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Effect of Paracrystalline Protein Surface Layers on Predation by *Bdellovibrio bacteriovorus*

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We determined that paracrystalline protein surface arrays (S layers) protected gram-negative eubacteria from predation by *Bdellovibrio bacteriovorus*. *Aquaspirillum serpens* VHA and MW5 and *Aquaspirillum sinuosum* were resistant to predation by *B. bacteriovorus* 6-5-S when fully covered by their S layers. The S layer of *Aeromonas salmonicida* A449 protected the cells from predation by *B. bacteriovorus* 109J. A predacious, plaque-forming vibrio that lysed an S-layer$^-$ variant of *Caulobacter crescentus* but was not predacious on the parental strain which possessed an S layer was isolated from raw sewage. Since S layers are stable components of many bacterial surfaces in nature, they can provide this protective function in both aquatic and terrestrial habitats where *Bdellovibrio* spp. are found.

Bdellovibrio bacteriovorus is a gram-negative bacterium which exhibits a morphologically and physiologically biphasic life cycle: a flagellated and free-swimming, nongrowing, predatory phase and a nonflagellated, reproductive phase in the periplasmic space within the cell wall of the prey (22). All *Bdellovibrio* strains, upon initial isolation, are dependent on intraperiplasmic growth in susceptible prey cells. The range of susceptible prey cells varies with the *Bdellovibrio* strain but is confined to gram-negative eubacteria. The wide host range remains one of the unsolved enigmas of *Bdellovibrio* spp. as intracellular organisms: the host range is presumed to be determined by some specificity for attachment, but a receptor has not been identified. To date, studies with *Escherichia coli* and *Salmonella typhimurium* have indicated that lipopolysaccharides or outer membrane proteins are important for attachment, depending upon the *Bdellovibrio* species (12, 24).

Most of the susceptible prey cells listed in the literature do not possess paracrystalline protein surface arrays (S layers). Strains of some organisms reported to possess S layers may be susceptible to predation, but only if they are naked (see Discussion) (5). In nature, S layers are ubiquitous, and they are represented in every major taxonomic group of eubacteria (both gram positive and gram negative) and archaebacteria (13). S layers cover the entire cell and are noncovalently associated with the underlying components of the cell envelope (9). In nature, S layers must be important for the survival of cells, since the S-layer protein is a major component of the cell and can constitute up to 10% of the total cellular protein. For a structure that is found across so many taxonomic boundaries, a single functional principle cannot exist. However, one function that many S layers share is that of a barrier. S layers may prevent attack by predators such as lytic bacteriophages or *Bdellovibrio* spp.

There are only two reports on the protective role of S layers against predation by *B. bacteriovorus* (2, 11). An abstract by Buckmire (2) states that *Aquaspirillum serpens* VHA, when fully covered by an S layer, was resistant to predation by *B. bacteriovorus* 6-5-S. When the S-layer protein was removed from intact cells by guanidine hydrochloride treatment, the cells were susceptible to predation. When the S-layer protein was reassembled onto the cell surface, predation was inhibited. It was concluded that resistance was due to the inability of *B. bacteriovorus* to attach to cells covered by an S layer.

This paper describes the effects of S layers on predation by *Bdellovibrio* isolates, making use of strains that have provided S-layer$^-$ variants.

MATERIALS AND METHODS

Organisms and growth conditions. For these studies, we chose three genera of gram-negative eubacteria of which the ultrastructure and chemistry of the S layers have been extensively studied for one or more species or strains (Table 1).

A. serpens VHL, VHA, and MW5 were maintained on slants of yeast extract-peptone-acetate medium (YPSC) (10) at room temperature. *Aquaspirillum sinuosum* was maintained on slants of peptone-succinate-salts-yeast extract medium (1) at room temperature. All *Aquaspirillum* cultures were from our laboratory collection. *E. coli* ML35 was obtained from E. G. Ruby, University of Southern California, and was maintained on slants of Trypticase soy agar (TSA) (BBL, Becton Dickinson and Co., Cockeysville, Md.) at 4°C. *Aeromonas salmonicida* A449 and A449-3 were obtained from W. W. Kay, University of Victoria, Victoria, British Columbia, Canada. They were maintained on slants of TSA at 15°C. Congo red agar plates were used to differentiate S-layer$^+$ and S-layer$^-$ strains of *A. salmonicida* (6). Filter-sterilized Congo red (catalog no. 86,095-6; Aldrich Chemical Company, Inc., Milwaukee, Wis.) was added to TSA to a final concentration of 30 μg/ml. *Caulobacter crescentus* CB2A and CB2NY66R were obtained from J. Smit, University of British Columbia. The original source of *C. crescentus* CB2NY66R is the strain collection of J. Poindexter. It was obtained and used by Smit (16) when it was discovered that strain CB2A was S layer defective. The *C. crescentus* strains were maintained on slants of PYE (0.2% peptone–0.1% yeast extract–0.02% $MgSO_4 \cdot 7H_2O$–0.02% $CaCl_2 \cdot 2H_2O$ [pH 7.0] with 1.5% agar) and stored at 4°C.

All cultures were transferred monthly. For experimental purposes, broth cultures of the bacteria described above were grown in the same medium on which they were

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TABLE 1. Prey cells used in this study

Organism	No. of S layers	Symmetry	Source	Host for *Bdellovibrio* strain
A. serpens VHA	1	p6	This laboratory	6-5-S
A. serpens VHL			This laboratory	
A. serpens MW5	2	p6	This laboratory	
A. sinuosum	2	p4 p6	This laboratory	
A. salmonicida A449	1	p4	W. W. Kay	109J
A. salmonicida A449-3			W. W. Kay	
C. crescentus CB2NY66R	1	p6	J. Smit (from J. Poindexter)	JSS
C. crescentus CB2A			J. Smit	

maintained. Cultures were incubated with shaking at 30°C for 24 h except for the *A. salmonicida* strains, which were incubated at 22°C for 30 to 40 h.

B. bacteriovorus 6-5-S, from our culture collection, was maintained by adding 1 ml of a lysate and 3 ml of a 24-h culture of *A. serpens* VHL to 50 ml of YPSC (3). The cells were incubated with shaking at 30°C for 24 to 48 h to obtain complete lysis of the prey cells. Lysates were transferred to sterile screw-cap tubes and kept at 4°C for up to 1 month. *B. bacteriovorus* 109J was obtained from E. G. Ruby, University of Southern California. It was maintained as described above, but *E. coli* ML35 was used as the prey and 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (Sigma Chemical Co., St. Louis, Mo.) plus 2 mM $CaCl_2$ at pH 7.8 (HEPES plus Ca^{2+}) was used as the buffer for lysate production. A *Bdellovibrio*-like organism, strain JSS, isolated as described below, was maintained on prey cells of *C. crescentus* CB2A in HEPES plus Ca^{2+} as described for *B. bacteriovorus* 109J.

Experimental procedure for two-membered culture systems. (i) PFU. PFU were determined by using the double-layer agar technique described by Stolp (20). Development of *Bdellovibrio* plaques is favored by use of media of low nutrient content. Twenty-four-hour cultures of the prey organisms were centrifuged and resuspended in YPSC to give 1/10 of the original volume. Ten drops of the concentrated host cells was added to 3.5 ml of YPSC agar (0.6%) at 50°C, along with 0.1 ml of the appropriate dilution of the *Bdellovibrio* lysate or two-membered system, and the mixture was vortexed and poured over the surface of prewarmed (37°C) fresh plates of YPSC agar (1.5%). Plaques were counted after 3 to 4 days of incubation at 30°C. In some cases, plates were incubated at 22°C, in which case 6 or more days of incubation were needed before the plaques were large enough to be counted.

(ii) Buffers and dilute medium. The buffer or dilute medium used was determined experimentally to provide optimal conditions for the maintenance of the prey cell and predation by *Bdellovibrio* spp. *Aquaspirillum* spp. and *B. bacteriovorus* 6-5-S were set up in HEPES plus Ca^{2+} as described by Crothers and Robinson (3). *A. salmonicida* and *B. bacteriovorus* 109J were set up in 10 mM Tris buffer plus 10 mM $CaCl_2$ and 10 mM $MgSO_4$ at pH 7.5 (Tris plus Ca^{2+} plus Mg^{2+}). *C. crescentus* and the *Bdellovibrio*-like strain JSS were set up in 1/10-strength YP broth (0.03% yeast extract–0.006% peptone–5 mM Tris, pH 7.5 [20]) (YP/10) supplemented with 2 mM $CaCl_2$ (YP/10 plus Ca^{2+}).

(iii) Two-membered systems. Twenty-four-hour cultures of prey organisms were grown in 50 ml of medium as described above, harvested, washed three times, and then resuspended in the appropriate buffer or dilute medium to give a final volume of 50 ml. The required amount of a fresh *Bdellovibrio* lysate was added, either undiluted or diluted in the same buffer or dilute medium, to give lysis of the control prey within 48 h. This amount of *Bdellovibrio* lysate corresponded to an initial concentration of 10^6 to 10^7 PFU/ml. The two-membered systems were incubated at 30 or 22°C with shaking, and predation was monitored by phase-contrast microscopy and by assessing a decrease in turbidity and an increase in PFU over the course of the experiment.

(iv) Klett readings. Turbidity measurements were taken on a Klett-Summerson photoelectric colorimeter fitted with a green filter (no. 54).

(v) Isolation of *Bdellovibrio*-like strain JSS. A 200-ml raw-sewage sample (Greenway sewage treatment plant, London, Ontario, Canada) was supplemented with 50 ml of YPSC, incubated with shaking at 30°C for 1 h, and centrifuged at 3,000 × *g* for 10 min, and the supernatant was then added to an equal volume of pregrown *C. crescentus* CB2A in PYE. The enrichment was incubated for 48 h with shaking at 30°C and centrifuged at 7,000 × *g* for 15 min, and the supernatant was then filtered through a series of 0.45-μm-pore-size membrane filters (Millipore Corp.). The filtrate was diluted and plated for plaques as described above with *C. crescentus* CB2A as host. The titer of this filtrate was 3.5×10^3 PFU/ml. Plaques were purified three times by resuspending the central area of the plaque in 2 ml of YP/10 plus Ca^{2+}, filtering the suspension (0.45-μm-pore-size membrane filter), and replating dilutions of the filtrate as described above. Plaques were examined for the presence of *Bdellovibrio* cells by negative-staining electron microscopy. In some cases, the numbers of *Bdellovibrio* cells in the filtrate were too low to continue purifying by the plaque purification method, and further enrichment of the *Bdellovibrio* strain with *C. crescentus* CB2A in YP/10 plus Ca^{2+} medium was necessary before plaque purification was resumed as described above.

Electron microscopy. To examine plaques for the presence of *Bdellovibrio* cells, a small drop of distilled water was placed on a Formvar carbon-coated 400-mesh copper grid and the grid was inverted onto a well-isolated plaque. After 5 min, the grid was lifted gently with forceps, and the liquid was removed by touching the grid to the torn edge of filter paper. The grid was immediately stained with 0.5% uranyl acetate, pH 4.4. The integrity and form of S layers on strains were monitored by electron microscopy of negatively stained envelopes and whole cells, as previously described (8, 10, 17, 18). Specimens were examined with a Philips EM300 electron microscope, operating at 60 kV.

RESULTS

B. bacteriovorus strains 6-5-S and 109J can multiply in the presence of viable but nonproliferating prey cells suspended in buffer supplemented with calcium and/or magnesium (3, 5). This experimental medium is very useful for studies on intraperiplasmic growth, since the metabolic activities of the prey cell are minimal (22). It is also an ideal environment for studying the predator-prey interaction. Modifications of nutrient medium as a result of growth of different prey organisms are maintained at a minimum. Huang (4) found a narrower spectrum of activity of *B. bacteriovorus* 6-5-S when YPSC, rather than buffer, was used as the medium for host range studies. Washed-cell suspensions of the potential

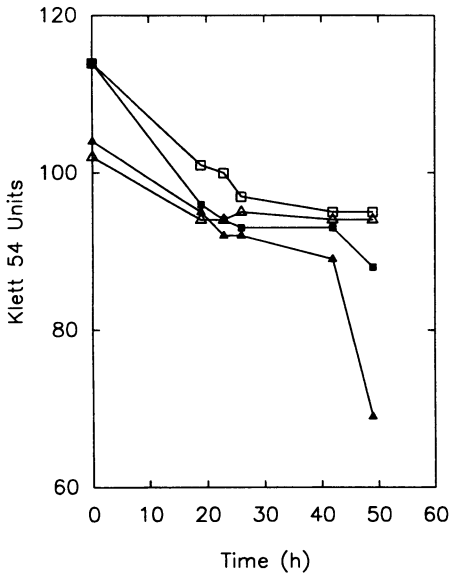

FIG. 1. Predation on *A. serpens* VHA by *B. bacteriovorus* 6-5-S. Symbols: □ and ■, VHA stock culture completely covered with S layers; △ and ▲, VHA culture with patches of S layers. Open symbols, Cells in HEPES plus Ca^{2+} buffer alone; closed symbols, cells in buffer plus *B. bacteriovorus*.

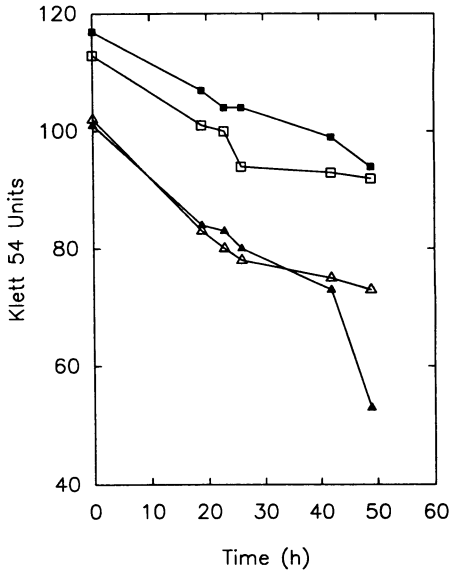

FIG. 2. Predation on *A. serpens* MW5 by *B. bacteriovorus* 6-5-S. Symbols: □ and ■, MW5 stock culture completely covered with S layers; △ and ▲, MW5 culture with patches of S layers. Open and closed symbols are as described for Fig. 1.

prey in buffer gave a consistently wide host spectrum. For our studies, the additional cations stabilized the structural integrity of S layers and their association with the outer membrane (13).

B. bacteriovorus 6-5-S was used to test the predation on *Aquaspirillum* spp., since this strain of *B. bacteriovorus* had been isolated from lawns of *A. serpens* (5). Two-membered culture systems in HEPES plus Ca^{2+} buffer were used, as described (3), to assess predation. Control cultures of *A. serpens* VHA and MW5 alone showed an initial decrease in turbidity (Fig. 1), but the cells retained their shape and form in HEPES plus Ca^{2+} buffer. *A. serpens* VHA cells possess one hexagonally arranged S layer (10), and this strain was not parasitized by *B. bacteriovorus*. At 48 h, the decrease in turbidity of the two-membered culture was not significantly different from that of prey cells alone in buffer (Fig. 1). There was no further decrease in turbidity of this two-membered culture after 5 days of incubation (data not shown). No bdelloplasts were seen by microscopy, and there was no increase in PFU per milliliter (Table 2). A culture of *A. serpens* VHA (patchy) which, in the absence of selection pressure and as a result of long-term subculturing, did not fully cover many of its cells with S layers, was slowly (compared with strain VHL) parasitized by *B. bacteriovorus* (Fig. 1). A spontaneous isogenic S-layer$^-$ variant of *A. serpens* VHA, strain VHL, which has been used routinely as the propagating host for *B. bacteriovorus* 6-5-S (3, 5), was used in the control system in our experiments (see Fig. 3 and Table 2). Another isogenic S-layer$^-$ variant of *A. serpens* VHA, strain SK12, was also parasitized by *B. bacteriovorus* 6-5-S (data not shown).

A. serpens MW5, which possesses two superimposed hexagonally arranged S layers (8), was not parasitized by *B. bacteriovorus* 6-5-S (Fig. 2), but, as described above, cultures with patchy S layers were eventually parasitized (Fig. 2 and Table 1). No isogenic S-layer$^-$ variant of *A. serpens* MW5 was available for study, nor did we manage to generate one.

A. sinuosum possesses two superimposed S layers of different symmetries which are not readily lost upon subculturing (18) and provided no patchy or S-layer$^-$ variants. This organism was not parasitized by *B. bacteriovorus* 6-5-S (Fig. 3 and Table 2). We can conclude from all these experiments that S layers protect *Aquaspirillum* spp. from predation by *B. bacteriovorus*.

In order to extend these observations to other systems, we obtained two other gram-negative bacteria with well-characterized S layers, for which isogenic S-layer$^-$ variants were available (Table 1). Unfortunately, although *B. bacteriovorus* 6-5-S has a wide host range (4), neither *A. salmonicida* A449-3 nor *C. crescentus* CB2A cells were suitable prey cells. However, *B. bacteriovorus* 109J, another well-studied strain, did parasitize *A. salmonicida* but not *C. crescentus*.

A. salmonicida A449 possesses one tetragonally arranged S layer (7). *A. salmonicida* cells in HEPES plus Ca^{2+} buffer were not stable, and there was a significant decrease in turbidity of the culture. Cells were more stable in Tris plus Ca^{2+} plus Mg^{2+}, and this buffer system was used. Assays of the predation on *A. salmonicida* by *B. bacteriovorus* 109J were performed at 22°C, because at temperatures above 24°C, *A. salmonicida* cells spontaneously lose the S layer

TABLE 2. Increase in *B. bacteriovorus* 6-5-S PFU/ml in two-membered culture systems after 48 h

Prey cell	Presence or extent of S layer	PFU/ml increase[a]
A. serpens VHL	−	1.2×10^2
A. serpens VHA	+	0
A. serpens VHA	Patchy	6.2×10^1
A. serpens MW5	+	0
A. serpens MW5	Patchy	10^2
A. sinuosum	+	0

[a] Data from experiments whose results are presented in Fig. 1 through 3.

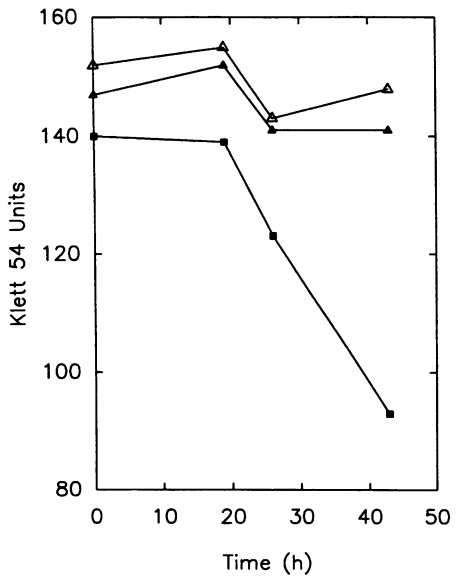

FIG. 3. Predation on *Aquaspirillum* spp. by *B. bacteriovorus* 6-5-S. Symbols: △ and ▲, *A. sinuosum*; ■, *A. serpens* VHL. Open and closed symbols are as described for Fig. 1.

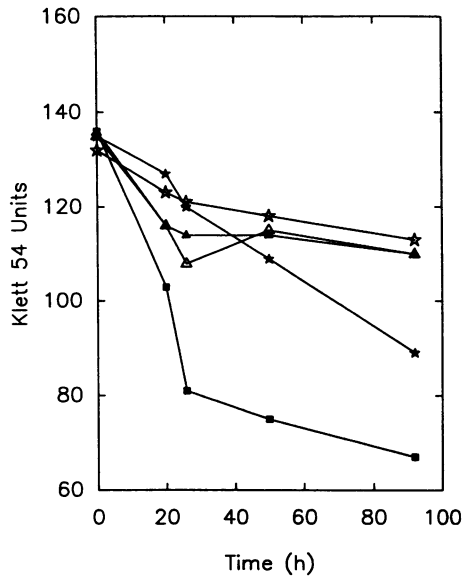

FIG. 4. Predation on *A. salmonicida* and *E. coli* by *B. bacteriovorus* 109J. Symbols: △ and ▲, *A. salmonicida* A449; ☆ and ★, *A. salmonicida* A449-3; ■, *E. coli* ML35. Open symbols, Cells in Tris plus Ca^{2+} plus Mg^{2+} buffer alone; closed symbols, cells in buffer plus *B. bacteriovorus*.

(7). To confirm this, *A. salmonicida* cultures with or without S layers were rapidly lysed by *Bdellovibrio* strains at 30°C (data not shown). We therefore had to conduct the experiments under conditions in which the prey cells would still have intact S layers. To monitor the presence of S layers, we plated cultures of *A. salmonicida* 449 and A449-3 grown at 20°C on TS-Congo red agar. Cells possessing S layers bind Congo red, and the colonies are brick red (6).

We confirmed that *B. bacteriovorus* 109J was able to lyse *E. coli* ML35 in Tris plus Ca^{2+} plus Mg^{2+} at 22°C, the conditions required for *A. salmonicida* experiments, although the process was slow. Predation at 30°C usually occurred in 24 to 36 h, but at 22°C, predation took 40 to 50 h for complete lysis, although the parasitization was well advanced at 24 h (Fig. 4). *A. salmonicida* A449-3 (S layer$^-$) was slowly parasitized by *B. bacteriovorus* 109J (Fig. 4) and, at 48 h, showed a 10^2 increase in PFU per milliliter, compared with 3.2×10^2 for predation on *E. coli* ML35. *A. salmonicida* A449 (S layer$^+$) was not parasitized by *B. bacteriovorus* 109J (Fig. 4). We plated *A. salmonicida* A449 and A449-3 in Tris plus Ca^{2+} plus Mg^{2+} (Fig. 4) on TS-Congo red agar at 0 h and at 48 h, when the counts (PFU per milliliter) were done, as further controls in the study of the predation on *A. salmonicida* by *B. bacteriovorus* 109J. At both time points, all A449 colonies (S layer$^+$) were brick red, and all A449-3 colonies (S layer$^-$) were a paler pink. Thus, the wild-type, S-layer$^+$ cells did not lose their S layers under the test conditions. Plaques were formed at 22°C on *A. salmonicida* A449-3 and on *E. coli* ML35 but not on *A. salmonicida* A449. This incubation temperature led to slow development (5 to 7 days) and small plaques. We conclude that S layers protect *A. salmonicida* from predation by *B. bacteriovorus*.

Neither *B. bacteriovorus* 6-5-S nor 109J parasitized an S-layer$^-$ strain of *C. crescentus*. Therefore, we sought a competent strain in raw sewage. An isolate, designated strain JSS, was a predacious vibrio that possessed a single, polar sheathed flagellum (diameter, 29 nm), a characteristic of *Bdellovibrio* species (Fig. 5). It formed small plaques on lawns of *C. crescentus* CB2A at 30°C but not on lawns of *A. serpens* VHL, *E. coli* ML35, or *A. salmonicida* A449-3. We did not observe any intraperiplasmic growth of strain JSS on *C. crescentus*, an essential character for *Bdellovibrio* species, and shall refer to this isolate as *Bdellovibrio*-like until it can be further characterized.

HEPES and Tris buffers plus cations were not suitable for two-membered culture systems involving *Caulobacter* strains. *C. crescentus* CB2A was stable in both buffer systems without *Bdellovibrio* cells, but the stalked cells of strain CB2NY66R (which possess one hexagonally arranged S layer) eventually formed spheroplasts. The *C. crescentus* CB2NY66R cells were more stable in YP/10 plus Ca^{2+}, which allowed some initial growth of the *Caulobacter* cells, but, in the presence of *Bdellovibrio*-like strain JSS, *C. crescentus* CB2A was eventually lysed (Fig. 6). After 66 h, there was a 10^2 increase in PFU per milliliter, an increase which is comparable to that produced in the other systems described. Cultures of *C. crescentus* CB2NY66R with or without *Bdellovibrio*-like strain JSS showed a decrease in turbidity with time. However, no parasitization was observed by light microscopy, and there was no increase in PFU per milliliter.

DISCUSSION

B. bacteriovorus 6-5-S was isolated in 1965 in London, Ontario, Canada, by using *Spirillum* (*Aquaspirillum*) *serpens* MW5 as a host culture (5). It was known at the time that *A. serpens* MW5 possessed an S layer, but the susceptibility of this host strain to predation was attributed to incomplete coverage of the cells by the S layer or to instability of the S layer under certain conditions of growth. Our results have confirmed the suggestion (4, 19) that cells possessing complete S layers are protected, and those with incomplete S layers are eventually parasitized when exposed to competent *Bdellovibrio* strains. Unfortunately, lack of knowledge about

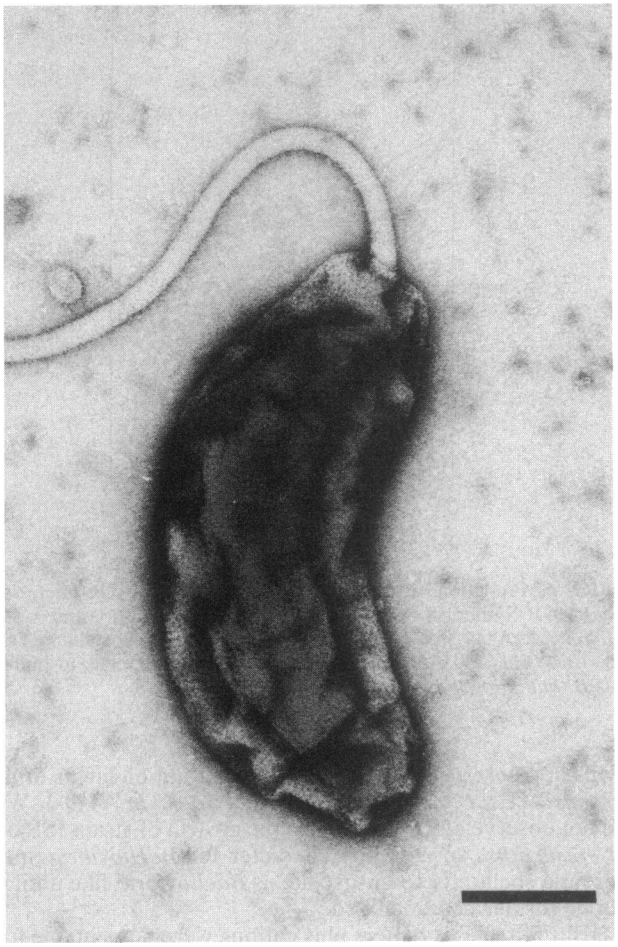

FIG. 5. Electron micrograph of negatively stained (0.5% uranyl acetate) preparation of the predacious vibrio which forms plaques on lawns of *C. crescentus*. Bar, 200 nm.

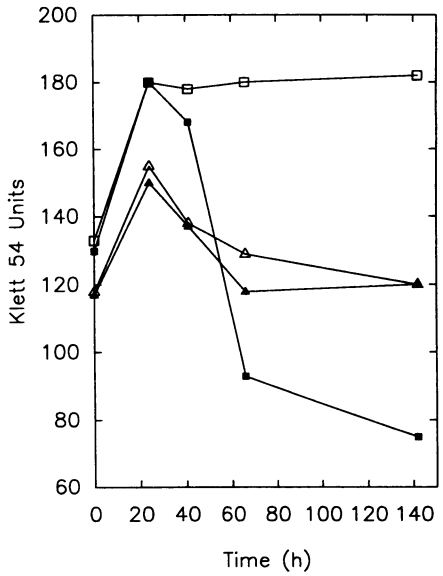

FIG. 6. Predation on *C. crescentus* by *Bdellovibrio*-like strain JSS. Symbols: □ and ■, *C. crescentus* CB2A; △ and ▲, *C. crescentus* CB2NY66R. Open symbols, Cells in YP/10 plus Ca^{2+} alone; closed symbols, cells in medium plus *Bdellovibrio*-like strain JSS.

S layers led Torrella et al. (23) to consider *A. serpens* MW5 a universal host because all *Bdellovibrio* isolates tested successfully parasitized this prey cell. The *A. serpens* MW5 culture used from the International Collection of Phytopathogenic Bacteria (University of California, Davis) must have had an incomplete S layer. For some unknown reason, although *B. bacteriovorus* 6-5-S was isolated on *A. serpens* MW5, most subsequent work with this *Bdellovibrio* strain was done with *A. serpens* VHL, an S-layer$^-$ variant of strain VHA.

In an initial description of *B. bacteriovorus* (21), *C. crescentus* CB15 was not found to be suitable for the isolation of *Bdellovibrio* strains, nor was it susceptible to any of the *Bdellovibrio* strains that had been isolated up to 1963. This is probably because *C. crescentus* CB15 (17) and most *Caulobacter* isolates from nature possess S layers (15).

The elucidation of the functional significance of S layers is still fragmentary (9), and our studies provide one of the few direct assays of a function. The protective function of S layers is not limited to one genus of gram-negative bacteria or to one *Bdellovibrio* isolate. This protective role must be important in vivo for survival of gram-negative bacteria in many ecosystems, since *Bdellovibrio* spp. are found in most aquatic and terrestrial habitats (25). However, if eubacteria with S layers are so ubiquitous in nature (9), then how do *Bdellovibrio* spp. find enough susceptible prey cells for growth and multiplication? One possibility is that the wide host range that most *Bdellovibrio* strains have on gram-negative bacteria may help to compensate for the high proportion of gram-negative bacteria with S layers in nature and also for the limited viability of *Bdellovibrio* cells in search of prey. During the attack phase, *Bdellovibrio* cells have a high respiratory rate, necessary to provide the energy for the extremely rapid motility of these cells (up to 100 cell lengths per second), and their viability decreases significantly under nongrowing conditions (25). There is genetic heterogeneity with respect to starvation. For example, the viable number (PFU) of strain 6-5-S decreased 5.5×10^3 after storage at 10°C for 6 months in dilute nutrient broth, whereas the viable count of strain 109J decreased 5×10^7 PFU under the same conditions (25). Bacteriophages, on the other hand, can exist with a very narrow, defined (taxonomically) host range, since they are not metabolically active autonomously and can survive independently of host cells for long periods. S layers probably also serve to protect gram-negative cells from attack by lytic bacteriophages. However, two S-layer-specific bacteriophages have been described (9).

Cells with patchy S layers have arisen in laboratory cultures in the absence of selection pressure for maintenance but probably do not occur in nature. These cultures serve to illustrate, however, that once an area of outer membrane is exposed, because of the absence of the S layer, *Bdellovibrio* organisms will randomly find this site and attach. The pores in the protein meshwork of S layers (sizes between 2 and 6 nm have been estimated [14]) are not sufficiently large to expose an outer membrane site or receptor for *Bdellovibrio* attachment. Because of their crystalline nature, S layers exhibit uniform pore morphologies (13, 14).

One of the unexpected findings during these studies was the isolation of a *Bdellovibrio*-like organism with a prey cell host range limited to *C. crescentus*. A significant differential

characteristic of this isolate, strain JSS, is the absence of intraperiplasmic growth, although that site of growth is a definitive character in the description of the genus. For strain JSS, the predator cells remain attached to the cell envelope, digest a localized region of the peptidoglycan, but do not enter into the periplasmic space (unpublished observations). We are currently investigating more characteristics of this predacious vibrio and comparing it with other plaque-forming eubacteria.

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