, MOI, etc.) contributed to increased bdellocyst production, some encystment occurred under all conditions tested. Whether other *Bdellovibrio* strains are capable of producing bdellocysts under appropriate conditions is presently unknown. We have tested 15 other strains of *Bdellovibrio* in this laboratory and have found that none will encyst under the conditions tested. Existing isolation procedures for *Bdellovibrio* depend heavily on differential filtration or centrifugation. Such methods may select against bdellocyst producers due to the larger size of the encysted cells.

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