Isolation and Characterization of Temperature-Sensitive Mutants of Host-Dependent *Bdellovibrio bacteriovorus* 109D

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A variety of temperature-sensitive mutants of host-dependent *Bdellovibrio bacteriovorus* 109D were selected after ethyl methane sulfonate mutagenesis. Mutants that demonstrated plaque-forming ability reversion frequencies of $10^{-4}$ to $10^{-8}$ were chosen for further study. Representatives of these mutants were then characterized by phase-contrast and electron microscopy, temperature-shifted one-step growth experiments, attachment kinetics, and macromolecular capabilities. Representative mutants demonstrate various types of blockage corresponding to the previously described morphological stages of *Bdellovibrio* predacious life cycle, i.e., attachment blockage (109D153), penetration blockage (109D3 and 109D48), and blockage of intracellular growth (109D4 and 109D152). The time of release from temperature repression for the mutant classes was found to correspond to the apparent morphological stage of blockage via temperature-shifted, one-step growth experiments. Mutants characterized as exhibiting blockage in the penetration or intracellular stages of the infection cycle exhibited, at the permissive and nonpermissive temperatures, kinetics of attachment to *Escherichia coli* WP2 similar to those of the wild type. One mutant, 109D153, exhibited depressed attachment at the restrictive temperature even though the *Bdellovibrio* cells were motile. The extent of 38.5°C attachment of 109D153 to *E. coli* is at the same level as that of wild-type 109D to *Bacillus subtilis*, a gram-positive, non-host organism. Subsequent detachments were revealed in the wild-type 109D-B. subtilis or mutant 109D153-*Escherichia coli* (38.5°C) cultures. These studies reveal a biphasic attachment phenomenon in the early interaction of *Bdellovibrio* with its host. It appears that, at the restrictive temperature, 109D153 is capable only of the initial, nonspecific type of attachment.

Members of the genus *Bdellovibrio* possess a life cycle unique among the bacteria. Their predacious interaction with sensitive, gram-negative bacteria initiates a complex sequence of events which, as characterized recently by Huang and Starr (3), involves "recognition" by the *Bdellovibrio* of a congenial host, forceful physical contact of the vigorously motile *Bdellovibrio* cell with the other bacterial cell, attachment, penetration, digestion of the host cell intracellular menstruum, intramural growth, and release of *Bdellovibrio* progeny. The mechanisms, controls, and sequence of events at play during this predacious interaction are at present only summarily delineated and are undoubtably much more elaborate. In the belief that studies of appropriate mutant *Bdellovibrio* strains will aid in the further delineation and understanding of these events, we have undertaken the isolation and initial characterization of temperature-sensitive mutants of host-dependent (H-D) *B. bacteriovorus* 109D. The purpose of this communication (presented in part at the Annual Meeting of the American Society for Microbiology, Miami, Fla., 6 to 11 May, 1973) is to describe some of the mutants isolated presently and explore the implications of the nature of the blockage to the overall *Bdellovibrio* life cycle.

**MATERIALS AND METHODS**

**Bacterial strains.** H-D *B. bacteriovorus* strain 109D and its temperature-sensitive mutant derivatives were studied. *Spirillum serpens* was used in the preparation of lysates and plaque-forming unit (PFU) determinations. *Escherichia coli* WP2 was used as the host strain in temperature-shift experiments and attachment kinetics experiments. *Bacillus subtilis* strain 168 was used in those attachment experiments noted.
Preparation of lysates and PFU enumeration. The culture medium used (designated YPS) was a modified YPSC broth (7) but prepared without cysteine and supplemented with 2 mM MgSO$_4$ and 3 mM CaCl$_2$. The stock medium (x1) contains (per liter): sodium acetate, 2 g; yeast extract (Difco), 4 g; and peptone (Difco), 4 g. The PFUs were determined, after serial dilution in x1, by using 0.8% bottom agar and 0.6% top agar of x1 YPS. Plates were incubated at 28 C. For the preparation of lysates, 5 ml of an overnight S. serpens x4 YPS culture was added to 30 ml of x1 YPS and incubated with 0.5 ml of Bdellovibrio. Before any Bdellovibrio culture was used for experimental purposes, a fresh lysate was prepared and transferred at least two times at 12-h intervals at 28 C. Such 12-h lysates contain short, highly motile forms which are a necessity for synchronous Bdellovibrio development. Liquid cultures were shaken at 28 C in a New Brunswick Scientific control environment incubator shaker at 200 rpm.

Selection of temperature-sensitive mutants. Fresh 12-h Bdellovibrio lysates were mutagenized with ethyl methane sulfonate, at a final concentration of 0.01%, for 2 to 2.5 h at 34 C, and then plated for isolated plaques. Replica plates were incubated at 28 and 38.5 C. Lysates were made of mutants demonstrating growth at 28 C but not 38.5 C, and those mutants exhibiting PFU reversion frequencies of $10^4$ or $10^5$ were chosen for further study.

Temperature-shift experiments. A series of one-step growth experiments was run in which it was attempted to determine the time of temperature repression for each mutant. A fresh 12-h Bdellovibrio lysate was centrifuged at 200 x g for 10 min at 4 C, sequentially filtered through a series of 5-, 3-, and 1.2-µm nitrocellulose membrane filters, and centrifuged at 25,500 x g for 15 to 20 min at 4 C. Equal volumes of Bdellovibrio, resuspended in x1 to a density of $10^6$ PFU/ml, and washed log-phase E. coli WP2, resuspended in x1 to a density of $10^5$ cells/ml, were mixed (multiplicity of infection [MOI], 0.2) at 28 C. After the infection cycle was permitted for some predetermined time at the permissive temperature (28 C), a sample was diluted 1:10 in x1, filtered through a 1.2-µm filter, and washed with 20 ml of nutrient broth (Difco) to remove any unattached Bdellovibrio, followed by 20 ml of x1. The filter pad was inverted over a sterile filter assembly, and cells were recovered after being washed with 10 ml of x1. A 4-ml amount of this cell suspension was added to 16 ml of x1 temperature-equilibrated at 28 or 38.5 C in a bubble tube. PFUs per milliliter were then followed hourly for 6 h, and the burst size and percentage of increase were determined as described previously (6).

Attachment kinetics determinations. Attachment kinetics were studied by using a modification of the procedure described by Varon and Shilo (9). In our experiments, $10^4$ Bdellovibrio and 5 x $10^9$ E. coli as described above, or 2 x $10^9$ washed and x1-resuspended log-phase B. subtilis, were mixed after preincubation for 10 min at the appropriate temperature. Immediately after mixing and at time intervals thereafter a sample was withdrawn and diluted 1:100 in chilled x1. After the last sample was withdrawn, 1 ml of each dilution was filtered through a 1.2-µm filter and washed with 9 ml of x1, and the filtrate PFUs were determined. Measurement of macromolecular capabilities. The ability of a particular S. serpens-Bdellovibrio combination to synthesize ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) was followed by measuring the incorporation of $[^3]_H$ uracil or $[^3]_H$ thymidine into cold trichloroacetic acid-precipitable counts. For these assays, a 0.1-ml sample of the cell suspension was mixed at the time indicated with 1 ml of 5% cold trichloroacetic acid and allowed to stand at 4 C for 30 min. The precipitates formed were collected and washed on a 1.2-µm nitrocellulose membrane filter with 10 ml of cold 5% trichloroacetic acid, followed by 10 ml of cold 70% ethanol. In control experiments, S. serpens was found not to incorporate extracellular $[^3]_H$ thymidine. Therefore, for the assay of DNA synthesis, 1.2 x $10^4$ S. serpens cells that were washed and suspended in a tri(hydroxymethyl)aminomethane (0.001 M at pH 7.5)-calcium/magnesium salts (2 and 3 mM, respectively) buffer (TCM buffer) were mixed with three times as many filtered and washed Bdellovibrio cells in TCM buffer containing either 0.2 or 0.4 µCi of $[^3]_H$ thymidine per ml. For the assay of RNA synthesis, 1.2 x $10^4$ washed and resuspended S. serpens cells were preincubated for 30 min in TCM buffer containing 0.2 or 0.4 µCi of $[^3]_H$ uracil per ml prior to the addition of the Bdellovibrio cells. Such $[^3]_H$ uracil-preincubated S. serpens cells did incorporate some cold trichloroacetic acid-precipitable counts, but in control experiments this incorporation was shown to cease after 15 min of incubation in the absence of Bdellovibrio. The release of extracellular, Bdellovibrio-specific proteases was followed via Azocollase activity by the method of Gloor et al. (in press). Increase in extracellular proteolytic activity is attendant with intracellular Bdellovibrio growth and represents the release of protease unit values far and above those found in host cell cultures or host cell extracts and, as such, is taken to represent the induction of a Bdellovibrio-specific enzyme system.

Electron microscopy. Two-membered cultures of wild-type 109D, 109D, and 109D48 were prepared (at an MOI of 3) with E. coli WP2 as host. After being shaken at the nonpermissive temperature for 60 min, each culture was fixed by the modified Rytter and Kellenberger procedure of Brock and Edwards (1). Cell pellets were dehydrated through a graded series of water-ethanol mixtures followed by propylene oxide. They were infiltrated with a propylene oxide-Epon-Araldite mixture and embedded in Epon-Araldite by conventional methods. Sections were double-stained with uranyl acetate and lead citrate and examined in a Phillips EM 200 electron microscope at 60 kV.

RESULTS

In one-step growth experiments at 28 C with E. coli WP2 as host and H-D B. bacteriovorus strain 109D at an MOI of 0.2 in x1 YPS, attachment is 50% complete in 4 min and
virtually complete in 8 min. By phase-contrast microscopy, host cell spheroplasts appear within 15 min, and penetration of Bdellovibrio between the host cell wall layers and membrane is completed by about 1 h. Bdellovibrio PFUs begin to increase after 3 to 4 h. According to this information, then, there are five major developmental subdivisions of the Bdellovibrio predatory growth cycle: (i) attachment, (ii) penetration, (iii) elongation, (iv) fragmentation, and (v) burst (6). Based upon this sequence of events, several morphological types of growth-repressed Bdellovibrio mutants should be expressed. We have isolated such temperature-sensitive Bdellovibrio mutants and characterized them as to the nature of the developmental stage blocked.

Preliminary characterizations of those mutants selected for further study were made by microscope observation of synchronously infected host cells incubated at 38.5 C. A mutant designated 109D153 was motile at the nonpermissive temperature but failed to produce host cell spheroplasts. Some apparent attachments were observed but at a frequency less than normal. Mutants designated 109D3 and 109D48 were motile at the nonpermissive temperature and appeared to attach normally to host cells, but failed to produce host cell spheroplasts. Mutants designated 109D4 and 109D152 were motile and appeared to attach normally at the nonpermissive temperature. Host cell spheroplasts were produced but no increase in PFUs was observed.

**Time of temperature repression.** A series of temperature-shifted, one-step growth experiments was run for each mutant to delineate the time of release from temperature repression. E. coli WP2 and the Bdellovibrio mutant under investigation were mixed at an initial MOI of 0.2 at the permissive temperature. At some predetermined time into the infection cycle, portions of this culture were removed and transferred to the permissive and nonpermissive temperatures under conditions which allow for one-step growth (6). Failure of the portion shifted to the nonpermissive temperature to yield a significant burst (less than 100% PFU increase) was taken as evidence that the time of the temperature shift was prior to the completion of the temperature-sensitive event. The onset of an increased burst was taken to denote the time period required for release from temperature sensitivity.

The percent increases exhibited by the 28 C control portions of the one-step growth experiments did vary from mutant to mutant, but never yielded percent increases less than a cell doubling (100% in Fig. 1). Mutant 109D4 demonstrated the smallest (at permissive temperature) percent increase observed—167%. Mutants 109D3, 109D48, and 109D152 demonstrated percent increase values of 270, 290, and 330% respectively.

Figure 1 summarizes the growth increments observed at the nonpermissive temperature for the mutants examined. Release from temperature repression to yield PFU increases greater than a doubling is illustrated by 109D3 at 20 min, but not at 15 min, into its infection cycle. Mutant 109D48 is capable of greater than a cell doubling when shifted to 38.5 C at 45 min into its infection cycle. When shifted at 15 min, only a 70% increase occurs, whereas at 8 min a slight drop in PFUs is recorded. Both 109D3 and 109D48 were identified by phase-contrast microscopy as mutants capable of attaching to, but not able to penetrate, the host cell wall.

Nonpermissive cultures of 109D3 and 109D48, the two penetration-deficient mutants described, were examined by electron microscopy. Temperature-equilibrated mutant and E. coli WP2 cultures were mixed at an MOI of 3, shaken at 38.5 C for 60 min, and then fixed as described. Figure 2 (A, B, C) illustrates the failure of 109D3 and 109D48 to initiate host cell penetration after 60 min at the nonpermissive temperature. Wild-type 109D cells have...
pletely invaded the host (Fig. 2D). It is of interest to note the extent of host cell dissolution which is evident in both mutant preparations. It is impossible to tell from the present study whether this dissolution is due to *Bdellovibrio* lytic systems or host cell autolytic enzymes. It should be noted, however, that although 109D3 and 109D48 fail to exhibit PFU increases at the nonpermissive temperature, they obviously are capable of causing host cell death.

Mutant 109D152 is not able to multiply even when shifted to 38.5°C at 90 min into its infection cycle, but when shifted at 180 min it is capable of a significant burst (Fig. 1). When shifted to the nonpermissive temperature as late as 90 min into its infection cycle, mutant 109D4 is capable of only an 85% increase in

![Fig. 2. *Bdellovibrio*-E. coli two-membered mixtures (38.5°C) incubated for 60 min and then fixed for electron microscopy. Bar represents 0.5 μm in all micrographs. (A, B) 109D3: Note extracellular position of *Bdellovibrio*. Host cell wall rupture exhibited in A is probably in part due to the advanced degree of host degradation demonstrated. The majority of host cells appeared to be undergoing dissolution (A, C), although some host cells still retain a more organized state (B). Magnification, ×50,500. (C) 109D48: Note failure of *Bdellovibrio* to penetrate. Magnification, ×50,500. (D) Wild-type 109D: Note fully penetrated, intracellular position of *Bdellovibrio* cells. Magnification, ×26,000.](http://jb.asm.org/)

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**Bdellovibrio** PFUs. Both 109D4 and 109D152 were identified by phase-contrast microscopy as mutants blocked at some intracellular developmental stage.

**Attachment kinetics.** The ability to follow **Bdellovibrio** attachment to **E. coli** via differential filtration through a membrane filter has been demonstrated (9) and was used in this study to follow the attachment kinetics of 109D wild-type and mutant **Bdellovibrio**. Figure 3 illustrates the attachment kinetics of wild-type **B. bacteriovorus** strain 109D to **E. coli** WP2 at 38.5 C. The kinetics observed compare favorably with those recorded by Varon and Shilo (9). By 10 min, the drop in PFUs obtained in the filtrate has essentially stabilized. If the average of the points taken after the 10-min sample is used in the calculation, attachment is shown by 79% of the **Bdellovibrio**. Penetration mutant 109D3 (Fig. 3) demonstrates attachment similar to that of the wild type, yielding values of 74 and 88%, respectively, at the permissive and nonpermissive temperatures. Another penetration mutant, 109D48, demonstrated similar kinetics, with the final attachment calculations yielding values of 73 and 72% (Fig. 3). Two mutants which possess stages of blockage relatively late in the infection cycle, 109D4 and 109D152, demonstrate unimpaired attachment kinetics at the nonpermissive temperature. The curves are not illustrated since the attachment kinetics observed were virtually identical to those of Fig. 3. The attachment calculations yield values of 77 and 65%, respectively.

Some degree of difficulty was experienced in attempting to isolate mutants which would fit our preconceived notion of an attachment-negative mutant. When assessed by phase-contrast microscopy at the nonpermissive temperature, all mutants demonstrated visible attachment. With 109D153 it was noted, however, that attachment was less efficient than normal, and further investigations of this strain were initiated. Figure 4 illustrates the attachment kinetics of 109D153 to **E. coli** and **B. subtilis** at the permissive and nonpermissive temperatures. Attachment to **E. coli** at the nonpermissive temperature follows wild-type kinetics and yields a final attachment calculation value of 76%. The attachment kinetics of 109D153 to **E. coli** at the nonpermissive temperature are significantly depressed, and incubation of the chilled attachment mixture appears to facilitate detachment. When the final attachment calculations as described above are applied to 109D13-**E. coli** mixtures at the nonpermissive temperature, a value of 37% is obtained.

Microscope observations have shown that **Bdellovibrio** exhibits attachment to infected spheroplasts, gram-positive bacteria, cover slips, etc. These observations lead to the conclusion that **Bdellovibrio** is capable of a degree of nonspecific attachment. The application of this conclusion to 109D153 led us to examine the attachment kinetics of 109D153 to **B. subtilis** at the permissive and nonpermissive temperatures. These attachment kinetics illustrated in Fig. 4 are strikingly similar to the attachment kinetics of 109D153 to **E. coli** at the nonpermissive temperature. The attachment values are 56 and 47% for the permissive and nonpermissive temperatures, respectively. Note also that incubation of the attachment mixture in ice appears to reveal detachments of the nonspecific type; thus, filtrate PFUs rise in the latter portions of the **B. subtilis**-109D153 and **E. coli**-109D153 nonpermissive attachment curves but remain...
flat in the other attachment curves. These observations we believe, reveal a biphasic attachment phenomenon involving an initial nonspecific, reversible process followed by a specific, irreversible attachment event.

In an attempt to support the contention that the *B. subtilis* attachment represents a reversible event, the attachment kinetics of wild-type 109D to *B. subtilis* were followed, and the detachments were revealed by inverting each washed filter pad over a second sterile filter assembly and washing off the entrapped cells with a volume (50 ml) of \( \times 1 \) buffer. This suspension was stored at ambient temperature for 1 h, after which time 1 ml was refiltered and washed with 9 ml of \( \times 1 \) and the resultant filtrate PFUs were titered (Fig. 5). The initial value of the detachment curve is not the theoretically desirable zero, but is a more elevated value representing the degree of attachment advanced before the withdrawal of the first sample. Point-by-point summation of the attachment and detachment curve can be seen to yield an essentially constant value. Thus, the attachment levels and subsequent detachment of wild-type 109D to *B. subtilis* appear analogous to that of 109D153 to *E. coli* at the nonpermissive temperature.

**Macromolecular capabilities of 109D153.**

Experiments were initiated to investigate the macromolecular capabilities of 109D153 during the infection cycle at the permissive and nonpermissive temperatures. RNA, DNA, and *Bdellovibrio* specific enzyme synthesis were followed. *S. serpens*, preincubated in the presence of \([^{1}H]\)uracil until uptake was complete or *S. serpens* plus extracellular \([^{1}H]\)thymidine, were the sole source of nutrients. The MOI used was sufficiently large to ensure complete infection of all hosts (Table 1).

During the first 5 min of infection in the presence of \([^{1}H]\)uracil, the net increase in trichloroacetic acid-precipitable counts at both the permissive and nonpermissive temperatures were identical. At later time intervals, the restrictive counts either dropped to the preincubation level or shut off at the 5-min level. Permissive temperature trichloroacetic acid-precipitable counts continued to rise.

Mutant 109D153 failed to incorporate any \([^{1}H]\)thymidine at the nonpermissive temperature, whereas incorporation at the permissive temperature was normal. Similarly, *Bdellovibrio*-specific proteases (Gloor et al., in press; 3, 5) were not induced at the nonpermissive temperature, whereas at the permissive temperature induction of this proteolytic capacity followed wild-type kinetics.

**DISCUSSION**

The mutants reported upon here demonstrate reversion frequencies of \(10^{-8}\) or \(10^{-9}\) with the lawn overlay technique described. They also all fail to yield PFU increases when the 38.5 C, two-membered cultures are prepared by mixing temperature-equilibrated host and *Bdellovibrio* cells. Mutants were, however, isolated that met the first criterion but not the second. This class of organisms apparently represents mutants which can initiate at least partial rounds of replication before the effects of the deficiency are sufficiently felt to prevent further replication. It is to be emphasized that the low levels of PFU percent increases seen at some time periods of the temperature-shifted, one-step growth experiments probably does not represent this type of growth but is, more probably, a

![Fig. 5. Attachment kinetics of wild-type 109D to Bacillus subtilis at 38.5 C. When the B. subtilis and attached Bdellovibrio cell population for each point in the attachment kinetics curve are suspended in a large volume and allowed to stand at room temperature for 1 h and the number of unattached Bdellovibrio cells then determined, the resultant detachment curve is demonstrated. Point-by-point summation of these two curves yields a relatively constant value. The points presented represent the average of three experiments.](http://jb.asm.org/)
reflection of *Bdellovibrio* infection cycle asynchrony.

Penetration-deficient mutants 109D3 and 109D48 fail to multiply at the nonpermissive temperature. The manifestation of this outcome occurs early in the infection cycle as a failure of the *Bdellovibrio* mutant to invade the host cell periplasmic compartment. It is not possible with the data presently at hand to determine whether the failure of 109D3 and 109D48 to penetrate the host cell is directly responsible for their subsequent failure to multiply or whether their failure to penetrate is a secondary manifestation of other defects.

109D4 and 109D152 fail to demonstrate PFU increases at the nonpermissive temperature although their infection cycle proceeds normally until the later stages of intracellular growth. The events occurring during the intracellular stages of the *Bdellovibrio* infection cycle are undoubtedly complex, and our data do not attempt to differentiate between the various intracellular developmental stages (elongation, fragmentation, and burst). Electron microscope studies are in progress on these mutants elsewhere and should help distinguish further the specific nature of the block.

The isolation of temperature-sensitive *Bdellovibrio* mutants that are blocked at different morphological stages of the infectious lifecycle is indicative of a physiological function unique to each stage of development. The mutants that are, morphologically, blocked early (such as 109D153) fail to initiate physiological functions normally associated with later intracellular development (DNA and protease synthesis). This observation, and the fact that mutants blocked specifically at one of several morphological stages can be isolated, suggests that normal *Bdellovibrio* development must proceed in a sequential manner, that is, a developmental event(s) supplies the trigger for later event(s).

The observations on mutant 109D153 and the investigations of 109D nonspecific attachment to *Bacillus subtilis* may provide insights relevant both to the survival of *Bdellovibrio* in nature and to previous studies on the early stage interaction of *Bdellovibrio* with its host (9, 10).

The effect of temperature on the burst size of *B. bacteriovorus* 109D growing in *E. coli* B, as reported by Seidler and Starr (6), indicates that *Bdellovibrio* reproduction, as measured by burst size, was greatest at the lowest incubation
temperature studied, 25 C. (Note that these experiments did not distinguish between differences in the degree of Bdellovibrio snake fragmentation and total progeny mass.) These findings and the ease of isolation of Bdellovibrio from soil and water at ambient temperatures (B. bacteriovorus 109D was originally isolated by Stolp from soil) would seem to indicate an ecological niche for Bdellovibrio at these temperatures.

Varon and Shilo (9) examined the relationship between temperature and the extent of attachment to E. coli of a B. bacteriovorus 109J population. The degree of population attachment at the end of a 20-min period was found to increase with temperature elevation increments of 5 C and to demonstrate an optimum at 35 C, with subsequent temperature elevation increments yielding decreased values. As reported by Varon and Shilo, a population of Bdellovibrio which demonstrates approximately 70% attachment at 35 C demonstrates only slightly higher than 40% attachment at 20 C, approximately 12% attachment at 15 C, and even less significant attachment at lower temperatures. In another experiment in the same report, Varon and Shilo examined the effect of shift in the incubation temperature on the attachment of B. bacteriovorus 109J to E. coli. A shift in the incubation temperature of the parasite-host mixture from 20 C (conditions which allow only part of the Bdellovibrio cells to attach) to 30 C was found to result in a "new wave" of attachment up to the level achieved in mixtures incubated at 30 C from the start.

These attachment experiments may reflect values resulting from the manifestation of two distinct attachment mechanisms. Peak attachment values may represent an initial nonspecific interaction shifted towards completion by a specific, irreversible event. The attachment values obtained by Varon and Shilo for temperatures in the range of 15 to 20 C seem to reflect ranges of attachment more compatible to our nonspecific attachment values (% A value of B. bacteriovorus 109D attachment to B. subtilis is 21%), which probably represent an equilibrium situation established between free swimming and attached Bdellovibrio forms. Lower attachment values at even lower temperatures may reflect a shift in this equilibrium by secondary changes in the physiology of the initial nonspecific parasite-host interaction event; most notably a change in Bdellovibrio motility.

Bdellovibrio attachment would thus appear to involve an initial nonspecific, reversible interaction which is followed by a temperature-stimulated, specific, irreversible event. Correlation of this view of attachment with the apparent preference of the Bdellovibrio burst size for less elevated temperatures would seem to lead to competition between the two processes. The net effects resolve at some optimal temperature for Bdellovibrio parasitism of E. coli.

The degree to which the Bdellovibrio nonspecific, reversible attachment phenomenon may be operating at environmental temperatures has led us to speculations on the possible ecological-evolutionary advantages of such an attachment mechanism. This predation-divorced attachment to noncongenial hosts could function in effect as a reversible holdfast. The widespread distribution of holdfast organelles would seem to attest to their ecological-evolutionary potential and speculation in print on the possible ecological role of the Caulobacter (4) and Pasteuria ramosa (8) holdfasts are brought to mind. The importance of such a device to so motile an organism as Bdellovibrio at first, however, appears moot. But observations have shown that Bdellovibrio lysates yield immobile, yet viable forms within an hour. To such an organism, the presence of a holdfast-like mechanism might be very beneficial, and the analogy to the Caulobacter situation becomes stronger.

This mechanism might thus form a basis for continued species endurance under conditions of individual hardship. As pointed out by Pointdexter in a review on the genus Caulobacter (4), such attachment can be interpreted as a means for establishing a close association with a continuous source of nutrients excreted by other organisms. Thus, a microenvironment is established which is richer in nutrients than the surrounding milieu. Such an association would not be indefinitely beneficial but the sparing effect of glutamate, acetate, and amino acid mixtures upon Bdellovibrio endogenous respiration demonstrated recently by Rittenberg et al. (2) would appear to enhance the favorability of such an association.

Varon and Shilo (10), in their study on the attachment of B. bacteriovorus to cell wall mutants of Salmonella and E. coli, suggest that Bdellovibrio populations may be heterogeneous, consisting of (i) "cells capable of efficient attachment to both rough and smooth strains," and (ii) "cells capable of efficient attachment only to rough strains." They do point out, however, that the efficiency of plating in their experiments was the same when two Bdellovibrio strains were plated on the seven cell wall variant host strains used. Our modified
view of Bdellovibrio attachment, as representing a biphasic phenomenon comprised of reversible and irreversible stages, offers an alternative view for the proposed culture heterogeneity. In this case, variations in cell wall composition would be seen as influencing the rapidity of onset of the second, irreversible stage and thus effecting the rapidity and degree to which the attached, unattached equilibrium population is shifted towards completion.

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LITERATURE CITED