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CHAPTER 3.4.2

The Genus *Bdellovibrio*

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Introduction

Bdellovibrio spp. are Gram negative, motile, and uniflagellated bacteria. What characterizes this bacterial genus as unique is its predatory behavior: Bdellovibrios attack other Gram negative cells, penetrate their periplasm, multiply in their cytoplasm, and finally burst their cell envelopes to start anew (Figure 1). Although many predator bacteria may lyse other bacteria, and many "micropredators" have been described, these *Bdellovibrio*-like organisms can grow without bacterial prey and do not divide inside the periplasm (Varon and Shilo, 1978).

Bdellovibrio research began with their serendipitous discovery by Stolp and Petzold (1962). The physiology, ecology, taxonomy, interactions with prey, and cell cycle of *Bdellovibrio* were established in the 1960's and 70's mainly by the groups of Shilo and Varon, Stolp and Starr, Rittenberg, Hespell, Diedrich, Ruby, Tudor, Thomashow, and more recently Williams.

Bdellovibrios can be described as predators, or parasites, or symbionts (Starr, 1975). In this review, the terms predator-prey and parasite-host will be used interchangeably.

Bdellovibrios are found in wet, aerobic environments. As described in other sections, their importance in shaping or influencing bacterial community dynamics and structure is still unknown. Similarly, the way these predators interact with potential prey, their survival mechanisms, and their preferred habitats in natural settings or man-made biotops are poorly understood.

Particularly fascinating is the two-phased cell cycle of bdellovibrios, which are dimorphic. During the "attack phase," small cells (0.5 to 1.5 μm long, 0.5 μm in diameter, Figure 2) swim at speeds up to one hundred body lengths·sec^{-1} (Stolp, 1967) to find prey. Prey-encounter is apparently by random collision, as evidence of chemotaxis has not been detected. After encountering and attaching to a substrate cell, a predatory cell penetrates the periplasm, shedding its long-sheathed flagellum in the process. The "growth phase" can then be initiated. The invaded bacterium usually rounds into the ensemble predator-prey, called a bdelloplast (Figure 3). Within this confined space *Bdellovibrio* engages in restricted development, turning the short cell into an unseptated filament at the expense of the host's cytoplasm (Figure 4). After a lag phase of about 45 min, DNA begins to replicate within the bdelloplast and proceeds for a couple of hours along with cell growth, and then the filament divides by multiple fission into progeny attack cells, the number determined by the size of the host. Progeny bursts from the ghost cell, ready for another attack cycle. Within the bdelloplast, the bdellovibrio cell is protected from photooxidation (Friedberg, 1977) and phage attack (Varon and Seijffers, 1975) and shows increased resistance to pollutants (Varon and Shilo, 1981). Host-independent mutants growing axenically can be isolated. The cell cycle can be extended to include the development of a cyst-like stage called a bdellocyst, which, however, has been seen in only two strains.

Basic biological questions such as predator-prey interactions, host recognition, regulation of a simple developmental program, and cell-cell signaling can be addressed by using this two-membered model. We shall deal with each of these issues in the linked sections.

Taxonomy

The taxomony of *Bdellovibrio* is still fragmentary. Historically, microorganisms that have been assigned to the genus *Bdellovibrio* exhibit characteristic features: flagellum-driven high motility, attachment and penetration through the outer membrane of Gram-negative hosts, intraperiplasmic growth, cell multiplication at the expense of host cytoplasm, lysis of prey, and finally release of *progeny*. Their wide habitat and heterogeneity can be interpreted in terms of development of a common lifestyle by convergent evolution or increased heterogeneity by divergent evolution from a common very ancient ancestor (Varon and Shilo, 1978).

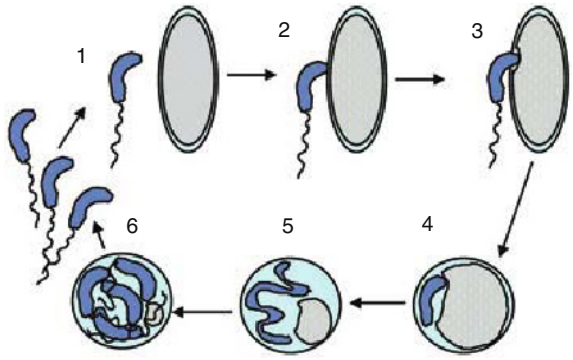

Fig. 1. The life cycle of *Bdellovibrio*. 1. Attack-phase cells; 2. Attachment to the prey's outer membrane; 3. Penetration; 4. Initiation of growth; 5. Intraperiplasmic growth; 6. Differentiation into attack cells and lysis of the prey envelope.

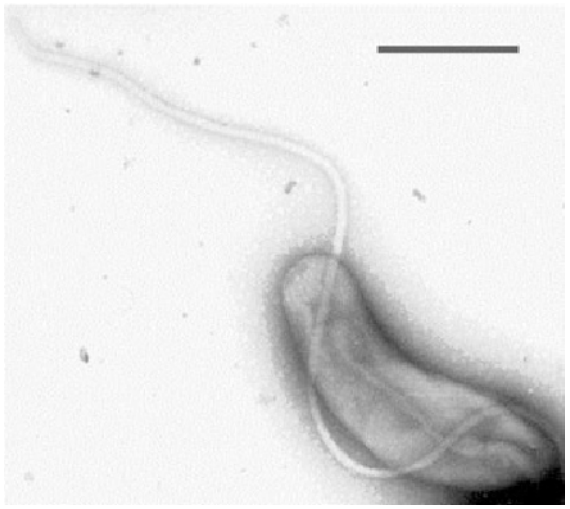

Fig. 2. A *Bdellovibrio* cell in the attack phase. The flagellum is wrapped around the cell. Bar: 0.5 µm.

The genus has been placed within the Proteobacteria (Ribosomal Database Project), myxobacteria and sulfate-reducing bacteria being their closest relatives. Based on G+C content, DNA/DNA (Table 1) and DNA/rRNA homology, and enzyme migration patterns, the genus was divided into three species: *B. bacteriovorus*, *B. stolpii* and *B. starrii* (Siedler et al., 1972). *Bdellovibrio* strain W, one of the two bdellocyst-forming isolates (Hoeniger et al., 1972), and the marine isolates were left undefined. The ion requirements of these halophilic isolates have been described (Taylor et al., 1974; Marbach and Shilo, 1978). Based on the similarities in host ranges, Taylor et al. (1974) subdivided the 13 analyzed isolates into three groups. Mole %G+C of three isolates from one group was similar (38.5–38.8) and much lower than that of any of the terrestrial isolates, whereas G+C content of

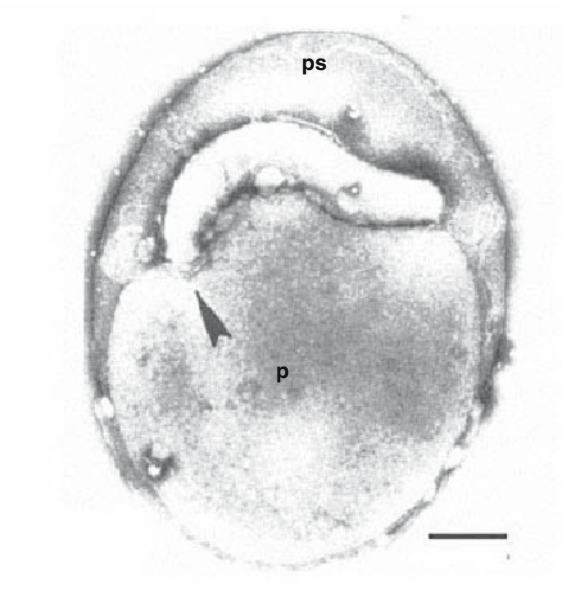

Fig. 3. An *E. coli* cell with a *B. bacteriovorus* 109J parasite in the intraperiplasmic space. The parasite at its entry pole is associated with the host cytoplasmic membrane (arrow). ps: periplasm. p: protoplasm. Bar: 0.2 µm. (From Abram et al., 1974.)

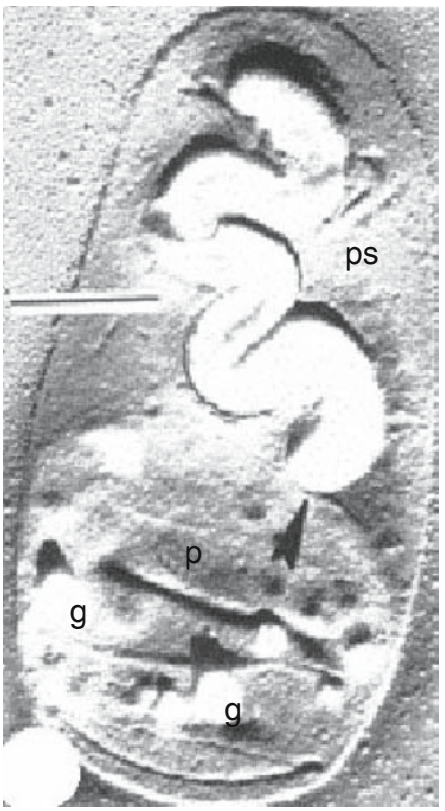

Fig. 4. Intraperiplasmic growth of *B. bacteriovorus* 109J in *Spirillum serpens*. p: protoplast. ps: periplasmic space. g: polymetaphosphate granules. Bar: 1 µm. (From Abram et al., 1974.)

Table 1. Genetic characteristics of *Bdellovibrio*.

Species	Genome size (kbp)	Mol% G+C	% DNA/DNA relative reassociation					
			109D	100	A3.12	UKi2	W	N6804
B. bacteriovorus 109D	2060	51	100	103				
B. bacteriovorus 100	2170	49.5		100	1	0		
B. starrii A3.12	2610	43.5			100	16	23	0
B. stolpii UKi2	2290	41.8				100	28.5	37
Strain W	2030	43.7					100	32
N 6804 (ICPB 3294)	2040	37.4						100

one isolate from another group was 43.5%. Marbach et al. (1976) separated halophilic bdellovibrios into 10 groups, based on prey range. However, no such grouping was found by Torrella et al. (1978) or by Schoeffield et al. (1991), and host range was shown to depend on the experimental conditions, thereby raising questions about the usefulness of this parameter as a taxonomic criterion. Bacteriophages of bdellovibrios (bdellophages) have been isolated (Sagi and Levisohn, 1976; Althauser et al., 1972; Varon and Levisohn, 1972; Hashimoto et al., 1970) and in some instances used to define taxomic groups of bdellovibrios (Althauser et al., 1972; Varon, 1974).

16S rRNA

Resolution of the phylogeny of *Bdellovibrio* has been increased by the analysis of the 16S rRNA molecule. Donze et al. (1991) analyzed 11 terrestrial strains and one marine isolate using partial sequences of the *16S rDNA* gene. Their results confirmed the placement of Bdellovibrio within the Proteobacteria and showed *Bdellovibrio* strains were related more closely to each other than to other bacteria, supporting the claim of common ancestry. The heterogeneity of the genus *Bdellovibrio* could be divided into two groups, *B. bacteriovorus* strains forming one and *B. stolpii*, *B. starrii* and the marine isolate the other (Figure 5). However, the large phenotypic and molecular (%G+C content, DNA/DNA reassociation) differences between the marine and terrestrial isolates, the scant molecular information available on the diversity of marine strains, and the heterogeneity observed within the species *B. bacteriovorus* call for further studies.

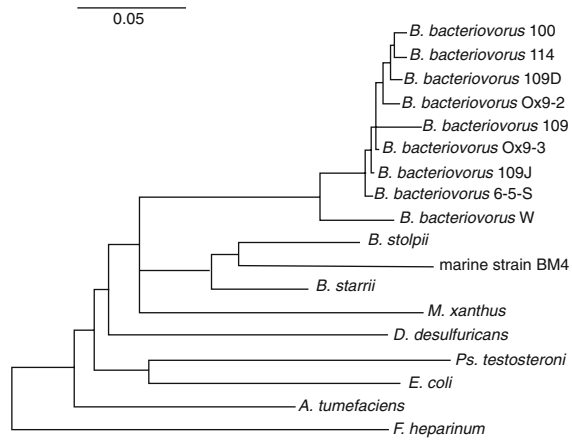

Fig. 5. Phylogenetic tree for 12 strains of *Bdellovibrio* based on the analysis of the 16S rRNA gene. Bar: fractional difference in nucleotide sequence. (From Donze et al., 1991.)

Serological Classification

Immunological approaches have been shown to offer a level of sensitivity high enough to resolve taxonomic groups of bdellovibrios (Schoeffield et al., 1991; Schelling et al., 1983; Kramer et al., 1977). Using techniques such as immunodiffusion, immunoelectrophoresis, agglutination, and indirect immunofluorescence, halophilic and nonhalophilic bdellovibrios can be separated into several serologically distinct subgroups: the terrestrial subgroups, sharing a common antigen (Schelling et al., 1983; Kramer et al., 1977); the halophilic isolates (Schoeffield et al., 1991), each identified by at least one common antigen; and the aquatic, salt-loving strains, sharing a common antigen with terrestrial strain W (Schoeffield et al., 1991). Thus, such immunology-based tools can help determine taxonomy.

Habitat and Ecology

Bdellovibrios have been isolated from a wide range of water systems: estuaries (brackish water), sea coasts and oceans, rivers, sewage, fish ponds, runoff (irrigation water), and man-made water supplies. Also, organisms have been found in the gills of crabs, in sediments, on submerged surfaces, in soils, in rice paddies, and in the rhizosphere of plants. Table 2 presents a summary of biotops in which *Bdellovibrio* were detected. Interestingly, the concentration of recovered *Bdellovibrio* in rivers correlated with water pollution. Whereas no predators could be detected

Table 2. *Bdellovibrio* in natural and man-made biotops.

Origin	Isolation steps	Prey	*Bdellovibrio* PFU·g^{-1}, ml^{-1} or cm^{-2}	Reference
Oyster shells in brackish water	Differential centrifugation	*Vibrio parahaemolyticus*	Up to 9.4×10^2	Kelley et al. 1997
Coastal sea water and aquaculture farms	Filtration	Various Gram-negatives	0 to 3.8×10^2	Pan et al. 1997
Soil and sewage	Filtration	*Escherichia coli* O157:H7; Salmonella spp.	N.D.	Fratamico and Cooke 1996
Rhizosphere of Chinese cabbage	Filtration	*Pseudomonas fluorescens*	Up to 10^5	Elsherif and Grossmann 1996
Aquatic plant surfaces	Differential centrifugation	*Vibrio parahaemolyticus*	Up to 1.2×10^2	Williams et al. 1995
Gills of blue crab (Callinecus sapidus)	Differential centrifugation	*Vibrio parahaemolyticus*	2×10^3	Kelley and Williams 1992
Brackish tidal pond*, saltwater aquarium**	Direct plating	*Vibrio parahaemolyticus*	40*, 10^2**	Schoeffield and Williams 1990
Man-made water supplies[a]	Concentrated samples	*Legionella* spp.	Positive, N.S.	Richardson 1990
Rice paddies, Japan	1.2µm filtration	*Xanthomonas oryzae*	0 to 10^3	Uematsu 1980
River water, Great Britain	Dilution to extinction	*Escherichia coli*	0–3×10^3;[b] 2×10^4 to 5×10^4;[c]	Fry and Staples 1976
Osaka Bay, Japan	N.S.	*Vibrio parahaemolyticus*	2×10^4 to 1.5×10^6	Miyamoto and Kuroda 1975
Oahu, Hawaii, USA	Filtration	Various Gram-negatives	1 to 2×10^2	Taylor et al. 1974
Sewage, Great Britain	Homogenization	Achromobacter spp.	9×10^2	Staples and Fry 1973
Soil, Australia	Filtration	*Rhizobium* spp.; *Agrobacterium* spp.	2 to 1.2×10^3	Parker and Grove 1970
Soil, USA	Direct plating	*Escherichia coli*	4.5×10^4	Klein and Casida 1967
Soil, USA	Filtration	Various Gram-negatives	4×10^1 to 2×10^2	Stolp and Starr 1963

N.S.: not specified; [a]: hospital water, shower unit, industry, etc; [b]: unpolluted water; [c]: polluted water.

in pure well waters (Varon and Shilo, 1978), *Bdellovibrio* counts increased from 10 to 3500 PFU·mL^{-1} (plaque-forming units) at the source and near an urbanized section of the river, respectively (Lambina et al., 1974). Although a higher total bacterial load in the sample may promote *Bdellovibrio* growth, the predator has been shown sensitive to environmental pollutants such as heavy metals, detergents, and pesticides (Varon and Shilo, 1978). Bdellovibrios are aerobic, multiplying under oxic conditions, but they are able to survive anoxic periods as attack-phase cells or as bdelloplasts (Schoeffield et al., 1991). Furthermore, halo-tolerant predators have been shown to grow under microaerobic conditions. Spells of low oxygen tension occur in soils and in water, and the parasite seems to be adapted to these conditions. The range of possible niches for bdellovibrios to survive and be active, therefore, may not be restricted to permanently aerobic biotas.

Another environment in which bdellovibrios have been found is biofilms on surfaces. In the continuous space between the solid phases of biofilms, dissolved chemicals, suspended particles, and cells move freely (Wanner, 1989). It is therefore plausible that bdellovibrios due to their small size, high motility, and mode of multiplication may strongly influence the bacterial composition of their associated biofilms. Recently, an isolate that effectively reduces the level of biofilm *E. coli* cells on stainless steel has been reported (Fratamico and Cooke, 1996). In some instances, biofilms seem to offer better conditions for the multiplication and survival of bdellovibrios as they have been found in the fabric of natural marine biofilms but not always recovered from the surrounding water (Kelley et al., 1997; Kelley et al., 1992; Williams et al., 1995). In these specific econiches, bdellovibrios might become entrapped in the gel-matrix of biofilms, benefiting from higher concentrations of prey cells that ensure survival, a greater multiplication rate, and also physical protection. Surface-associated bdellovibrios were shown to survive various environmental insults but free-living cells died rapidly (Markelova et al., 1998).

A high density of prey has often been shown to be necessary for *Bdellovibrio* survival. Various authors have reported that prey concentrations of at least 10^5 to 10^6 CFU·g^{-1} soil or mL^{-1} are required for *Bdellovibrio* survival (Keya and Alexander, 1975; Uematsu, 1980). Calculations based on the Lotka-Volterra predator-prey model (Varon and Zeigler, 1978) indicate that at least 3×10^6 prey cells are needed to give bdellovibrios a 50% chance of survival. From this type of calculation, the general conclusion was that *Bdellovibrio* can survive only in special ecological niches. However, low-level, steady-state bdellovibrio populations were sustained for long periods in continuous cultures at low host density (10^4 cells·mL^{-1}).

As seen above, biofilms can potentially provide a richer habitat for bdellovibrios. Biofilms may provide an environment more conducive to bdellovibrio development in low microbial density biotas, the predator expanding beyond that realm during bacterial population surges. Notably, the calculated minimal prey levels usually relate to bdellovibrio predation upon a defined bacterial species in pure culture. Because bdellovibrios are usually not stringently host-range specific, the concentration of substrate cells in natural settings may well be high enough to sustain bdellovibrio populations. Moreover, only a fraction (>1 to a few percent) of the bacterial cells contained in environmental samples are typically amenable to cultivation. This fact has to be considered (Amann et al., 1995), as did Rice et al. (1998), who quantified the number of bdellovibrio-susceptible bacteria in an estuarine environment. Seventy to 85% of recovered bacteria were found susceptible to bdellovibrios isolated from the same sampling sites. Assuming that almost 10% of the bacteria in samples were retrieved on culture, the authors calculated that the susceptible populations were sufficient to ensure survival of the predators. Therefore, *Bdellovibrio* spp. may have a modulatory role in nature (Rice et al., 1998), eradication being prevented by natural oscillations in a predator-prey system (Varon, 1979).

Survival of bdellovibrios in nature has been the subject of conjecture because cell composition in bdellovibrios is known to change rapidly in starving conditions, and bacterial suspensions quickly lose viability (Marbach et al., 1976; Hespell et al., 1974), signifying that a free swimming cell must find a prey rapidly. However, bdellovibrios were shown to survive longer periods in nutrient-poor environments (Fry and Staples, 1976; Daniel, 1969), possibly due to population heterogeneity (Varon and Shilo, 1978) or better resilience of bdelloplasts (certainly as bdellocysts, although the number of strains able to develop this morphology seems to be small). The molecular responses to starvation are unknown.

Isolation

The peculiar cell cycle of *Bdellovibrio* dictates its method of isolation, whether from sewage, plant parts, submerged surfaces, or water samples. Basically, bdellovibrios and phages are isolated in a similar manner. The sample, or serial dilutions of it, is mixed with a potential prey bacterium in melted soft agar and poured onto an agar

plate containing a diluted growth medium. The bacterial predator forms plaques that have to be differentiated from those formed by protozoa or bacteriophages.

Relatively low levels of bdellovibrios are usually retrieved from environmental sources that contain much higher levels of unsusceptible bacteria and "blurring" through overgrowth results, which can interfere with the detection of bdellovibrio plaques. Moreover, bdellovibrio growth can be hindered by bacteria-excreted compounds as well as by bacteriophages. A number of protocols have been developed based on the physical separation of *Bdellovibrio* cells from the rest of the bacterial population, with the purpose of obtaining enriched fractions of the predator while reducing contaminating protozoa, bacteria, or viruses.

The number of bdellovibrio plaques detected in any environmental sample depends on the host bacterium chosen, the processing of the sample, and the isolation protocol. *Bdellovibrio* shows variation in host range, and no single bacterial species can potentially support the growth of all isolates. This problem is compounded by the lack of information on the structure and diversity of *Bdellovibrio* populations. However, *Vibrio parahaemolyticus* was shown to be an effective host for the retrieval of *Bdellovibrio* from estuarine environments (Schoeffield and Williams, 1990). *Pseudomonas* spp., *Aquaspirillum serpens*, a number of phytopathogens such as *Erwinia carotovora* sspp. *carotovora*, *E. amylovora*, *Xanthomonas oryzae* and *P. syringae* have been used to isolate soil and water bdellovibrios (Stolp and Starr, 1963; Scherff, 1973; Uematsu, 1980; Epton et al., 1989; Elsherif and Grosman, 1996).

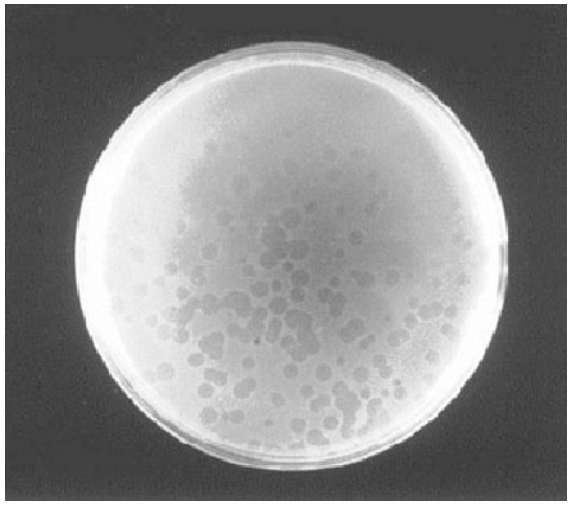

Fig. 6. Lytic plaques of *Bdellovibrio* on *Pseudomonas corrugata*.

Direct Isolation of *Bdellovibrio* from Environmental Samples

The most common approach is based on the use of one or more filtration steps with or without differential centrifugation of the sample analyzed. The compositions of the agar and soft agar can also vary. *Bdellovibrio* plaque development is favored in low-nutrient media, resulting in a higher plaque yield (Staples and Fry, 1973), but growth of contaminating bacteria is inhibited in plain agar medium (Uematsu, 1980).

Based on Stolp (1981): A water sample or 50-g soil in 500 mL of sterile buffer is shaken vigorously for 1 h, then centrifuged for 5 min at 2000g to remove gross particles. The supernatant is passed through a series of membrane filters of decreasing pore size (3.0, 1.2, 0.8 and 0.45 μm). Filtrates from the last two steps are serially diluted, and 100-μL aliquots are mixed with approximately 109 cells of the prey bacterium in molten soft top agar. The mixture is poured onto an agar plate and incubated at 28–32°C. Rapidly developing lytic regions visible after 24 h are the result of bacteriophage multiplication. These plaques are usually small and do not grow further. They should be marked to differentiate them from the slower growing bdellovibrios. Plaques becoming visible within two to three days and showing further expansion for a few more days are potentially bdellovibrios (Figure 6). Small and highly motile *Bdellovibrio* cells can be detected by examination of crushed plaque material in wet-mounts with a phase-contrast microscope.

Because only a fraction of the bdellovibrios is detected using this method, a number of variations have been proposed that increase the efficiency of recovery, thereby enabling more accurate quantification. From Uematsu (1980): Samples are collected, filtered through a 1.2-μm membrane filter, mixed with a phage-resistant host in molten top plain agar and plated on bottom plain agar. From Varon and Shilo (1970): The sample is centrifuged at 800 g for 5 min or passed through filter paper to remove coarse particles, then the bacteria are concentrated by centrifugation at 27,000 g for 20 min. The supernatant, which contains free bacteriophages, is discarded. The pellet is resuspended in a few mL and passed through a 1.2-μm membrane filter. Serial dilutions of the filtrate are then mixed with the prey in molten agar and plated on solid DN.

Sixty to 70% of bdellovibrios may be recovered with less than 1% of prey cells passing through the filter, as compared with 0.1% recovery with sequential filtration.

A second method, using a Ficoll gradient for purifying bdellovibrios from mixed samples, was also proposed by Varon and Shilo (1970): Samples are prepared as above but instead of being filtered, the resuspended pellet is layered on top of a 14-mL linear Ficoll gradient of 1 to 15% (Mw = 400,000) in DN, in 1.5 × 9.5 cm centrifugation tubes. The tubes are centrifuged horizontally in the cold at 1,620 g. The upper 3 mL contains 80 to 90% of the *Bdellovibrio* with less than 2% contamination, whereas the other bacteria concentrate lower along the gradient.

Low-nutrient media:

DN: (Diluted Nutrient, Seidler and Starr, 1969a) 0.08% Difco Nutrient Broth is supplemented with $CaCl_2 \cdot 2H_2O$, 2 mM, and $MgCl_2 \cdot 6H_2O$, 3 mM, after autoclaving, and pH is adjusted to 7.2 with 0.1 N NaOH.

YP (Yeast Extract Peptone, Stolp and Starr, 1963) contains 0.3% yeast extract, 0.06% peptone in 0.05 M Tris buffer, pH 7.5.

Plain agar (Uematsu, 1980): $MgCl_2 \cdot 6H_2O$, 20 mM; $CaCl_2 \cdot 2H_2O$, 30 mM, pH 6.5–8. Bottom agar: 1.2 to 1.5%. Top agar: 0.6%.

Plating: Aliquots of 4-mL molten top agar are kept at 42°C in a water bath prior to mixing with prey and sample suspensions.

Isolation of Marine Bdellovibrios

Halophilic or halotolerant bdellovibrios have been isolated from marine environments such as estuaries, sediments, coastal waters, open sea and submerged surfaces (Williams et al., 1995; Williams and Falkler, 1984; Williams, 1988; Taylor et al., 1974; Marbach and Shilo, 1978). The chemical composition of sea water has to be considered for isolating marine bdellovibrios. These organisms have a different G+C content than the terrestrial strains (Taylor et al., 1974) and their requirements for Na^+, K^+, and Ca^{2+} dictate the use of adapted media for their isolation (Marbach and Shilo, 1978). Marine bacteria such as *Vibrio parahaemolyticus* P-5 can be used as prospective hosts. Moreover, the relative low abundance of *Bdellovibrio* in these biotas may require concentration of large sample volumes.

From Marbach et al. (1976): Both direct and enrichment approaches can be used. If needed, the water samples are concentrated by centrifugation (10,000 g, 30 min) or collected on a 0.1-μm membrane. The concentrate is then mixed with a potential host bacterium in soft agar and poured on bottom agar. Alternatively, 5 mL of the sample is added to 3.3 mL of molten top agar having 0.7 mL of the prey suspension for a final agar concentration of 0.65%. The mixture is then poured onto large Petri dishes and incubated at 25°C (Schoeffield and Williams, 1990).

The medium used for isolating marine strains of *Bdellovibrio* should contain at least 25% sea water or appropriate salts.

Polypeptone (Pp 20) medium (Williams et al., 1982): Filtered ocean water, 1 L; Polypeptone, 1 g; agar, 15 g for bottom agar and 6.5 g for top agar; pH 7.7–7.8.

Synthetic marine salt solution (Marbach and Shilo, 1978): NaCl, 500 mM; KCl, 10 mM; $MgSO_4$, 25 mM; $MgCl_2$, 25 mM; $CaCl_2$, 10 mM.

Specific Enrichment for *Bdellovibrio*

Stolp (1968) devised a method yielding large numbers of *Bdellovibrio* plaques, thereby greatly facilitating the isolation of the predator on a specific host bacterium whenever quantification is not needed. This approach has been applied to obtain bdellovibrios lytic to *Rhizobium* and *Agrobacterium* (Parker and Grove, 1970), *Legionella* (Richardson, 1990) and *Azospirillum brasilense* from 2-years-stored air-dried soils (Germida, 1987).

Based on Stolp, modified by Ruby (1991): One-hundred milliliter aliquots of a dense suspension of the prospective prey bacterium (10^{10} cells·mL^{-1}) are prepared in DN medium or HM buffer in Erlenmeyer flasks. The sample (soil, 100 mg; sewage, 0.5 mL; river water, 1 mL) is added and the flasks are incubated on a rotary shaker. The suspension is examined daily over 2 to 4 days for lysis (reduction in optical density) and for the presence of small, highly motile presumptive bdellovibrio cells or bdelloplasts by phase contrast microscopy. If no bdellovibrios are apparent, the incubation can be prolonged or a 1-mL aliquot can be transferred into a fresh suspension of substrate bacterium. When bdellovibrios are detected, the enrichment culture is centrifuged for 5 min at 2000g (250g; Germida, 1987) and the supernatant filtered through a 0.45-μm membrane. Serial dilutions are plated on the prospective prey bacterium. Developing plaques are checked microscopically for bdellovibrios.

HM buffer: Hydroxyethyl piperazine-N′-2-ethanesulfonic acid, 25 mM, adjusted to pH 7.6 with NaOH and supplemented with $CaCl_2 \cdot 2H_2O$, 1 mM, and $MgCl_2 \cdot 6H_2O$, 1 mM.

Isolation of Host-Independent-Saprophytic *Bdellovibrio* Mutants

Wild-type bdellovibrios isolated from natural habitats are obligate parasites. However, host-independent (H-I) mutants can be recovered from all wild-type strains at a low frequency of 10^{-5} to 10^{-8} (Cotter and Thomashow, 1992a; Varon and Seijffers, 1975; Seidler and Starr,

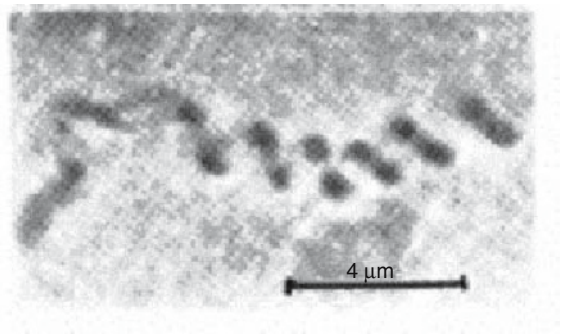

Fig. 7. Growth phase of a host-independent mutant of *B. bacteriovorus* 10^9D growing in enriched nutrient broth (From Eksztejn and Varon, 1977.)

1969b; Stolp and Starr, 1963). This suggests that the H-I phenotype results from a single mutational event. Although these axenic mutants are able to lyse substrate cells, they do so less efficiently than their wild-type counterparts (Cotter and Thomashow, 1992b; Varon and Seijffers, 1975; Stolp and Starr, 1963), and they tend to lose this ability completely after numerous transfers, seemingly the result of a second mutational event (Cotter and Thomashow, 1992b; Gray and Ruby, 1991). Host-independence may have pleiotropic effects as only partial phenotypic complementation can be achieved (Cotter and Thomashow, 1992b) and the mutants, contrary to the colorless wild-type cells, produce pigments protective against photooxidative damage (Friedberg, 1977).

The axenic mutants show strong proteolytic activities and are oxidase positive; most are catalase positive and possess a cytochrome *a* and *c* component (Seidler and Starr, 1969b; Diedrich et al., 1970; Stolp and Starr, 1963). Strikingly, the cell cycle of H-I mutants still shows the dimorphic pattern of filamentous growth, division, and differentiation (Figure 7; Seidler and Starr, 1969a). This observation has triggered many investigations of the possible involvement of prey-derived signals in the initiation or completion of the specific growth phases of the parasite.

From Shilo and Bruff (1965): A *Bdellovibrio* suspension (10^9 cells·mL^{-1}) is filtered through a 0.45-µm membrane. The filtrate is transferred to nutrient broth (NB) or DN broth (containing 10^8 heat-killed host cells) and incubated at 30°C with vigorous shaking. For growth on plates, solid NB or PY (peptone, 10 g; yeast extract, 3 g, pH 6.8) is supplemented with 1% autoclaved culture supernatant fluid of the same bdellovibrio strain grown in NB. In that case, a large (10^4 cells per plate) inoculum is required.

Although more exacting, the method described by Seidler and Starr (1969b) was successful with all isolates tested, contrary to the protocol of Shilo and Bruff (1965), which did not always yield axenic mutants:

1. Selection of Sm^r *Bdellovibrio*. The host-dependent (H-D) strain is grown in DN broth on a Sm^r host. After 24 h, streptomycin is added (50 µg·mL^{-1}) and the culture is incubated for another 12 h. Next, the H-D strain is heavily (15%, v/v) inoculated in DN broth with the Sm^r host in the presence of streptomycin. When a lysate is obtained, it is washed from the antibiotic by centrifugation, resuspended in DN broth, and filtered through a 0.45-µm membrane.

2. A Sm^s host suspension is inoculated with the filtrate. Five to 10 mL of the resulting lysate are transferred to the selection medium (DN or PY broth amended with 10^9 cells·mL^{-1} heat-killed prey cells and with streptomycin) and incubated for 3 to 6 days. Cultures are transferred twice in PY broth with a heavy inoculum (15 to 25%) and the H-I isolates can then be plated on solid PY.

Preparation of Pure Cultures

Pure cultures of *Bdellovibrio* are obtained in the manner of bacteriophages. A single plaque of the original isolation event is lifted, resuspended in HM buffer or in dilute medium, passed through a 0.45-µm filter, and serially diluted. Plating of the dilutions on the original host results in growth of plaques. The procedure is performed at least three times.

Identification

Bdellovibrios are best identified by morphological features along with the particular growth requirements of the bacteria, i.e. a cell cycle featuring an obligate, intraperiplasmic, developmentally separated stage inside the substrate bacterium, leading to the generation of progeny cells. The physiology of both host and parasite is intimately linked to the interaction between the two and is dealt with separately. No molecular markers have been developed to date that enable the detection and identification of bdellovibrios in samples without relying on isolation, cultivation, and morphological identification.

Morphology and Ultrastructure

After isolation, plaques have to be detected before filtrates can be examined under phase contrast for small, highly motile cells, swimming at a speed of up to 100 body-lengths per second (Stolp, 1967). Electron microscopy of subsequent lysates reveals small, vibrioid or rod-shaped bacteria. The size of bdellovibrio can

vary between 0.25 and 0.40 μm in width and 1 and 2 μm in length (Ruby, 1991), but smaller cells (0.2 μm, width; 0.8 μm, length) have been reported (Pan et al., 1997). The cell is propelled by a long (up to 30 μm), thick-sheathed polar flagellum. The sheath is an extension of the outer membrane but is of different biochemical composition (Thomashow and Rittenberg, 1985). To date and under the proper conditions, only two isolates have been shown to enter the bdellocyst stage.

The *Bdellovibrio* Cell Cycle

Chemotaxis Attack-phase bdellovibrios in liquid culture have been shown to lose viability rapidly (Hespell et al., 1973). Therefore, the steps leading to attachment and penetration within the host cell are crucial. No chemotactic responses towards potential hosts, their exudates, or their lysates have been detected (Straley and Conti, 1977; LaMarre et al., 1977). However, positive and negative chemotaxis was measured for numerous compounds including organic and amino acids, sugars and inorganic ions, but very different responses were obtained with different *Bdellovibrio* strains (Straley et al., 1979). All the tested strains showed aerotaxis. Because nonhalophilic and halophilic bdellovibrios have been shown not to grow or to grow less, respectively, in microaerobic conditions (Schoeffield et al., 1996), aerotaxis lets the cells find optimal niches. Similarly, the chemotactic responses may drive the predator towards ecological niches rich in prey (LaMarre et al., 1977) or may help the cells find compounds that alleviate starvation in the extracellular environment (Varon and Shilo, 1978).

Because there is no active "marauding," the process of finding prey appears to be based on random collisions and therefore on the concentration of both predator and prey cells. At low prey density (10^4 cells·mL^{-1}), a high initial predator : prey ratio (1 : 250) is necessary to obtain significant predation (Varon and Zeigler, 1978).

In pelagic habitats, many bacteria are attached to particles, and clusters of bacteria continuously form and disperse around patches centered on the lysis of a large cell or protozoal excretion (Caldwell, 1977; Blackburn et al, 1998). Chemotaxis towards organic compounds may indicate bacteria-rich niches and therefore be helpful in bringing bdellovibrios in proximity to potential prey.

Attachment Attachment to substrate cells is affected by many factors such as the composition and the pH of the medium, oxygen tension, and temperature (Varon and Shilo, 1968). It is reversible within the first minutes of an encounter, but agitation is unable to separate attached bdellovibrios after 20 to 30 min (Varon and Shilo, 1968). Reversible attachment to non-hosts as well as to inorganic surfaces has been reported (Varon and Shilo, 1978). Although completely different in their mechanisms, two-stage attachment processes (the first reversible, the second irreversible) are required in many bacteria-host systems: *Agrobacterium tumefaciens* for DNA transfer into a plant cell; *Rhizobium* for cell invasion (Vande Broek and Vanderleyden, 1995) and colonization of plant surfaces (Romantschuk, 1992); and pathogenic *E. coli* for penetration into epithelial cells (Mulvey et al., 1998). Recognition sites on the surface of the bacterial prey of bdellovibrios are still uncharacterized. *B. bacteriovorus* 109D and *B. stolpii* UKi2 interacted differently with various components of the host cell's outer membrane. Whereas strain 109D specifically required core sugar of the host's lipopolysaccharide for irreversible attachment to occur, *B. stolpii* UKi2 seemed to require specific proteins to interact with its prey (Schelling and Conti, 1986).

Corroborating the involvement of specific moieties in the recognition process, detailed electron microscopy revealed that, upon attachment, the nonflagellated pole has strands believed to mediate the attachment of parasite to host protoplast, even before a pore is created. The parasite is subsequently anchored to the host protoplast (Figure 8; Abram et al., 1974).

Two extracellular components of bacterial cells have been shown to exert opposite effects on the attachment of bdellovibrios. Bacterial capsules did not hinder *B. bacteriovorus* 109J to infect *E. coli* cells (Koval and Bayer, 1997), but the proteinaceous S-layer of *Aquaspirillum ser-*

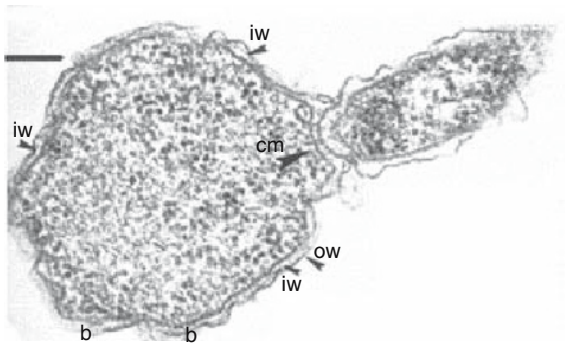

Fig. 8. Penetration of *B. bacteriovorus* 10^9 into an *E. coli* cell. The penetration pole is flattened against the host cytoplasmic membrane. b: bulges in the host membranes. cm: cytoplasmic membrane. iw: peptidoglycan layer. ow: outer membrane. The penetrating parasite shows constriction at the entry pore. (From Abram et al., 1974.)

pens, *A. sinuosum*, and *Aeromonas salmonicida* played a protective role, rendering the bacteria resistant to penetration by bdellovibrios (Koval and Hynes, 1991).

Penetration Penetration is completed within 5 to 20 min after attachment. It may involve mechanical "drilling" through the prey's membranes (Stolp, 1981), although enzymatic action is essential (Physiology). The inhibition of invasion by streptomycin (Varon and Shilo, 1968) supports the involvement of a tightly regulated response to initial attachment and production of enzymes. On the other hand, penicillin has no effect on invasion but inhibits intraperiplasmic multiplication (Varon et al., 1969). The driving force for entry may be generated by fluxes of water entering the infected host and causing a differential expansion of the host protoplast and cell wall, leading to their separation. The bdellovibrio cell, which is bound to the cytoplasmic membrane, is then passively pulled into the periplasm, shedding its flagellum in the process. During attack and penetration, the prey-cell's membrane-derived oligosaccharides are lost to the extracellular medium and are not used by the predator (Ruby and McCabe, 1988).

Intraperiplasmic Growth The invaded periplasm offers a protected environment for the predator to enter a new developmental stage characterized by elongation into a spiraling cell, DNA replication, and finally fragmentation of the filament by the formation of septa and the biosynthesis of one flagellum per progeny cell. The ghost host cell is then lysed by the action of lytic enzymes, and the progeny is released. The filamentous cell developing at the expense of the host's cytoplasmic content grows in proportion to the size of the prey (Kessel and Shilo, 1976) and exhibits a very efficient energy metabolism. Therefore, the number of progeny varies, from 5.7 per *E. coli* (Seidler and Starr, 1969b) to 20 to 30 cells in *Aquaspirillum serpens* (Stolp, 1967). In liquid culture, the initial cell cycle is completed within 2 to 4 h, depending on the age of the bdellovibrios (Varon et al., 1969). The signals leading to initiation of growth within the periplasm, cell elongation, division, and differentiation into attack phase cells have been sought, but a consistent picture of their mode of action has not been obtained.

Bdellocysts Under special growth conditions, bdellovibrios enter a cyst-like stage. The formation of bdellocysts has been reported in two strains, one of which (strain W) is the subject of a number of investigations (Burger et al., 1968; Hoeniger et al., 1972; Tudor and Conti, 1977; Tudor and Conti, 1978). Bdellocysts of strain W developing on *Rhodospirilum rubrum* mature within 4 h. The predator enlarges into a kidney-shaped cell enclosed by a structureless, amorphous outer layer. A finely particulate inner layer surrounds the more particulate plasma membrane of the predator cell. Structures resembling storage granules are present (Figure 9). Bdellocysts are more resistant than vegetative cells to dessication, high temperatures, and sonication (Tudor and Conti, 1977), and their germination is favored by L-glutamate, K^+, and NH_4^+ (Tudor and Conti, 1978).

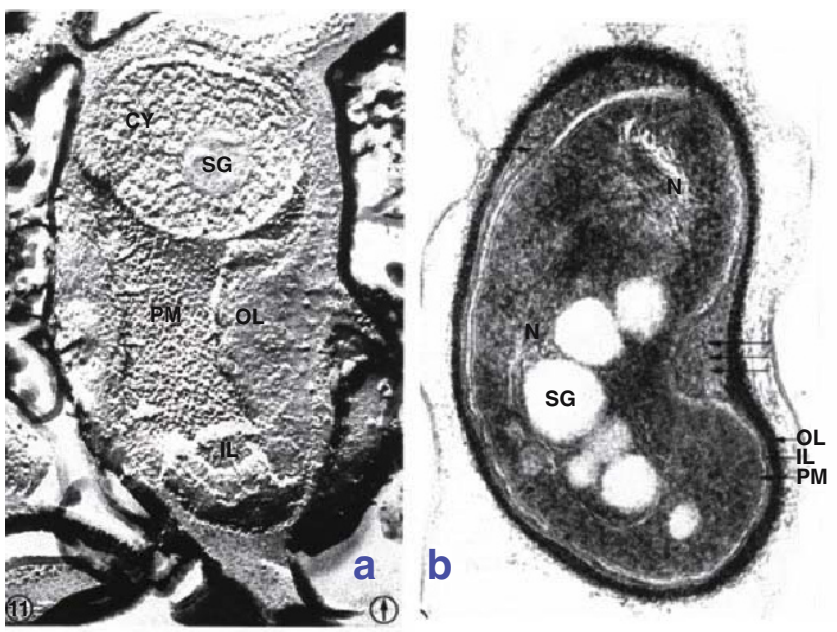

Fig. 9. Mature resting body of *Bdellovibrio* sp. strain W in *Rhodospirillum rubrum*. Panel a: Freeze-etching, × 173,000. Panel b: Negative staining, × 150,000. OL: outer layer. IL: inner layer. PM: particulate plasma membrane. CY: granular cytoplasm. SG: storage granule. Arrows: infolding of the inner layer. N: fibrillar nucleoplasm. (From Hoeniger et al., 1972.)

Cultivation

The cultivation of bdellovibrios in a diluted broth or buffer suspension of host cells routinely yields high concentrations of the predator (10^9 cells·ml^{-1}). However, these suspensions rapidly lose their viability because bdellovibrios have very high endogenous respiration rates (Hespell et al., 1973). Therefore, aerating cultures on a rotary shaker and filling flasks only to one third of their volume is strongly recommended. Viability of the predators can be augmented by adding 5 mM glutamate to the fresh lysates.

Propagation of *Bdellovibrio*

One single plaque with a small piece of its surrounding unlysed prey cell lawn is excised from a Petri dish, resuspended in 10 to 15 mL of a diluted medium, such as DN broth or YP, and shaken at 30°C for 24 to 48 h. The prey cells first multiply, creating a suspension dense enough to enable a high yield of bdellovibrios. This first lysate can then be subcultured.

Routine Culture

HM buffer or DN broth (30–40 ml) is inoculated with freshly grown, stationary phase host suspension to a final concentration of 10^8 to 10^9 cells·ml^{-1} and 100 to 200 µl of lysate. Overnight incubation on a rotary shaker usually lyses all the prey cells. The resulting bdellovibrio suspension can then be supplemented with 5 mM glutamate and stored at 4°C.

Large Scale Culture

According to Ruby (1991): In a 500-mL Erlenmeyer flask, 50 to 100 µl of a fresh *B. bacteriovorus* 109J lysate are added to 200 ml of DN broth amended with 40 ml of an *Escherichia coli* ML35 stationary phase culture, totalling 1.6×10^{11} cells. The flask is shaken overnight and total lysis is observed under a phase-contrast microscope. The culture is cooled on ice, centrifuged at 700g for 15 min, and the pellet discarded. The supernatant, containing most of the attack-phase bdellovibrios, is concentrated at 10,000g for 15 min. The pellet is resuspended in HM buffer, harvested again by centrifugation, and resuspended in HM buffer. The yield should be about 6×10^{11} cells.

Single-Cycle Growth of Synchronous Cultures

Synchronous cultures are tools essential for investigating developmentally regulated events in the cell cycle of bdellovibrios. A 2 : 1 attack-phase predator-to-prey ratio enables concomitant attack of all host cells within minutes. By separating the bdelloplasts from excess attack phase cells, a uniform synchronous single-cycle suspension is obtained. The developing intraperiplasmic bdellovibrio cells can be prematurely released from the bdelloplast for further analysis. This technique can be used to study the cell cycle of wild type cells, which are known to differ from their host-independent variants.

From Thomashow and Rittenberg (1978a) and McCann et al. (1998): *E. coli* cells are grown into stationary phase in Luria or nutrient broth, centrifuged at 5,000g for 10 min, washed once with cold HM buffer, and then resuspended in the same buffer. A fresh lysate of attack phase *B. bacteriovorus* 109J is harvested in the cold by centrifugation at 10,000g for 20 min and treated as the host suspension. Combining 10^{10} predator with 5×10^9 prey cells·ml^{-1} results in a synchronous, single-cycle culture in which host penetration occurs in less than 30 min and the cycle is complete in 3.5 h. Because the culture is very dense, proper aeration has to be ensured by vigorous shaking of the flask.

Premature Release from Bdelloplasts

From Ruby (1991): Bdelloplasts of *B. bacteriovorus* 109J can be obtained from a synchronous culture at any time in its development by cooling the culture on ice, removing the unattached, attack-phase cells by centrifugation at 1,100g for 5 min at 0°C, and resuspending the pellet by vigorous vortexing in cold HM buffer. This step is repeated twice to yield a virtually attack-phase bdellovibrio-free bdelloplast pellet as observed by phase-contrast microscopy. The pellet is warmed to room temperature, resuspended in 2 volumes of 10 mM EDTA in 120 mM Tris-hydrochloride (pH 7.6), and incubated at 30°C for 3 min to permeabilize the outer membrane. The bdelloplasts become transparent and the envelopes are lysed when 10 volumes of lytic enzyme concentrate are added and then incubated at 30°C for 15 to 20 min. The suspension is cooled to 0°C, centrifuged at 10,000g for 20 min, and resuspended in 1 ml HM buffer. The released bdellovibrios can then be purified on a Ficoll gradient in HM buffer. The band is removed from the tube and washed three times in HM buffer at 0°C (Ruby and Rittenberg, 1983). The use of a diaminopimelic acid (DAP)-deficient mutant host results in higher yields of released bdellovibrios. Culture of this mutant host in DAP-deficient medium for one doubling before harvest will weaken its envelope.

Preparation of a Lytic Enzyme Concentrate

From Ruby and Rittenberg (1983): Progeny bdellovibrios from 3 to 4 liters of a synchronous culture (300 ml in a 2-liter flask) are separated from the dithiothreitol (50 $\mu g \cdot ml^{-1}$) amended lytic solution by two successive centrifugations at 10,000g for 20 min. The supernatant is concentrated 60 times by pressure filtration using a membrane with a molecular cut-off of 30,000 daltons. The concentrate is centrifuged at 10,000g for 20 min and 0.5% polyethylene glycol is added. Aliquots are frozen in liquid nitrogen and stored at –70°C.

Preservation

Bdellovibrios can be kept as plaques on solid medium at 4°C for 1 to 2 weeks. Lysates amended with 5 mM glutamate and conserved at 4°C should be transferred every few weeks. For long-term conservation, aliquots of a fresh lysate are concentrated to half their original volume in freeze-resistant tubes and glycerol is added to a final concentration of 25%. The tubes are quickly frozen in liquid nitrogen and kept at –80°C. To revive the culture, thawed aliquots are serially diluted and plated on double agar seeded with the appropriate host. Alternatively, storage as bdelloplasts also maintains viability for long periods (Stolp, 1981): one ml of host bacteria is added to 5 ml of fresh lysate; the final concentrations of bdellovibrio and host bacteria are equal. The mixture is incubated for 30 min on a shaker to produce a high density of invaded prey cells. Glycerol is added to a final concentration of 10% and the vials are conserved in a deep freezer at –70°C.

Physiology

Upon attachment of attack phase bdellovibrios, the host cell is rapidly immobilized (Stolp and Starr, 1963). Major physiological features change quickly in the host, following an orchestrated sequence, even before the initiation of intraperiplasmic growth by the predator: the prey cell stops respiring, its peptidoglycan changes (Thomashow and Rittenberg, 1978a; 1978b; 1978d), the cell loses control over uptake, secretion, and synthesis (Varon et al., 1969; Rittenberg and Shilo, 1970), and its nucleic acids are degraded (Matin and Rittenberg, 1972; Hespell and Odelson, 1978; Hespell et al., 1976). Bdellovibrios, in contrast to viruses and most intracellular parasites, do not depend on the metabolism of their host: they normally proliferate in ultraviolet irradiated- or heat-killed prey cells (Varon et al., 1969). However, the use of prey is highly organized: degradation of the substrate cell leads to precursors that are used directly as building blocks, which drives an efficient coupling between anabolic and catabolic processes. The result is a highly efficient energy metabolism.

Lytic-Enzymatic Activities

Host morphology changes dramatically upon penetration by bdellovibrio: the host becomes a bdelloplast, which is usually round. Penetration of noncovalently bound outer-membrane components can be explained by the action of mechanical forces. However, for pore formation and passage through the peptidoglycan to occur, covalent bonds must be ruptured by enzymes (Fackrell and Robinson, 1973; Huang and Starr, 1973). The lytic activities must permit penetration on the one hand and prevent premature lysis of the host on the other. Rapid solubilization of peptidoglycan, which is the result of differentially regulated glycanase and peptidase activities, has been demonstrated during penetration, the free diaminopimelic acid (DAP) and the amino sugar being released to the extracellular medium (Thomashow and Rittenberg, 1978a). Free DAP is then rapidly incorporated into the peptidoglycan of the bdelloplast by a soluble enzyme activity only present during intraperiplasmic growth (Araki and Ruby, 1988). A lipopolysaccharidase is also active during invasion. The glycanase is inactivated rapidly by peptidoglycan deacetylation, while peptidase activity continues at a reduced pace throughout the bdelloplast stage (Thomashow and Rittenberg, 1978a; 1978c). Prevention of bdelloplast superinfection by other bdellovibrios has been attributed to deacetylation of the peptidoglycan backbone (Thomashow and Rittenberg, 1978d). Acylation of the host's peptidoglycan with long-chain fatty acids may stabilize the outer membrane of the bdelloplast, although the peptidoglycan is not essential for its osmotic stability (Thomashow and Rittenberg, 1978b; 1978c). Many hosts do not round up. Because strain W infected cells and heat-killed substrate cells, which do not round, have no detectable glycanase, Tudor et al. (1990) concluded that glycanase activity is responsible for the formation of a round bdelloplast, but deacetylation is not required for exclusion. A second burst of lytic activity takes place at the end of the intraperiplasmic growth phase, the newly synthesized enzymes degrading the remaining peptidoglycan (Thomashow and Rittenberg, 1978a; Fackrell and Robinson, 1973; Huang and Starr, 1973).

Energy Metabolism

At least some strains of bdellovibrios have a very high endogenous respiration rate, seven times the rate of *E. coli*, that increases little during the growth phase (Rittenberg and Shilo, 1970; Hespell et al., 1973).

Unlike most bacteria, free swimming and intraperiplasmically growing bdellovibrios have their endogenous respiration partially or totally shut-off by acetate and glutamate, respectively, and by mixtures of amino acids, and without increase in respiration rate (Hespell et al., 1973). This may be due to saturation of the tricarboxylic acid cycle or oxidative phosphorylation capacities (Hespell, 1976: Hespell et al., 1973). These findings and the inability of bdellovibrios to utilize (and possibly transport) sugars (Ruby, 1989) suggest substrate-level phosphorylation is not a major energy source (Rittenberg and Hespell, 1975a). Notably, bdellovibrios can use nucleoside monophosphates and ATP from the substrate cell or an exogenous source directly, and they may also use prey-derived phospholipids (Ruby and McCabe, 1986; Ruby et al., 1985; Rittenberg and Langley, 1975b). During the intraperiplasmic stage, the parasite DNases degrade the substrate cell's DNA in a timely regulated manner, providing the parasite with most of the nucleosides needed for growth (Rosson and Rittenberg, 1979; Matin and Rittenberg, 1972). The other nucleosides come from RNA breakdown and ribonucleotide and deoxyribonucleotide conversions (Rosson and Rittenberg, 1981), inasmuch as bdellovibrios seem unable to synthesize nucleotide denovo (Engelking and Seidler, 1974).

Rittenberg and Hespell (1975a) calculated that 16% of the total energy requirement for growth could be saved if the predator used intact prey fatty acids instead of denovo synthesizing fatty acids from acetate. Indeed, bdellovibrios growing intraperiplasmically are capable of totally or partially conserving substrate cell fatty acids and possibly phospholipids, thereby greatly reducing their energy need (Kuenen and Rittenberg, 1975).

Similarly, fatty acids and hexosamines from the lipopolysaccharide (LPS) of the prey's outer membrane were retrieved in large proportions from the LPS of intracellularly growing bdellovibrios (Nelson and Rittenberg, 1981a). Thin layer chromatography resolved lipid A isolated from the predator into two fractions shown to contain an unequal distribution of fatty acids originating from both substrate and bdellovibrio cells (Nelson and Rittenberg, 1981b).

Another peculiar feature of the bdellovibrio physiology is its unbalanced growth. During the growth phase, the DNA concentration increases faster than the protein concentration, the original ratio being restored by protein synthesis, which continues after DNA replication stops (Gray and Ruby, 1989). This property may permit the generation of the largest number of progeny with a minimal cell mass (Gray and Ruby, 1989).

This extraordinarily efficient metabolism, combining both scavenging and a finely regulated degradation of the substrate cell, can explain the outstanding energy efficiency of intraperiplasmically growing bdellovibrios, which were shown to achieve a YATP (biomass yield per ATP molecule expended) of 26 when compared with 10.5 for bacteria grown on rich medium (Rittenberg and Hespell, 1975a).

Transport

Outer Membrane Proteins Little is known about the transport capacities of *Bdellovibrio*, including its export pathways that involve breakdown of prey cytoplasm by predator proteases and nucleases and its import pathways that use the breakdown products and other solutes. Secretion of bdellovibrio products may involve export pathways designated as types I, II and III (Genin and Boucher, 1994; Schatz and Dobberstein, 1996) or other mechanisms. Interestingly, type III secretion pathways are found in plant and animal pathogens and are involved in the secretion of peptides directly into the host's cytoplasm (Finlay and Cossart, 1997; Baker et al., 1997).

More data are available on uptake routes. As described in energy metabolism, bdellovibrios use phosphate-ester containing molecules obtained from the prey. The molecules are transferred to the parasite, and in the case of phosphate nucleosides, transport seems to involve two different systems, one specific for the growth and the other for the free-living stage (Ruby and McCabe, 1986; Ruby et al., 1985). Inorganic phosphorus transport is low and reflects the lack of need for this element, which is obtained in organic form directly from the prey (Ruby and McCabe, 1986). Sugar transport pathways are similarly lacking (Ruby and McCabe, 1988). Mechanisms of fatty acid and lipopolysaccharide incorporation into bdellovibrio molecules are not known.

The very efficient metabolism of bdellovibrios is epitomized by the transfer of molecules that move solute: outer membrane proteins (OMPs) are routed to the outer membrane of the predator and cytoplasmic membrane of the prey (Diedrich et al., 1983; 1984; Talley et al., 1987; Tudor and Karp, 1994). The structure of the relocated proteins seems to be conserved in that

OmpF protein of *E. coli* transferred to the outer membrane of *B. bacteriovorus* 109D during intraperiplasmic growth had unaltered function in colicin A bdellovibrio-killing experiments (Diedrich et al., 1983). The process of acquiring outer membrane porin-like molecules from the substrate cell has limited adaptability. In OmpF mutants, the OmpC protein was transferred to the predator, although a clear preference for OmpF was detected when wild-type strains were the prey (Diedrich et al., 1984). However, other porin-like proteins were not found in the bdellovibrio outer membrane. Furthermore, it could be demonstrated that the protein relocalization of bdellovibrios is influenced by both the prey (smooth versus rough strains produce different results) and the history of the cultured predator (Talley et al., 1987).

The acquired proteins may play a role in the passive uptake of nutrients in the free-living phase of *Bdellovibrio* (Diedrich et al., 1983). Alternatively, proteins relocated to the host's cytoplasmic membrane may be responsible for the increased permeability of this membrane to small hydrophilic molecules and ions (Rittenberg and Shilo, 1970; Cover et al., 1984a). Thus, breakdown products from the cytoplasm might diffuse to the periplasmic space to be taken up by the predator (Tudor and Karp, 1994).

Properties of Bdelloplast and of Predator Membranes Change in the cell wall structure of the prey upon attack by *Bdellovibrio* is not limited to its peptidoglycan. The cell surface becomes more hydrophobic (Cover and Rittenberg, 1984b), maybe as a result of peptidoglycan modification with long-chain fatty acids (Thomashow and Rittenberg, 1978b) or cleavage of glucosamine residues from the substrate cell lipopolysaccharide (Thomashow and Rittenberg, 1978a). The cytoplasmic membrane of the substrate cell becomes permeable to lactose and small hydrophilic molecules (Rittenberg and Shilo, 1970; Cover et al., 1984a). However, the permeability of the outer membrane layer to small hydrophilic molecules is not altered, and most periplasmic proteins are retained (Odelson et al., 1982; Cover et al., 1984b).

Signaling The dimorphic cell cycle of bdellovibrios, which include an obligatory intracellular cell multiplication stage, is highly ordered, and physiology involved in attachment, penetration, and intraperiplasmic growth is highly regulated. The transition between the phases of the cell cycle seems to involve signals, whose function and nature are still elusive. It is obvious that during the cell cycle important changes occur in gene expression and protein synthesis. It was recently demonstrated by two-dimensional gel analysis of synchronous cultures that protein synthesis is affected by the growth stage of the cell: more than 30 polypeptides falling into nine kinetic groups were detected (McCann et al., 1998).

Wild-type bdellovibrios cultured on complex media amended with extracts obtained from prey cells have a developmental cycle similar to the one occurring intraperiplasmically (Friedberg, 1978; Horowitz et al., 1974; Reiner and Shilo, 1969; Introduction) and in host-independent (H-I) mutants. Bdellovibrios, obtained by premature release from bdelloplasts before the onset of DNA replication, differentiate into attack cells but complete their DNA synthesis round only if DNA replication has been initiated. The addition of host cell extracts enables initiation of DNA replication (Ruby and Rittenberg, 1983; Gray and Ruby, 1990). In an extensive review on the subject, Gray and Ruby (1991) proposed a two-signal model, the first triggering the differentiation of attack-phase bdellovibrios into growth-phase cells, the second initiating and sustaining subsequent rounds of DNA replication. Attempts at purifying the early and late putative signal molecules from prey cells were not conclusive, although these molecules were shown to be nondialyzable, heat stable, DNAse resistant, and possibly proteinaceous (Gray and Ruby, 1990; Friedberg, 1978; Horowitz et al., 1974; Reiner and Shilo, 1969). Interestingly, axenic growth of prey-dependent bdellovibrios in a rich medium without prey extracts can be triggered by heat shock, eliminating the need for a growth inducing signal. Heat shock may trigger changes in gene transcription (Gordon et al., 1993).

In contrast to prey-derived signals, Eksztejn and Varon (1977) detected a cell-density dependent, division-promoting activity identified as an apparent cyclic peptide produced by bdellovibrios during the middle to late growth stages.

The influence of cell density was already noted by Shilo and Bruff (1965), who reported colony growth of H-I mutants of *B. starrii* only with a high density inoculum. More recently, Cotter and Thomashow (1992a) and (1992b) reported that H-I mutants could be differentiated into two classes, defined as type I and type II: type I develop small colonies growing close one to the other, and type II exhibit large, density-independent growth. Type I mutants arise at a frequency of 10^{-6} to 10^{-7} and type II at a frequency of 10^{-8} to 10^{-9}. The latter may result from a double mutation, lending support to the two-signal hypothesis (Thomashow and Cotter, 1992). Type II mutants could be partially com-

plemented by a 1 kilobase fragment (the hit locus) containing four putative open reading frames of unknown function and not homologous to any sequence in the databases (Cotter and Thomashow, 1992b).

Cell-cell communication may therefore exist in bdellovibrios but its nature, whether peptidic like in *Myxococcus* (Dworkin, 1996), N-acyl-L-homoserine lactone based, quorum-sensing (Dunlap, 1997), or other, is unknown.

Applications

The characteristic life-style of bdellovibrios makes them attractive candidates for a number of applications concerned with reducing or modulating bacterial populations, i.e. biological control of pathogens, water purification, and biofilm control.

Surprisingly little emphasis has been put on studying the possible role of *Bdellovibrio* in agricultural applications: Bdellovibrios efficiently eradicated *Xanthomonas oryzae* from rice paddy field water and caused rapid decline in populations of *E. carotovora* sspp. *carotovora* in soil (Uematsu, 1980). *Bdellovibrio* isolates from the rhizosphere of soybean were used to control bacterial blight caused by *Pseudomonas glycinea* (Scherff, 1973). Reduction in disease severity and systemic symptoms were significant.

An attempt was made to control soft rot and black leg of potato but mixed results were obtained (Epton et al., 1989).

That bdellovibrios might be deleterious to plant growth-promoting rhizobacteria was suggested by Germida (1987), who isolated bdellovibrios parasitic to *Azospirillum brasilense* from soils and by Elsherif and Grossman (1996), who could show an increase in bdellovibrio counts from the rhizosphere of plants inoculated with a beneficial strain of *Pseudomonas fluorescens*.

A role for *Bdellovibrio* in the self-purification of natural water systems has been suggested (Daniel, 1969; Fry and Staples, 1976). Because bdellovibrios are highly sensitive to certain chemicals and metals, they can be used as indicators for those compounds in water (Varon and Shilo, 1981).

A few attempts have been made to use bdellovibrios in animal models: Nakamura (1972) effectively treated *Shigella flexneri*-induced keratoconjunctivitis in rabbit with bdellovibrio, but bdellovibrio used as predator against pathogens in the intestinal tract of rabbits was unsuccessful (Westergaard and Kramer, 1977). It has been proposed that because of its unique physiology *Bdellovibrio* may be a source of novel chemicals and biochemicals (Ruby, 1991), although the evidence is unpublished. Undeniably, this intriguing bacterial group is a great model for the study of a myriad of subjects central to biology, e.g. predator-prey interactions, host recognition, development of prokaryotes, and cell-cell signaling.

Conclusion

Although much has been done to understand the ecology and the biology of bdellovibrios, one can still ask whether bdellovibrios play a role in determining the structure of bacterial populations. How do they survive? How is their cell cycle regulated, and what special functions are needed to complete it? Those are few of the very basic questions still left unanswered.

The advent of genomics should greatly improve our understanding of bdellovibrio biology and thereby its relation with the environment. Looking at the metabolic properties of this particular microbial predator, a few comparisons with other organisms can already be drawn: transport of exogenous phosphorylated nucleosides, sometimes accompanied by a marked reduction in de novo nucleotide biosynthesis capabilities, has been shown in intracellular parasites of eucaryotic cells such as *Rickettsia*, *Mycoplasma* and, *Chlamydia* (Stephens et al., 1998; Fraser et al., 1995; Moulder, 1991). This type of adaptation points to converging phenotypic evolution between intracellular parasites, although the biochemical mechanisms widely differ. Similarly, it will be possible to assess the dynamics of genome evolution and reduction by looking for "missing" genes and for the ones pointing to the particular lifestyle of this organism when the *Bdellovibrio* genome becomes available.

Literature Cited

Abram, D., Castro e Melo, J., and D. Chou. 1974. Penetration of Bdellovibrio bacteriovorus into host cells. J. Bacteriol. 118:663–680.

Althauser, M., Samsonoff, W.A., Anderson, C., and S.F. Conti. 1972. Isolation and preliminary characterization of bacteriophages for Bdellovibrio bacteriovorus. J. Virol. 10:516–524.

Amann, R., Ludwig, W., and K.H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual cells without cultivation. Microbiol. Rev. 59:143–161.

Araki, Y., and E.G. Ruby. 1988. A soluble enzyme activity that attaches free diaminopimelic acid bdelloplast peptidoglycan. Biochem. 27:2624–2629.

Baker, B., P. Zambrisky, B. Staskawicz, S.P. Dinesh-Kumar. 1997. Signaling in plant-microbe interactions. Science 276:726–733.

Burger, A., Drews, G., and R. Ladwig. 1968. Wirtskreis und infecktionscyclus eines neu isolierten Bdellovibrio bacteriovorus-stammes. Arch. Mikrobiol. 61:261–279.

Blackburn, N., Fenchel, T., and J. Mitchell. 1998. Microscale nutrient patches in planktonic habitats shown by chemotactic bacteria. Science 282:254–256.

Caldwell, D.E. 1977. The planktonic microflora of lakes. CRC Crit. Rev. Microbiol. 5:305–370.

Cotter, T.W. and M.F. Thomashow. 1992a. A conjugation procedure for Bdellovibrio bacteriovorus and its use to identify DNA sequences that enhance the plaque-forming ability of a spontaneous host-independent mutant. J. Bacteriol. 174:6011–6017.

Cotter, T.W. and M.F. Thomashow. 1992b. Identification of a Bdellovibrio bacteriovorus genetic locus, hit, associated with the host-independent phenotype. J. Bacteriol. 174:6018–6024.

Cover, W.H., Martinez, R.J., and S.C. Rittenberg. 1984a. Permeability of the boundary layers of Bdellovibrio bacteriovorus 109J and its bdelloplasts to small hydrophobic molecules. J. Bacteriol. 157:385–390.

Cover, W.H., and S.C. Rittenberg. 1984b. Change in the surface hydrophobicity of substrate cells during bdelloplast formation by Bdellovibrio bacteriovorus 109J. J. Bacteriol. 157:391–397.

Daniel, S. 1969. Etude de l'influence de Bdellovibrio bacteriovorus dans l'auto-epuration marine. Rev. Intern. Ocean. Med. 15–16:61–102.

Diedrich, D.L., Portnay Duran, C., and S.F. Conti. 1984. Acquisition of Escherichia coli outer membrane proteins by Bdellovibrio sp. strain 109D. J. Bacteriol. 159:329–334.

Diedrich, D.L., Portnoy, C.A., and S.F. Conti. 1983. Bdellovibrio possesses a prey-derived OmpF protein in its outer membrane. Curr. Microbiol. 8:51–56.

Diedrich, D.L., Denny, C.F., Hashimoto, T., and S.F. Conti. 1970. Facultatively parasitic strain of Bdellovibrio bacteriovorus. J. Bacteriol. 101:989–996.

Donze, D., Mayo, J.A., and D.L. Diedrich. 1991. Relationships among the bdellovibrios revealed by partial sequences of 16S ribosomal RNA. Curr. Microbiol. 23:115–119.

Dunlap, P.V. 1997. N-acyl-L-homoserine lactone inducers in bacteria: unity and diversity. Shapiro, J.A., and M. Dworkin. Bacteria as multicellular organisms. Oxford University Press. Oxford, 69–106.

Dworkin, M. 1996. Recent advances in the social and developmental biology of the myxobacteria. Microbiol. rev. 60:70–102.

Eksztejn, M., and M. Varon. 1977. Elongation and cell division in Bdellovibrio bacteriovorus. Arch. Microbiol. 114:175–181.

Elsherif, M., and F. Grossmann. 1996. Role of biotic factors in the control of soil-borne fungi by fluorescent pseudomonads. Microbiol. Res. 151:351–357.

Engelking, H.M., and R.J. Seidler. 1974. The involvement of extracellular enzymes in the metabolism of Bdellovibrio. Arch. Microbiol. 95:293–304.

Epton, H.A.S., Walker, N.M., and D.C. Sigee. 1989. Bdellovibrio: a potential control agent for soft rot and black leg of potato. Klement, Z.. Plant pathogenic bacteria – Part A. Akademia Kiado. Budapest, 207–212.

Fackrell, H.B., and J. Robinson. 1973. Purification and characterization of a lytic peptidase produced by Bdellovibrio bacteriovorus 6–5-S. Can. J. Microbiol. 19:659–666.

Finlay, B.B., and P. Cossart. 1997. Exploitation of mammalian host cell functions by bacterial pathogens. Science 276:718–725.

Fraser, C.M., Gocayne, J.D., White, O., Adams, M.D., Clayton, R.A., Fleischmann, R.D., Bult, C.J., Kervalage, A.R., Sutton, G., Kelley, J.M., Fritchman, J.L., Weidman, J.F., Small, K.V., Sandusky, M., Fuhrmann, J., Nguyen, D., Utterback, T.R., Saudek, D.M., Phillips, C.A., Merrck, J.M., Tomb, J.F., Dougherty, B.A., Bott, K.F. Hu, P.-C., Lucier, T.S., Peterson, S.N., Smith, H.O., Hutchinson III, C.A. and J.C. Venter. 1995. The minimal gene complement of Mycoplasma genitalium. Science 270:397–403.

Fratamico, P.M., and P.H. Cooke. 1996. Isolation of bdellovibrios that prey on Escherichia coli 0157:H7 and Salmonella species and application for removal of prey from stainless steel surfaces. J. Food Saf. 16:161–173.

Friedberg, D. 1978. Growth of host-dependent Bdellovibrio in host-free system. Arch. Microbiol. 116:185–190.

Friedberg, D. 1977. Effect of light on Bdellovibrio bacteriovorus. J. Bacteriol. 131:399–404.

Fry, J.C., and D.G. Staples. 1976. Distribution of Bdellovibrio bacteriovorus in sewage works, river water, and sediments. Appl. Environ. Microbiol. 31:469–474.

Genin, S., and C.A. Boucher. 1994. A superfamily of proteins involved in different secretion pathways in gram-negative bacteria: modular structure and specificity of the N-terminal domain. Mol. Gen.Genet. 243:112–118.

Germida, J.J. 1987. Isolation of Bdellovibrio spp. that prey on Azospirillum brasilense in soil. Can. J. Microbiol. 33:459–461.

Gordon, R.F., Stein, M.A., and D.N. Diedrich. 1993. Heat shock-induced growth of Bdellovibrio bacteriovorus. J. Bacteriol. 175:2157–2161.

Gray, K.M., and E.G. Ruby. 1991. Intercellular signalling in the bdellovibrio developmental growth cycle. Dworkin, M. Microbial Cell-Cell Interactions. ASM. Washington DC, 333–366.

Gray, K.M., and E.G. Ruby. 1990. Prey-derived signals regulating duration of the developmental growth phase of Bdellovibrio bacteriovorus. J. Bacteriol. 172:4002–4007.

Gray, K.M., and E.G. Ruby. 1989. Unbalanced growth as a normal feature of development of Bdellovibrio bacteriovorus. Arch. Microbiol. 152:420–424.

Hashimoto, T., Diedrich, D.L., and S.F. Conti. 1970. Isolation of a bacteriophage for Bdellovibrio bacteriovorus. J. Virol. 5:97–98.

Hespell, R.B. 1976. Glycolytic and tricarboxylic acid enzymes activities during intraperplasmic growth of Bdellovibrio bacteriovorus . J. Bacteriol. 128:677–680.

Hespell, R.B., and D.A. Odelson. 1978. Metabolism of RNA-ribose by Bdellovibrio bacteriovorus during intraperiplasmic growth. J. Bacteriol. 136:936–946.

Hespell, R.B., Thomashow, M.F., and S. C. Rittenberg. 1974. Changes in cell composition and viability of Bdellovibrio bacteriovorus during starvation. Arch. Microbiol. 97:313–327.

Hespell, R.B., Rosson, R.A., Thomashow, M.F., and S.C. Rittenberg. 1973. Respiration of Bdellovibrio bacteriovorus strain 109J and its energy substrates for intraperiplasmic growth. J. Bacteriol. 113:1280–1288.

Hoeniger, J., Ladwig, R., and H. Moor. 1972. The fine structure of "resting bodies" of Bdellovibrio sp. strain W developed in Rhodospirillum rubrum. Can. J. Microbiol. 18:87–92.

Horowitz, A.T., Kessel, M. and M. Shilo. 1974. Growth cycle of predaceous bdellovibrios in a host-free extract system and some properties of the host extract. J. Bacteriol. 117:270–282.

Huang, J.C.C., and M.P. Starr. 1973. Possible enzymatic basis of bacteriolysis by bdellovibrios. Arch. Microbiol. 89:147–167.

Kelley, J.I., Turng, B.F., Williams, H.N., and M.L. Baer. 1997. Effects of temperature, salinity, and substrate on the colonization of surfaces in situ by aquatic bdellovibrios. Appl. Environ. Microbiol. 63:84–90.

Kelley, J.I., and H.N. Williams. 1992. Bdellovibrios in Callinectus sapidus, the blue crab. Appl. Environ. Microbiol. 58:1408–1410.

Kessel., M., and M. Shilo. 1976. Relationship of Bdellovibrio elongation and fission to host cell size. J. Bacteriol. 128:477–480.

Keya, S.O., and M. Alexander. 1975. Regulation of parasitism by host density: the Bdellovibrio-Rhizobium interrelationship. Soil Biol. Biochem. 7:231–237.

Klein, D.A., and L.E. Casida, Jr. 1967. Occurence and enumeration of bdellovibrio bacteriovorus in soil capable of parasitizing Escherichia coli and indigenous soil bacteria. Can. J. Microbiol. 13:1235–1241.

Koval, S.F., and M. E. Bayer. 1997. Bacterial capsules: no barrier against Bdellovibrio. Microbiol. 143:749–753.

Koval, S.F., and S.H. Hynes. 1991. Effect of paracrystalline protein surfaces layers on predation by Bdellovibrio bacteriovorus. J. Bacteriol. 173:2244–2249.

Kramer, T.T., and J.M. Westergaard. 1977. Antigenicity of bdellovibrios. Appl. Environ. Microbiol. 33:967–970.

Kuenen, J.G., and S.C. Rittenberg. 1975. Incorporation of long-chain fatty acids of the substrate organism by Bdellovibrio bacteriovorus during intraperiplasmic growth. J. Bacteriol. 121:1145–1157.

LaMarre, A.G., Straley, S.C., and S.F. Conti. 1977. Chemotaxis toward amino acids by Bdellovibrio bacteriovorus. J. Bacteriol. 131:201–207.

Lambina, V.A., Chuvilskaya, N.A., Ledova, L.A., Afinogenova, A.V. and I.V. Averburg. 1974. Quantitative characteristics of distribution of Bdellovibrio bacteriovorus in river waters. Microbiologia 42:715–720.

Miyamoto, S., and K. Kuroda. 1975. Lethal effect of fresh sea water on Vibrio parahaemolyticus and isolation of Bdellovibrio parasitic against the organism. J. Microbiol. 19:309–317.

Marbach, A., and M. Shilo. 1978. Dependence of marine bdellovibrios on potassium, calcium, and magnesium ions. Appl. Environ. Microbiol. 36:169–177.

Marbach, A., Varoon, M., and M. Shilo. 1976. Properties of marine bdellovibrio. Microb. Ecol. 2:284–295.

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.

Markelova, N.Y., Shahamat, M., Levin, M.A., and R.R. Colwell. 1998. Effect of various pollutants on Bdellovibrio's contribution to the self-purifiaction processes. 8th Inter. l Symp. Microb. Ecol. 227.

McCann, M.P., Solimeo, H.T., Cusick, Jr., F., Panunti, B., and C. McCullen. 1998. Developmentally regulated protein synthesis during intraperiplasmic growth of Bdellovibrio bacteriovorus 109J. Can. J. Microbiol. 44:50–55.

Moulder, J.W. 1991. Interaction of Chlamydiae and host cells in vitro. Microbiol. Rev. 55:143–190.

Mulvey, M.A., Lopez-Boado, Y.S., Wilson, C.L., Roth, R., Parks, W.C., Heuser, S.J., and S.J. Hultgren. 1998. Induction and evasion of host defenses by type 1-piliated uropathogenic Escherichia coli. Science 282:1494–1497.

Nakamura, M. 1972. Alteration of Salmonella pathogenicity by other bacteria. Am. J. Clin. Nutr. 25:1441–1451.

Nelson, D.R., and S.C. Rittenberg. 1981a. Incorporation of substrate cell lipid A components into the lipopolysaccharide of intraperiplasmically grown Bdellovibrio bacteriovorus. J. Bacteriol. 147:860–868.

Nelson, D.R., and S.C. Rittenberg. 1981b. Partial characterization of lipid A of intraperiplasmically grown Bdellovibrio bacteriovorus. J. Bacteriol. 147:869–874.

Odelson, D.A., Patterson, M.A., and R.B. Hespell. 1982. Periplasmic enzymes in Bdellovibrio bacteriovorus and Bdellovibrio stolpii. J. Bacteriol. 151:756–763.

Pan, C-L., Hsu, Y-L., Tsai, G-J., Kuo, H-J, Chang, C-M., Wang, F-J., and C-S. Wu. 1997. Isolation and identification of Bdellovibrio from coastal areas of Taiwan. Fish. Sci. 63:52–59.

Parker, C.A., and P.L. Grove. 1970. Bdellovibrio bacteriovorus parasitizing Rhizobium in western Australia. J. Appl. Bacteriol. 33:253–255.

Reiner, A.M., and M. Shilo. 1969. Host-independent growth of Bdellovibrio bacteriovorus in microbial extracts. J. Gen. Microbiol. 59:401–410.

Rice, T.D. Williams, H.N. and B.F. Turng. 1998. Susceptibility of bacteria in estuarine environments to autochtonous bdellovibrios. Microb. Ecol. 35:256–264.

Richardson, I.R. 1990. The incidence of Bdellovibrio spp., in man-made water systems: coexistence with legionellas. J. Appl. Bacteriol. 53:134–140.

Rittenberg, S.C., and M. Shilo. 1970. Early host damage in the infection cycle of Bdellovibrio bacteriovorus. J. Bacteriol. 102:149–160.

Rittenberg, S.C. and R.B. Hespell. 1975a. Energy efficiency of intraperiplasmic growth of Bdellovibrio bacteriovorus. J. Bacteriol. 121:1158–1165.

Rittenberg, S.C., and D. Langley. 1975b. Utilization of nucleoside monophosphates per se for intraperiplasmic growth of Bdellovibrio bacteriovorus. J. Bacteriol. 121:1137–1144.

Romantschuk, M. 1992. Attachment of plant pathogenic bacteria to plant surfaces. Annu. Rev. Phytopathol. 30:225–243.

Rosson, R.A., and S.C. Rittenberg. 1979. Regulated breakdown of Escherichia coli deoxyribonucleic acid during intraperiplasmic growth of Bdellovibrio bacteriovorus 109J. J. Bacteriol. 140:620–633.

Rosson, R.A., and S.C. Rittenberg. 1981. Pyrimidine metabolism of Bdellovibrio bacteriovorus grown intraperiplasmically and anexically. J. Bacteriol. 146:108–116.

Ruby, E.G. 1991. The genus Bdellovibrio. A. Balows, H.G. Truper, M. Dworkin, W. Harder, and K.H. Schleifer. The Prokaryotes. Springer-Verlag. New York, NY. 3400–3415.

Ruby, E.G. 1989. Cell-envelope modifications accompaying intracellular growth of Bdellovibrio bacteriovorus. Moulder, J.W. Intracellular parasitism. CRC Press. Boca Raton, 17–34.

Ruby, E.G., and J. McCabe. 1988. Metabolism of periplasmic membrane-derived oligosaccharides by the predatory bacterium Bdellovibrio bacteriovorus 109J. J. Bacteriol. 170:646–652.

Ruby, E.G., and J.B. McCabe. 1986. An ATP transport system in the intracellular bacterium Bdellovibrio bacteriovorus 109J. J. Bacteriol. 167:1066–1070.

Ruby, E.G., McCabe, J.B., and J.I. Barke. 1985. Uptake of intact nucleoside monophosphate by Bdellovibrio bacteriovorus 109J. J. Bacteriol. 163:1087–1094.

Ruby, E.G., and S.C. Rittenberg. 1983. Differentiation after premature release of intraperiplasmically growing Bdellovibrio bacteriovorus. J. Bacteriol. 154:32–40.

Sagi, B., and R. Levisohn. 1976. Isolation of temperate bacteriophages for Bdellovibrio bacteriovorus. Isr. J. Med. Sci. 12:709–710.

Schatz, G., and B. Dobberstein. 1996. Common principles of protein translocation across membranes. Science 271:1516–1526.

Schelling, M.E., and S.F. Conti. 1983. Serotyping of bdellovibrios by agglutination and indirect immunofluorescence. J. Syst. Bacteriol. 33:816–821.

Schelling, M., and S. Conti. 1976. 1986. Host receptor sites in the attachment of Bdellovibrio bacteriovorus and Bdellovibrio stolpii. FEMS Microbiol. Let. 36:319–323.

Scherff, R.H. 1973. Control of bacterial blight of soybean by Bdellovibrio bacteriovorus. Phytopathol. 63:400–402.

Schoeffield, A.J., Williams, H.N., Turng, B.F., and W.A. Falkler, Jr. 1996. A comparison of the survival of intraperiplasmic and attack phase bdellovibrios with reduced oxygen. Microb. Ecol. 32:35–46.

Schoeffield, A.J., and H.N. Williams. 1990. Efficiencies of recovery of bdellovibrios from brackish-water environments by using various bacterial species as prey. Appl. Environ. Microbiol. 56:230–236.

Schoeffield, A.J., Falkler, Jr., W.A., Desai, D., and H.N. Williams. 1991. Serogrouping of halophilic bdellovibrios from Chesapeake Bay and environs by immunodiffusion and immunoelectrophoresis. Appl. Environ. Microbiol. 57:3470–3475.

Siedler, R.J., Mendel, M., and J.N. Baptist. 1972. Molecular heterogeneity of the bdellovibrios: evidence for two new species. J. Bacteriol. 109:209–217.

Seidler, R.J., and M.P. Starr. 1969a. Isolation and characterization of host-independent bdellovibrios. J. Bacteriol. 100:769–785.

Seidler, R.J., and M.P. Starr. 1969b. Factors affecting the intracellular parasitic growth of Bdellovibrio bacteriovorus developing within Escherichia coli. J. Bacteriol. 97:912–923.

Shilo, M., and B. Bruff. 1965. Lysis of Gram negative bacteria by host-independent ectoparasitic Bdellovibrio bacteriovorus isolates. J. Gen. Microbiol. 40:317–328.

Staples, D.G., and J.C. Fry. 1973. Factors which influence the enumeration of Bdellovibrio bacteriovorus in sewage and river water. J. Appl. Bacteriol. 36:1–11.

Starr, M.P. 1975. Bdellovibrio as a symbiont: The association of bdellovibrios with other bacteria interpreted in terms of a generalized scheme for classifying organismic associations. Symposia of the Society of Experimental Biology 29:93–124.

Stephens, R.S., Kalman, S., Lammel, C., Fan, J., Marathe, R., Aravind, L., Mitchell, W., Olinger, L., Tatusov, R.L., Zhao, Q., Koonin, E.V., and R.W. Davis. 1998. Genome sequence of an obligate intracellular pathogen of humans: Chlamydia trachomatis. Science 283:754–759.

Stolp, H. 1981. The genus Bdellovibrio. Starr, M.P., Stolp, H., Troper, H.G., Balows, A., and Schegel. The Prokaryotes. Springer-Verlag. Berlin, 618–629.

Stolp, H. 1968. Ein rauberischer backterienparasit. Naturwiss. 55:57–63.

Stolp, H. 1967. Lysis von bacterien durch den parasiten Bdellovibrio bacteriovorus. I.W.F., Gottingen Film C972.

Stolp, H., and M.P. Starr. 1963. Bdellovibrio bacteriovorus gen. et sp., a predatory, ectoparasitic, and bacteriolytic microorganism. Anton. van Leeuw. 29:217–248.

Stolp, H., and H. Petzold. 1962. Untersuchungenuber einen obligat parasitischen mikroorganismus mit lytischer aktivat fur Pseudomonas-bakterien. Phytopatho. Zeit. 45:364–390.

Straley, S.C., LaMarre, A.G., Lawrence, L.J., and S.F. Conti. 1979. Chemotaxis of Bdellovibrio bacteriovorus toward pure compounds. J. Bacteriol. 140:634–642.

Straley, S.C. and S.F. Conti. 1977. Chemotaxis of Bdellovibrio bacteriovorus toward prey. J. Bacteriol. 132:628–640.

Talley, B.G., and McDade, R.L. and D.L. Diedrich. 1987. Verification of the protein in the outer membrane of Bdellovibrio bacteriovorus as the OmpF protein of its Escherichia coli prey. J. Bacteriol. 169:694–698.

Taylor, V.I., Baumann, P., Reichelt, J.L., and R.D. Allen. 1974. Isolation, enumeration, and host range of marine bdellovibrios. Arch. Microbiol. 98:101–114.

Thomashow, M.F. and T.W. Cotter. 1992. Bdellovibrio host-dependence: the search for signal molecules and genes that regulate the intrapreriplasmic growth cycle. J. Bacteriol. 174:5767–5771.

Thomashow, L.S. and S.C. Rittenberg. 1985. Isolation and composition of sheathed flagella from Bdellovibrio bacteriovorus. J. Bacteriol. 161:1047–1054.

Thomashow, M.F., and S.C. Rittenberg. 1978a. Intraperiplasmic growth of Bdellovibrio bacteriovorus 109J: solubilization of Escherichia coli peptidoglycan. J. Bacteriol. 135:998–1007.

Thomashow, M.F., and S.C. Rittenberg. 1978b. Intraperiplasmic growth of Bdellovibrio bacteriovorus 109J: attachment of long-chain fatty acids to Escherichia coli peptidoglycan. J. Bacteriol. 135:1015–1023.

Thomashow, M.F., and S.C. Rittenberg. 1978c. Penicillin-induced formation of osmotically stable spheroplasts in nongrowing Bdellovibrio bacteriovorus. J. Bacteriol. 133:1484–1491.

Thomashow, M.F., and S.C. Rittenberg. 1978d. Intraperiplasmic growth of Bdellovibrio bacteriovorus 109J: N-deacetylation of Escherichia coli peptidoglycan amino sugars. J. Bacteriol. 135:1008–1014.

Torrella, F., Guerrero, R., and R.J. Seidler. 1978. Further taxonomic characterization of the genus Bdellovibrio. Can. J. Microbiol. 24:1387–1394.

Tudor, J.J., and M.A. Karp. 1994. Translocation of an outer membrane protein into prey cytoplasmic membranes by bdellovibrios. J. Bacteriol. 176:948–952.

Tudor, J.J., McCann, M., and I.A. Acrich. 1990. A new model for the penetration of prey cells by bdellovibrios. J. Bacteriol. 172:2421–2426.

Tudor, J.J., and S.F. Conti. 1978. Characterization of germination and activation of Bdellovibrio bdellocysts. J. Bacteriol. 133:130–138.

Tudor, J.J., and S.F. Conti. 1977. Characterization of bdellocysts of Bdellovibrio sp. J. Bacteriol. 131:314–322.

Uematsu, T. 1980. Ecology of Bdellovibrio parasitic to rice bacterial leaf blight pathogen, Xanthomonas oryzae. Rev. Plant Protec. Res. 13:12–26.

Vande Broek, A., and J. Vanderleyden. 1995. The role of bacterial motility, chemotaxis, and attachment in bacteria-plant interactions. Mol. Plant Microb. Inter. 8:800–810.

Varon, M. 1979. Selection of predation-resistant bacteria in continuous culture. Nature 277:386–388.

Varon, M. 1974. The bdellophage parasitic three-membered system. CRC Crit. Rev. Microbiol. 221–241.

Varon, M., and M. Shilo. 1981. Inhibition of the predatory activity of Bdellovibrio by various environmental pollutants. Microb. Ecol. 7:107–111.

Varon, M., and M. Shilo. 1978. Ecology of aquatic bdellovibrios. Droop, M.R., and H.W. Jannash. Advances in microbial ecology. Academic Press. London, 1–48.

Varon, M., and B. Zeigler. 1978. Bacterial predator-prey interaction at low prey density. Appl. Environ. Microbiol. 36:11–17.

Varon, M., and J. Seijffers. 1975. Symbiosis-independent and symbiosis-incompetent mutants of Bdellovibrio bacteriovorus 109J. J. Bacteriol. 124:1191–1197.

Varon, M., and M. Shilo. 1974. Isolation of host-dependent and nonparasitic mutants of the facultative parasitic Bdellovibrio UKi2. J. Bacteriol. 119:635–637.

Varon, M., and R. Levisohn. 1972. Three-membered parasitic systems: A bacteriophage, Bdellovibrio bacteriovorus, and Escherichia coli. J. Virol. 9:519–525.

Varon, M., and M. Shilo. 1970. Method for separation of bdellovibrio from mixed bacterial population by filtration through millipore or by gradient differential centrifugation. Rev. Intern. Oceanogr. 18–19:145–151.

Varon, M., Dickbuch, S., and M. Shilo. 1969. Interaction of Bdellovibrio bacteriovorus and host bacteria. II. Intracellular growth and development of Bdellovibrio bacteriovorus in liquid cultures. J. Bacteriol. 99:136–141.

Varon, M., and M. Shilo. 1968. Interaction of Bdellovibrio bacteriovorus and host bacteria: I. Kinetic studies of attachment and invasion of Escherichia coli B by Bdellovibrio bacteriovorus. J. Bacteriol. 95:744–753.

Wanner, O. 1989. Modeling of population dynamics. Characklis, W.G. and P.A. Wilderer. Structure and function of biofilms. Wiley & Sons. New York, NY. 91–110.

Westergaard, J.M., and T.T. Kramer. 1977. Bdellovibrio in the intestinal flora of vertebrates. Appl. Environ. Microbiol. 34:506–511.

Williams, H.N., Schoeffied, H.N., Guether, D., Kelley, J., Shah, D., and W.A. Falkler. 1995. Recovery of bdellovibrios from submerged surfaces and other aquatic habitats. Microb. Ecol. 29:39–48.

Williams, H.N., and W.A. Falkler. 1984. Distribution of bdellovibrios in the water column of an estuary. Can. J. Microbiol. 30:971–974.

Williams, H.N., Falkler, W.A., and D.E. Shay. 1982. Seasonal distribution of bdellovibrios at the mouth of the Patuxent River in the Chesapeake Bay. Can. J. Microbiol. 28:111–116.

Williams, H.N. 1988. A study of the distribution of bdellovibrios in estuarine sediment over an annual cycle. Microb. Ecol. 15:9–20.