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Identification of a *Bdellovibrio bacteriovorus* Genetic Locus, *hit*, Associated with the Host-Independent Phenotype

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Bdellovibrios invade and grow within the periplasmic space of suitable gram-negative bacteria. Wild-type bdellovibrios are obligately dependent on host cells for growth, but spontaneous host-independent (H-I) mutants that grow axenically on standard rich culture media can be isolated. Such mutants generally retain the ability to grow intraperiplasmically, although the plaques that they produce on lawns of host cells are smaller and more turbid than those produced by wild-type bdellovibrios. Here, we identify the first genetic locus associated with the H-I phenotype: *hit* (host interaction). We show that three individual H-I mutants suffered mutations at the *hit* locus and that recombination of wild-type *hit* sequences into the genomes of the H-I mutants greatly enhanced their plaquing ability. DNA sequence analysis localized the *hit* mutation in each of the H-I mutants to a 135-bp region of the genome. Mutations at *hit* may not fully account for the H-I phenotype, however, as recombination of wild-type *hit* sequences into the genomes of the H-I mutants had little effect on the axenic-growth phenotype of the mutants. Possible explanations for this result and potential roles for the *hit* locus are discussed.

Wild-type bdellovibrios are obligate intraperiplasmic (IP) parasites of other gram-negative bacteria (7, 24, 25). One of the fundamental goals of *Bdellovibrio* research is to determine the nature of the host-dependent (H-D) phenotype. At present, little is known about this aspect of *Bdellovibrio* biology. Biochemical studies have demonstrated that concentrated cellular extracts from hosts and other bacteria can induce wild-type bdellovibrios to multiply on rich media (6, 9, 15). However, no specific growth factors or signal molecules have been identified, and the mechanism by which the extracts stimulate bdellovibrios to grow and multiply is not known (24). Genetic studies have shown that spontaneous bdellovibrio mutants that are capable of growth on complex bacteriological media in the absence of hosts or host extracts can be isolated (4, 10, 20, 22, 26). However, the identity and function of the gene(s) affected in such host-independent (H-I) mutants are unknown.

A detailed characterization of the gene(s) involved in the H-I phenotype should provide significant insight into the molecular basis of *Bdellovibrio* host dependence. Until recently, however, such analysis has been hampered by a lack of genetic systems for manipulating the *Bdellovibrio* genome. In an accompanying paper (3), we have begun to right this situation by identifying vectors that can be used to conjugally transfer DNA from *Escherichia coli* to *Bdellovibrio bacteriovorus*. Moreover, we used the system to identify a 5.6-kb *Bam*HI fragment from wild-type *B. bacteriovorus* 109J that greatly enhanced the plaquing ability of an H-I mutant, *B. bacteriovorus* BB5. In this study, we show that *B. bacteriovorus* BB5 and two additional H-I mutants suffered mutations within the 5.6-kb *Bam*HI fragment of the genome and we define a locus, designated *hit* (host interaction), that affects the interaction that *B. bacteriovorus* has with host cells. Potential roles for the *hit* locus in the *Bdellovibrio*-host interaction are discussed.

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MATERIALS AND METHODS

Bacterial strains, plasmids, media, and culture conditions. The bacterial strains and plasmids used are listed in Table 1. *B. bacteriovorus* 109J and *E. coli* ML35 were obtained from S. C. Rittenberg. Antibiotic-resistant mutants and H-I mutants of *B. bacteriovorus* 109J were isolated as described by Seidler and Starr (20). All bdellovibrio strains were single-plaque or single-colony purified and stored in 15% glycerol at −80°C. Media and culture conditions used for the propagation of all *E. coli* and *B. bacteriovorus* strains were as described elsewhere (3).

Chemicals and reagents. Complex medium components were purchased from Difco. Restriction endonucleases and T4 DNA ligase were purchased from New England Biolabs. [α-^{32}P]dCTP (800 Ci/mmol) and α-^{35}S-dATP (500 Ci/mmol) were purchased from DuPont/New England Nuclear.

Matings. The conjugal transfer of plasmids from *E. coli* to *B. bacteriovorus* was done as described elsewhere (3). All of the genetic experiments described here involved the conjugal transfer of IncP plasmids containing *B. bacteriovorus* DNA and their integration into the recipient *B. bacteriovorus* genome via homologous recombination. All of the recipients were therefore merodiploid for the cloned sequences. Southern blot analysis has shown that integration of *B. bacteriovorus* DNA-containing constructs occurs via homologous recombination between the cloned *B. bacteriovorus* insert and the equivalent region of the recipient genome (3).

DNA manipulations and construction of pVKα-1. Most DNA purification and recombinant DNA methods were as described by Sambrook et al. (18). *B. bacteriovorus* genomic DNAs were purified by a CTAB (cetyltrimethylammonium bromide)-based extraction procedure (1).

The 5.6-kb *Bam*HI fragments that contain the *hit* locus in H-I mutants *B. bacteriovorus* BB3, BB4, and BB5 were

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description	Source or reference
B. bacteriovorus		
109J	Wild type	16
109J.1	Sm^r derivative of 109J	3
109J.2	Rf^r derivative of 109J.1	3
BB3	H-I derivative of 109J.1	This study
BB4	H-I derivative of 109J.1	This study
BB5	H-I derivative of 109J.2	3
E. coli		
ML35	B *lacI lacY*	17
DH5	F^- *endA1 recA1 hsdR17*(r_K^- m_K^+) *deoR thi-1 supE44 gyrA96 relA1*	8
DH5α	F^- φ80d*lacZ*ΔM15 Δ(*lacZYA argF*)U169 *endA1 recA1 hsdR17*(r_K^- m_K^+) *deoR thi-1 supE44 gyrA96 relA1*	Bethesda Research Laboratories
DH5αF′	F′ φ80d*lacZ*ΔM15 Δ(*lacZYA argF*)U169 *endA1 recA1* hsdR17(r_K^- m_K^+) *deoR thi-1 supE44 gyrA96 relA1*	Bethesda Research Laboratories
SM10	*supE44 hsdR thi-1 thr-1 leuB6 lacY1 tonA21 recA* Mu_c^+ RP4-2Tc::Mu; Km^r	23
pKC7	ColE1 Ap^r Km^r	14
pUC18	ColE1 Ap^r	27
pUC19	ColE1 Ap^r	27
pRK2013	ColE1 Km^r *tra*(RK2)	5
pVK100	IncP Tc^r Km^r	11
pVK102	IncP Tc^r Km^r	11
pVKα-1	pVK100 derivative containing the 445-bp *Hae*II fragment from pUC19 in *Eco*RI site	This study
pTC3	23.5-kb insert of *B. bacteriovorus* 109J DNA in *Eco*RI site of pVK100	3
pTC7	19.5-kb insert of *B. bacteriovorus* 109J DNA in *Eco*RI site of pVK100	3
pTC12	5.6-kb *Bam*HI insert from pTC7 in *Bgl*II site of pVK102	3
pTC20	5.6-kb *Bam*HI fragment from *B. bacteriovorus* BB5 in *Bam*HI site of pUC18	This study
pTC32	5.6-kb *Bam*HI fragment from pTC20 in *Bgl*II site of pVK102	This study
pTC35	2.5-kb *Bam*HI-*Xba*I fragment from pTC12 in *Bam*HI-*Xba*I site of pVKα-1	This study
pTC36	3.1-kb *Bam*HI-*Xba*I fragment from pTC12 in *Bam*HI-*Xba*I site of pVKα-1	This study
pTC37	2.8-kb *Eco*RI fragment from pTC12 in *Eco*RI site of pVKα-1	This study
pTC50	0.96-kb *Eco*RI-*Xba*I fragment from pTC12 in *Bam*HI site of pVKα-1	This study

cloned by isolating genomic *Bam*HI fragments that ranged from 5.0 to 6.0 kb in size, ligating them into pUC18, and transforming them into *E. coli*; *Bam*HI fragments in the correct size range were isolated by electroelution after agarose gel electrophoresis (18). Plasmids containing the 5.6-kb *Bam*HI fragment were identified by colony hybridization (18) with pTC12 as a probe. The mutated 5.6-kb *Bam*HI fragments from *B. bacteriovorus* BB3, BB4, and BB5 were subcloned into the *Bgl*II site of pVK102, yielding pTC28, pTC30, and pTC32, respectively, to allow for conjugal transfer into *B. bacteriovorus*.

pVKα-1 was constructed by cloning the 445-bp *Hae*II fragment of pUC19 that contains the polylinker and α-complementation sequences into the *Eco*RI site of pVK100. Prior to ligation, the overhanging termini of each fragment were removed by treatment with Klenow fragment. pVKα-1 allows the use of α-complementation for cloning and contains unique *Bam*HI, *Kpn*I, *Pst*I, *Sst*I, and *Xba*I restriction sites within the polylinker.

DNA sequencing. DNA sequences were determined by the dideoxy chain termination method (19) using Sequenase (United States Biochemical) with single-stranded M13 DNA as the template. The sequence of the wild-type *B. bacteriovorus* 959-bp *Eco*RI-*Xba*I fragment (see below) was obtained from both strands. The equivalent mutant-derived fragments were sequenced in their entirety on one strand by using primers that were designed from the wild-type sequence. Discrepancies with the wild-type sequence were confirmed by sequencing on the complementary strand. DNA and protein sequence analyses were performed with the GCG programs (version 6.0) developed by the University of Wisconsin Biotechnology Center (Madison, Wis.) and the PROsis and DNAsis programs of Hitachi (San Bruno, Calif.).

Nucleotide sequence accession number. The sequence of the *hit*-containing 959-bp *Eco*RI-*Xba*I fragment from wild-type *B. bacteriovorus* 109J has been deposited in GenBank under accession number M97807.

RESULTS

Identification of a mutation in H-I mutant *B. bacteriovorus* BB5. In an accompanying paper (3), we showed that recombination of pTC12 into the genome of *B. bacteriovorus* BB5 greatly enhanced the plaquing ability of this H-I mutant; whereas *B. bacteriovorus* BB5 formed small, turbid plaques (Fig. 1B), the recombinants carrying pTC12 produced plaques that were large and clear (Fig. 1C). A likely explanation for this result was that *B. bacteriovorus* BB5 contained a mutation within the region of the genome corresponding to the 5.6-kb *Bam*HI fragment cloned in pTC12 and that recombination of the wild-type sequences at this locus resulted in at least a partial correction of the original genetic lesion. To test this hypothesis, we isolated the 5.6-kb *Bam*HI fragment from *B. bacteriovorus* BB5, carried on pTC32, and compared it with the wild-type fragment at the physical and genetic levels. Comparative restriction analysis of wild-type and mutant-derived 5.6-kb *Bam*HI fragments did not reveal any obvious deletions or rearrangements. Genetic experiments, however, indicated that the fragment did indeed

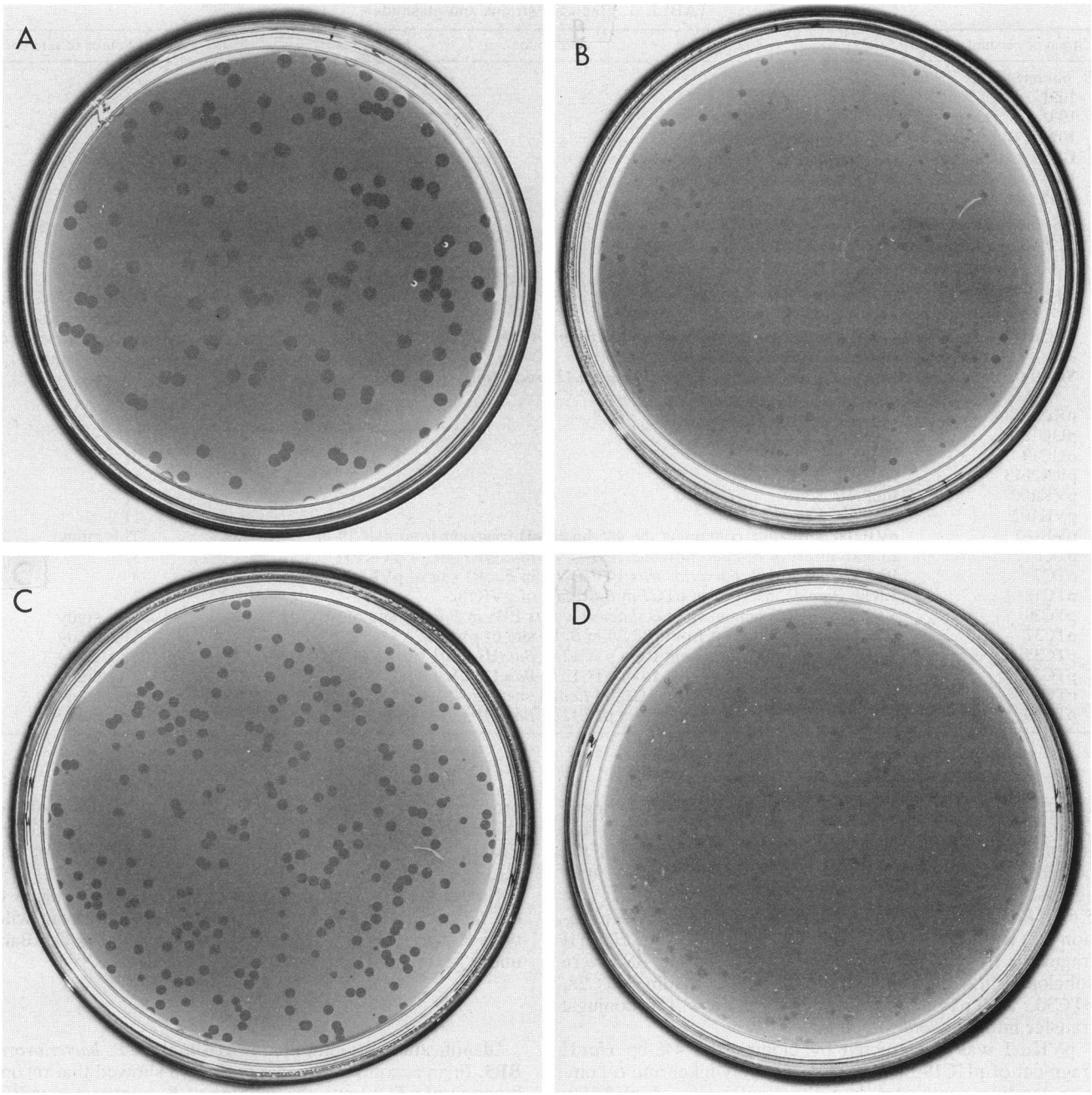

FIG. 1. Effects of pTC12 and pTC32 on the plaque phenotype of *B. bacteriovorus* BB5. Shown are plaques formed by *B. bacteriovorus* 109J.2(pTC3) (A), BB5(pTC3) (B), BB5(pTC12) (C), and BB5(pTC32) (D). pTC3 is used to confer Km^r; it does not affect the plaque phenotype of H-D or H-I strains.

contain a mutation: in contrast to pTC12, recombination of pTC32 into the *B. bacteriovorus* BB5 genome did not result in enhanced plaquing (Fig. 1D).

The probable site of the mutation within pTC32 was narrowed down by additional recombination experiments. The 5.6-kb *Bam*HI fragment contained in pTC12 was subcloned, yielding pTC35, pTC36, pTC37, and pTC50 (Fig. 2), and each of the plasmids was tested to determine whether it could enhance the plaquing ability of *B. bacteriovorus* BB5. The smallest active region was the 0.96-kb *Eco*RI-*Xba*I fragment cloned in pTC50. Presumably, *B. bacteriovorus* BB5 had suffered a mutation within this region of the genome. This supposition was confirmed by DNA sequence analysis (Fig. 3). The data indicated that the corresponding *Eco*RI-*Xba*I fragments from wild-type *B. bacteriovorus* 109J and its H-I derivative, *B. bacteriovorus* BB5, differed by 1 bp; the mutant had a single base pair deletion located 208 bp from the *Xba*I site (Fig. 3, closed circle). As this region of the genome had an effect on the bdellovibrio plaquing phenotype, the locus was designated *hit* (host interaction).

The *hit* locus is affected in other H-I mutants. The occurrence of a mutation within the *hit* locus of *B. bacteriovorus*

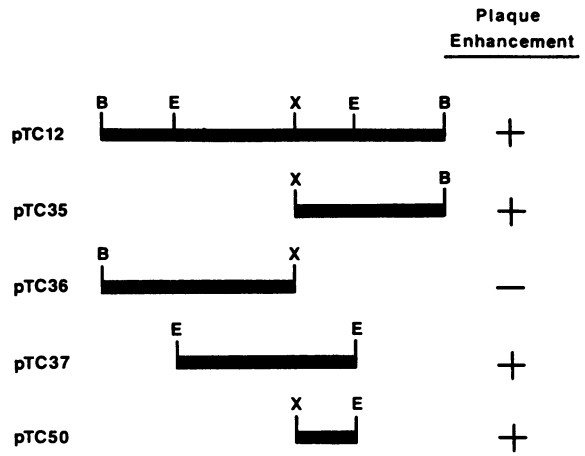

FIG. 2. Plasmid constructs containing *B. bacteriovorus* 109J DNA and their ability to enhance plaque formation by *B. bacteriovorus* BB5. The region of the *B. bacteriovorus* 109J genome cloned in various plasmids and the abilities of the plasmids to enhance the plaque-forming ability of *B. bacteriovorus* BB5 are indicated. Insert sizes (in kilobases): pTC12, 5.6; pTC35, 2.5; pTC36, 3.1; pTC37, 2.8; pTC50, 0.96. Restriction sites: B, *Bam*HI; E, *Eco*RI; X, *Xba*I.

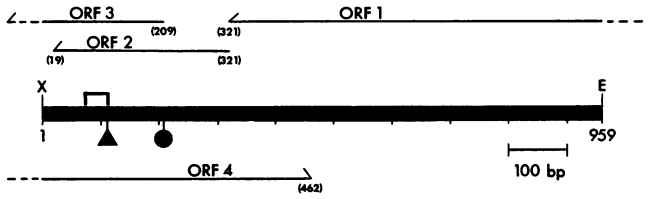

FIG. 4. Potential coding regions within the 959-bp *Eco*RI-*Xba*I fragment. The locations and orientations of four substantial ORFs are indicated. Numbers in parentheses, nucleotide positions in the 959-bp *Eco*RI-*Xba*I fragment of *B. bacteriovorus* 109J presented in Fig. 3. Triangle, bracket, and closed circle, sites of deletions in *B. bacteriovorus* BB3, BB4, and BB5, respectively. E, *Eco*RI; X, *Xba*I.

BB5 raised the question of whether this locus was commonly affected in H-I mutants derived from *B. bacteriovorus* 109J. This issue was addressed by isolating two additional H-I mutants, *B. bacteriovorus* BB3 and BB4, and subjecting them to the same analysis used for *B. bacteriovorus* BB5. Like *B. bacteriovorus* BB5, *B. bacteriovorus* BB3 and BB4 formed small, turbid plaques. Also, as was observed with *B. bacteriovorus* BB5, recombination of pTC12 or pTC50 into the genomes of *B. bacteriovorus* BB3 and BB4 conferred an enhanced-plaque phenotype (data not shown). These results were consistent with *B. bacteriovorus* BB3 and BB4 having suffered a mutation at the *hit* locus.

To confirm that both *B. bacteriovorus* BB3 and *B. bacteriovorus* BB4 had a mutation at *hit*, the *hit*-containing *Eco*RI-*Xba*I fragment from each of the mutants was cloned and subjected to DNA sequence analysis. The data indicated that both mutants had indeed suffered mutations within the *Eco*RI-*Xba*I fragment: *B. bacteriovorus* BB3 contained a single base pair deletion 108 bp from the *Xba*I site (Fig. 3, closed triangle), and *B. bacteriovorus* BB4 had a 42-bp deletion that overlapped the site of the mutation in *B. bacteriovorus* BB3 (Fig. 3, bracket). It is likely that the deletion in *B. bacteriovorus* BB4 resulted from recombination between the identical 10-bp direct repeats present in the wild-type genome beginning at positions 64 and 106 (Fig. 3, underlining).

The *hit* mutations affect multiple ORFs. Computer analysis indicated that the wild-type 959-bp *Eco*RI-*Xba*I fragment contained four substantial open reading frames (ORFs), only one of which, ORF2, was completely contained within the fragment (Fig. 4). ORF2 overlapped ORF1 by 1 bp, suggesting that these two ORFs might be part of the same operon. Comparison of the DNA sequence of the 959-bp *Eco*RI-*Xba*I fragment and the amino acid sequences of the polypeptides encoded by ORFs 1 to 4 with the GenBank (release 68.0), EMBL (release 27.0), and SWISS-PROT (release 18.0) data bases did not reveal extensive similarity with any previously sequenced genes or polypeptides.

The *hit* mutations suffered by each of the H-I mutants affected three of the ORFs, ORF2, ORF3, and ORF4, contained in the *Eco*RI-*Xba*I fragment (Fig. 4). A role for

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      XbaI
1     TCTAGACAGA TGGGATTACT GTCTTCCAGT CCCGGCTTTC TCAGATCCAC CAGGCGTCGC GCTGGAGCCT TTGATTTCTC TGGAAACGGT GTCGTCATGG
101   CGACGGGAGC CTTTGCCCGT GGTGGCGCCG GTACGGTAGG CAATACACTC ACGGCAGTCG CCCAAAGCCG ATGTCGTGGC TTCAAACGGA GTGGATCTGA
201   AGGCCTCATT AGGGTCTTCG CCAGGGTTTA CCGGGCGGTT GGCATTTTCG TCAGCAGAGG CTGTTCCCGC AAAGGAGAAG CCCAGGGTCA GCAAGATGGA
301   AAGGACCAAG AGTCTTTTCA TATAATCACC TTCTCCTTAC AGGTAAAGTT CCGCAGTTGC GGAGCAATGA TATCCAAGAG GTTATTAAAT GTGCTGGCTA
401   CCTGCATAGC TACAGATGGC CCCTCTTTGC TGTCCACCTT GTTGATTTCA CCATTTGGTT TATAGGATAC CACAATTGTG CCCAAACCGG GACGGGTAAC
501   TACAGATGGC TTCCCATAGC GCTCCTCGTA CTCGATTTTA ACCACTTTCT TGGCCTGATC TGTAATGGTC GCAATACGGC CGCGGTTATC ATATGTCATA
601   TTGATCTTCT GGCCATCGCT GTTCTGGGCG AAAGAAAGAT TCCCTTTGCC ATCGTATTTG AACTGAGCGG CCTTGGTGGC TACTTTGGCA CCCTTCTCGT
701   TAAAGTAGAT GCTGTTCACC GAACTGACCT TTTTGACCTT CGGATCGTAT TCAAAGGACA TCTTCATGTT CGGTGCGGCT TTCACCTTCA CCAGGCCATC
801   AGGATAGTAT TCGTAGGAAA TACGGTCGGC GTTACGACGG ATGGAAACCG GCTTGCCGAA GACCTCATGG TAAGTGATGT CGGTGACGTT GCCGTTCACA
901   GTCGTCATCA CACGCTGCAG ATAGTATTGA CCGTCTGCGC GCTGTTGATG CCAGAATTC
                                                                  EcoRI
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FIG. 3. DNA sequence of the 959-bp *Eco*RI-*Xba*I fragment from wild-type *B. bacteriovorus* 109J and *B. bacteriovorus* H-I mutants BB3, BB4, and BB5. The DNA sequence for the wild-type *B. bacteriovorus* 109J 959-bp *Eco*RI-*Xba*I fragment is given, and the locations of the deletion mutations in *B. bacteriovorus* BB3, BB4, and BB5 are indicated by a triangle, bracket, and closed circle, respectively (see text for details). Circle and triangle above the sequence, locations of single base pair deletions; bracket, location of a 42-bp deletion. Underline, 10-bp direct repeat located at the borders of the deletion in *B. bacteriovorus* BB4.

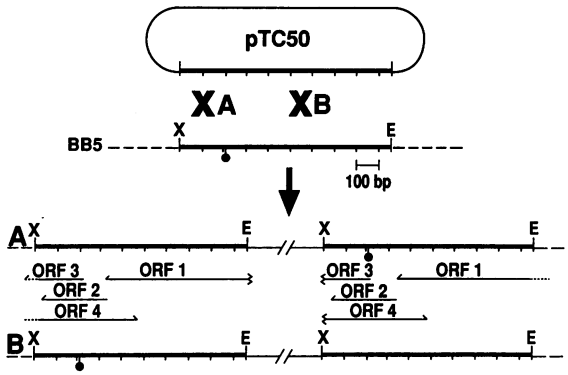

FIG. 5. Classes of *hit* recombinants. Products of single recombination events between pTC50 and the *B. bacteriovorus* BB5 genome at sites to the left (A) and right (B) of the mutation are diagrammed. Symbols: X, site of recombination; closed circle, site of mutation in *B. bacteriovorus* BB5; thick line, 959-bp *Eco*RI-*Xba*I fragment; thin line, pVKα-1; dashed line, *B. bacteriovorus* BB5 genome; zigzag line, ORF interruption by vector. Restriction sites: E, *Eco*RI; X, *Xba*I.

ORF2 in the enhanced-plaque phenotype was suggested by the *hit* recombination experiments. That is, recombination of the wild-type *hit* sequences on pTC50 into the H-I mutants would be expected to give two classes of recombinants, designated A and B in Fig. 5. Given the site of the *hit* mutations, most of the recombinants would presumably be of class B (in *B. bacteriovorus* BB4, there would be only 73 bp available for recombination on the left side of the mutation). Examination of the predicted ORFs in this class of recombinants indicates that only ORF2 is completely restored; ORF3 and ORF4 either contain the original spontaneous H-I mutation or are deleted at the 3′ (ORF3) or 5′ (ORF4) end. Thus, it seems probable that restoration of ORF2 is responsible for the enhanced-plaque phenotype observed in the *hit* recombinants.

A role for ORF2 in the *B. bacteriovorus*-host interaction is interesting, as ORF2 probably encodes an exported polypeptide. This is suggested by the fact that the N-terminal sequence of the deduced 10.6-kDa ORF2 polypeptide has properties consistent with its being a signal peptide (13): it has two positively charged amino acids at the N terminus, followed by a hydrophobic core of 20 amino acids which is predicted to assume an α-helical or β-sheet secondary structure (Fig. 6 and 7). In addition, at the end of the hydrophobic core there are three alanine residues (positions 18, 21, and 23) that are potential signal peptide cleavage sites (13). Cleavage of the ORF2 polypeptide at any of these sites

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          10        20        30        40        50        60        70
     MKRLLVLSILLTLGFSFAGTASADENANRPVNPGEDPNEAFRSTPFEATTSALGDCRECIAYRTGATTGK

HELIX hhhhhhhhhhhhhhhhhH
SHEET SSSSSSSSSSSSSSSSs                                  SSSSSSSSs    ssSSs   ss
TURN                          TTTT  TTTTTTTT                        TTTTTTT  TTTTTTTTT
COIL                    ccccccc    cc          ccccc

          80        90        100
     GSRRHDDTVSREIKGSSATPGGSEKAGTGRQ

HELIX
SHEET
TURN  TTTTTTTT     TTTTTTTTTTTT TTTT
COIL            cccc           c    c
```

FIG. 6. Deduced amino acid sequence and potential secondary structure of the ORF2 polypeptide. Secondary structure prediction is according to the method of Chou and Fasman (2).

would yield a mature hydrophilic polypeptide devoid of any extensive hydrophobic regions.

Recombination of the wild-type *hit* locus into the H-I mutants does not restore a wild-type phenotype. Recombination of pTC50, pTC12, or pTC7 (the original cosmid from which pTC12 and pTC50 were derived) into *B. bacteriovorus* BB5 did not restore a wild-type phenotype (Table 2). The primary difference between the recombinants and wild-type bdellovibrios was their axenic-growth characteristics. Plating wild-type *B. bacteriovorus* 109J or *B. bacteriovorus* 109J.2(pTC3) on peptone-yeast extract (PYE) medium gave colonies at a frequency of 10^{-6} to 10^{-7} (compared with PFU) that varied widely in their size and capacity to grow upon replating. Some colonies, such as those that gave rise to *B. bacteriovorus* BB3, BB4, and BB5, were relatively large and could be picked and restreaked without difficulty. Others were small and did not give growth upon restreaking unless they were pooled with other colonies to give a large inoculum, and then they generally grew only in the heavily streaked area of the plate. In contrast, *B. bacteriovorus* BB5(pTC7), BB5(pTC12), and BB5(pTC50) produced colonies on PYE medium at a frequency of about 10^{-4} (compared with PFU) that were indistinguishable from those formed by *B. bacteriovorus* BB5; they were relatively large and uniform in size and grew well upon replating. Thus, recombination of the *hit* locus into *B. bacteriovorus* BB5 had little effect on the axenic-growth phenotype of the H-I mutant. Similar results were obtained when pTC7, pTC12, or pTC50 was recombined into *B. bacteriovorus* BB3 or BB4.

DISCUSSION

Little is known about the nature of *Bdellovibrio* host dependence (24). Indeed, it has not been conclusively established whether the host cell requirement has a nutritional or a regulatory basis. It is possible, although perhaps unlikely, that the ability of H-I mutants to grow axenically on standard rich culture media results from spontaneous mutations that bring about a gain of biosynthetic capacity. Alternatively, attack-phase cells might be locked into a search mode, requiring a signal from the host to initiate IP growth. Such a negative regulatory model was initially suggested by Shilo (21). The model took into account the fact that mutations are generally more likely to result in a loss of function rather than a gain in function. Recently, Gray and Ruby (7) suggested that bdellovibrios require at least two distinct signals from the host to successfully complete the IP growth cycle. What these host signals are and how they might regulate *Bdellovibrio* growth and development are unknown.

The ability to isolate H-I mutants and define the nature of the genetic lesions offers a powerful approach to determining the molecular basis of *Bdellovibrio* host dependence. Here, we have initiated such efforts, identifying the first genetic locus associated with the H-I phenotype; *hit*. The fact that the locus suffered mutations in three individual H-I mutants strongly suggests that it has a fundamental role in the conversion of an obligate wild-type H-D *B. bacteriovorus* to an H-I mutant. Further, the data presented demonstrate that the small, turbid plaque phenotype of the H-I mutants was due primarily, if not exclusively, to the mutations at *hit*.

The challenge now is to determine why mutations at *hit* result in a small, turbid plaque phenotype and how this relates to the ability of *B. bacteriovorus* BB3, BB4, and BB5 to grow axenically on rich culture medium. At present, we have little information on these points, but the available data

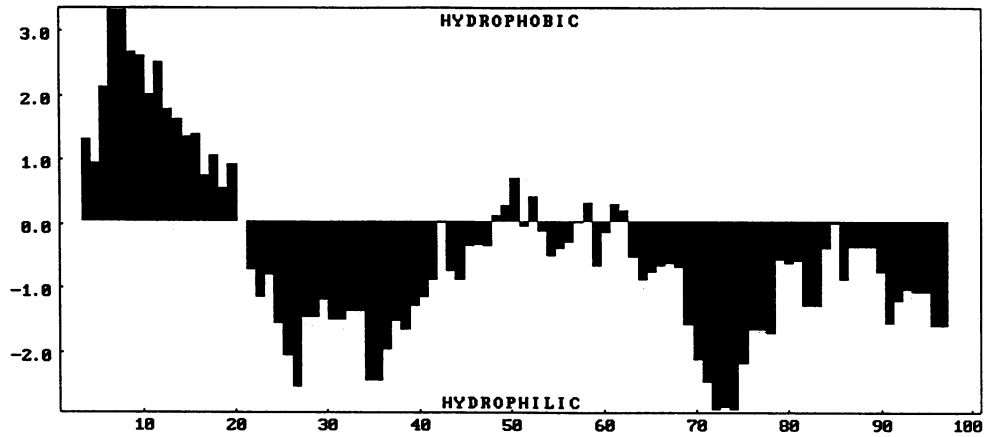

FIG. 7. Hydropathy profile of the deduced ORF2 polypeptide. Results of a Kyte and Doolittle (12) hydrophobicity analysis using a window of seven amino acids are shown. Ordinate, hydrophobicity index; abscissa, amino acid sequence residue numbers.

invite speculation. In particular, the data presented indicate that the 10.6-kDa hydrophilic polypeptide deduced to be encoded by *hit* ORF2 is probably required for *B. bacteriovorus* to produce a large, clear plaque. Of interest here is that the ORF2 polypeptide is predicted to have a signal sequence. Thus, the polypeptide is probably exported, most likely to the periplasm, although an outer membrane or extracellular location is not ruled out (13). This suggests that the ORF2 gene product may have a direct role in the *B. bacteriovorus*-host interaction. One possibility is that the polypeptide has a structural function or enzymatic activity required for host invasion. Mutations at *hit* might then result in poor penetration and thus poor plaque production.

An alternative possibility is that the ORF2 gene product might be part of a bdellovibrio-host signalling mechanism akin to what has been proposed by Gray and Ruby (7). Specifically, attack-phase bdellovibrios might be repressed for growth, as suggested by Shilo (21), with the ORF2 gene product being a part of the negative regulatory circuit. Upon encountering a host, a critical signal molecule might be bound by the ORF2 polypeptide, modifying its activity and relieving its negative effect on axenic growth; mutations at *hit* could have a similar effect. In this scenario, the small, turbid plaque phenotype of the H-I mutants would result from an increased capacity of the bdellovibrios to grow in the absence of hosts, not an inability to invade the host. Thus, this regulatory model is particularly satisfying as it offers a potential link between the plaquing and plating phenotypes of the H-I mutants.

While it is clear that mutations at *hit* account largely, if not completely, for the small, turbid plaque phenotype of the H-I mutants, the role of *hit* in the axenic-growth phenotype is uncertain. This is because recombination of wild-type *hit* sequences into the genomes of the H-I mutants did not abolish axenic growth (Table 2). There was, however, an effect on the CFU-to-PFU ratio: whereas the value for *B. bacteriovorus* BB5 was about 1, that for the *B. bacteriovorus* BB5 *hit* recombinants was about 10^{-4}. It is conceivable that mutations at *hit* are sufficient to give the H-I phenotype and that the intermediate axenic-growth phenotype of the *hit* recombinants was due to the nature of the recombination events. The *hit* recombinants are merodiploid at the *hit* locus, and thus their intermediate axenic-growth phenotype might have resulted from allelic interactions between the wild-type and the mutant *hit* loci. Another formal possibility is that ORF3 or ORF4 or both are involved in the H-I phenotype and that wild-type versions of these loci were not restored and/or expressed in the *hit* recombinants. This situation seems improbable, however, given that the intermediate phenotype was observed even with pTC7, a cosmid which has sequences spanning some 5 kb to the right and 13 kb to the left of the *hit* mutations.

An alternative explanation for the phenotype of the *hit* recombinants would be that *B. bacteriovorus* BB3, BB4, and BB5 are double mutants. In this case, the *hit* recombinants would retain a second, undefined mutation responsible for the intermediate phenotype. Support for this double-mutation hypothesis is discussed in detail elsewhere (24). While the hypothesis is speculative, it is attractive given the results of previous biochemical studies. As has been discussed by Gray and Ruby (7), the available data suggest that at least two distinct host signal molecules must be supplied to the bdellovibrio during IP growth in order for it to complete the entire IP growth cycle. Perhaps the *hit* locus is involved in

TABLE 2. Plating characteristics of *B. bacteriovorus hit* recombinants[a]

Recombinant	PFU		CFU	
	No./ml	Phenotype	No./ml	Phenotype
109J.2(pTC3)	10^9	Large, clear	10^2–10^3	Variable[b]
BB5(pTC7), BB5(pTC12), or BB5(pTC50)	10^9	Large, clear[b]	10^5	Large
BB5(pTC3)	10^9	Small, turbid	10^9	Large

[a] Overnight cultures of *B. bacteriovorus* grown on *E. coli* were plated either on dilute nutrient broth plates containing lawns of host cells or on PYE plates, and the number of PFU or CFU, respectively, was determined.
[b] See text.

the reception or processing of one of the two signals and a second locus, one that is also mutated in *B. bacteriovorus* BB3, BB4, and BB5, receives or processes the other. The fact that *hit* ORF2 probably encodes a polypeptide that is targeted to the periplasm is consistent with this model.

Determining the molecular bases for the H-D growth phenotype of wild-type bdellovibrios and the regulation of the switch between search and growth phases are essential to an overall understanding of the bdellovibrio-host cell interaction. In this and the accompanying paper (3), we have developed a rudimentary system for the genetic analysis of H-I mutants and have identified a locus, *hit*, that has a fundamental role in the bdellovibrio-host interaction. Future efforts will be directed at determining the function of *hit* and the identification of other loci with central roles in *Bdellovibrio* growth and development.

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