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Identification of a Divided Genome for VSH-1, the Prophage-Like Gene Transfer Agent of *Brachyspira hyodysenteriae*▽

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The *Brachyspira hyodysenteriae* B204 genome sequence revealed three VSH-1 tail genes, *hvp31*, *hvp60*, and *hvp37*, in a 3.6-kb cluster. The location and transcription direction of these genes relative to those of the previously described VSH-1 16.3-kb gene operon indicate that the gene transfer agent VSH-1 has a noncontiguous, divided genome.

Gene transfer agents (GTAs) have been described for diverse bacterial species (2, 5, 13, 19, 22). These GTAs resemble small tailed bacteriophages in ultrastructure and package short, random fragments of their bacterial host genomes. Gene transfer ability and "avirulence," i.e., an inability of GTA particles to lyse bacteria or form plaques, are GTA traits (1, 11, 14, 20, 21, 24).

VSH-1 is a GTA produced by the anaerobic, pathogenic spirochete *Brachyspira hyodysenteriae* strain B204 (11). VSH-1 particles contain 7.5-kb fragments of *B. hyodysenteriae* DNA and transfer genes, including antibiotic resistance genes, between strains of the spirochete (11, 15, 20, 21). VSH-1-like elements are widespread in *Brachyspira* species (3, 4, 22, 26).

Previously, head (capsid) and tail proteins of VSH-1 particles were sequenced, and the sequences were used to establish the VSH-1 genome (15). VSH-1 structural and escape (lysis) proteins are encoded by contiguous clusters of VSH-1 head, tail, and lysis genes (Fig. 1; Table 1) occupying 16.3 kb of the B204 chromosome (15). In those studies, the gene encoding Hvp60, a tail-associated protein with the sequence N′-M_K_MPYHFLRNKIYKLPPAPYINE was not found within the 16.3-kb VSH-1 genome cluster (15). Two additional tail-associated proteins, Hvp31 and Hvp37, with sequence ambiguities at several amino acid positions, also could not be located (unpublished observations).

A draft sequence of the *B. hyodysenteriae* B204 genome was recently generated in our laboratory by pyrosequencing (Roche FLX). (The *B. hyodysenteriae* B204 draft genome is currently undergoing closure and annotation. During completion of this effort, web access to a pseudogenome assembly of the DNA contigs will be provided upon request. The VSH-1 genes described in this paper are contained within contig00046 in the draft assembly.) One open reading frame (ORF) was predicted to encode a 62.5-kDa protein with an N-terminal sequence matching the Hvp60 sequence. This ORF, designated *hvp60*, was flanked by two ORFs, designated *hvp31* and *hvp37* (Fig. 1). The predicted sequences of proteins encoded by these ORFs, N′-MAFDFKLIVRDKYSEEIFDI . . . and N′-M NNNLNKNISDTNKELQNIE . . ., were discernible in the ambiguous sequences previously obtained for Hvp31 and Hvp37, respectively (unpublished observations). Predicted tail proteins Hvp31, Hvp60, and Hvp37, encoded by a 3.6-kb gene cluster, share no significant amino acid identity with any of the proteins in the GenBank database.

The VSH-1 3.6-kb and 16.3-kb gene clusters are oriented in opposite transcriptional directions (Fig. 1). Six genes (gene numbers 3 to 8 [Fig. 1]) encoding putative proteins with various predicted functions (Table 1) comprise a 16.7-kb region that separates the 3.6-kb and 16.3-kb clusters. None of the six hypothetical proteins resemble other GTA or phage-related proteins, and several are likely to have bacterial physiological functions.

The sequence ACTTATA was previously identified as a heptanucleotide repeat upstream of *hvp45*, the first gene in the VSH-1 16.3-kb cluster, and was proposed as a possible binding site for proteins involved in transcription regulation of VSH-1 expression (16). The repeat is more likely to be a hexanucleotide sequence, in that a matching six-nucleotide repeat sequence (. . . CTTATAAATCCTTATATTTTTTA . . . [repeat sequence is underlined]) was also detected upstream of the VSH-1 3.6-kb cluster (Fig. 1). A string of six T's follows the second CTTATA repeat upstream of both VSH-1 gene clusters. Direct-repeat recognition sequences have been identified for both bacterial and bacteriophage promoters (7, 8, 17, 23). In the draft B204 genome, similar "recognition" sequences are present upstream of two other single ORFs at least 100 kb from the VSH-1 genes. The identities of hypothetical proteins encoded by those genes are uncertain. Their possible involvement in VSH-1 production is currently being investigated.

Quantitative real-time PCR was used to measure transcription of VSH-1 genes *hvp38* and *hvp60* and putative bacterial gene numbers 4 and 7 (Fig. 1) during the induction of VSH-1 by the antimicrobial carbadox. Quantitative real-time PCR conditions and specific primers for *hvp38* have been described (20). Primers for *hvp60* mRNA were 5′-AC AAATAACAATGTCATTCAGCG and 5′-TATCCGTCAA AATCTACTCCCC, and the quencher-reporter (Q-R) probe was 5′-CGAGGTAGAAGAAAGTTTATCAGATA CTTGGTGC. Primers for gene number 4 mRNA were 5′-G

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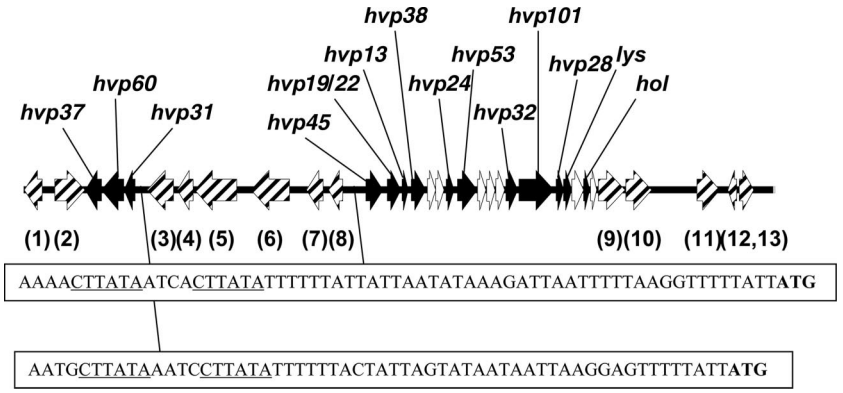

FIG. 1. VSH-1 gene map based on this and previous studies. VSH-1 genes (black arrows), *B. hyodysenteriae* genes (striped arrows), and ORFs greater than 240 bp with no GenBank homologs (white arrows) are oriented according to their direction of transcription. Descriptions of identified or putative proteins encoded by the genes are included in Table 1. At the bottom of the figure, sequences immediately upstream of the VSH-1 16.3-kb and 3.6-kb gene clusters are given. (For convenience, the reverse complement sequence upstream of the 3.6-kb gene cluster is provided.) **ATG** represents start codons of the respective genes encoding Hvp31 and Hvp45 proteins. CTTATA hexanucleotide repeat sequences representing potential transcription control regions are underlined.

GATCATGTAATGGAAGGTGCTGC and 5′-TTCCTTTAC CAGTACCGCCGAA, and the Q-R probe was 5′-AGGAGG AGGAAGCAGTATGGACTCTT. The primers for gene number 7 were 5′-CTGCTGCAACTTTGCCTGTAGCTT and 5′-ATAGTGCCAATAGCAGGAAGCTGG, and the Q-R probe was 5′-GGGAGCAACTATACACTTATGCGGA TCAGT.

Transcription of both VSH-1 genes *hvp38* and *hvp60* increased by 200- to 300-fold within 5.5 h after *B. hyodysenteriae* cultures were treated with 0.5 μg carbadox/ml. In contrast, transcription of bacterial gene number 4 decreased 10- to 20-fold to undetectable levels in treated versus control cultures, and transcription of bacterial gene number 7 was undetectable in both treated and control cultures. These results and the finding of common upstream sequences with identical hexanucleotide repeat sequences suggest that the VSH-1 16.3-kb and 3.6-kb gene clusters, although noncontiguous, are regulated by a common mechanism. Transcription of the intervening bacterial-type genes is not detectably induced and, thus, those genes are not essential for VSH-1 production.

TABLE 1. Genes and proteins of VSH-1 and *B. hyodysenteriae* B204 identified in this and previous studies[a]

Gene(s)	ORF in B204 draft genome	Protein identity	Identification basis	Closest protein match (GenBank accession no.) and E value
VSH-1				
hvp45 to *hvp24*	02481 to 02474	Head proteins	Protein sequencing	None
hvp53 to *hvp28*	02472 to 02467	Tail proteins	Protein sequencing	None
lys/*hol*	02465/02463	Endolysin/holin lysis proteins	Enzyme activity/predicted properties	*Salmonella* phage ϵ15 endolysin (AAO06088); $E = 5 \times 10^{-29}$
hvp31 to *hvp37*	02494 to 02497	Tail proteins	Protein sequencing	None
B. hyodysenteriae B204[b]				
1	02501	AdSS	Conserved domains	*Thermosinus carboxydivorans* adenylosuccinate synthase (ZP_01666816.1); $E = 10^{-146}$
2	02498	Unknown		*Paramecium* TPR protein (XP_001427675); $E = 7 \times 10^{-50}$
3	02493	Mcp	Conserved domains	*B. hyodysenteriae* methyl-accepting chemosensory protein (AAAX81982); $E = 10^{-92}$
4	02491	Adh	Conserved domains	*Caldicellulosiruptor saccharolyticus* Fe-alcohol dehydrogenase (YP_001179521); $E = 10^{-116}$
5	02490	Unknown		*Tetrahymena* TPR protein (XP_001026739); $E = 10^{-22}$
6	02487	Trep		*Treponema denticola* unknown function protein (NP_972591); $E = 8 \times 10^{-34}$
7	02485	AA transport	Conserved domains	*Fusobacterium nucleatum* Ser/Thr transporter (NP_604045); $E = 10^{-105}$
8	02484	Fe-S oxidoreductase	Conserved domains	*Clostridium kluyveri* Fe-S oxidoreductase (YP_001393555); $E = 10^{-39}$
9	02461	Mcp	Conserved domains	*B. hyodysenteriae* methyl-accepting chemosensory protein (AAP58978); $E = 4 \times 10^{-94}$
10	02460	Mcp	Conserved domains	*B. hyodysenteriae* methyl-accepting chemosensory protein (AAP58978); $E = 3 \times 10^{-112}$
11	01437	NhaC	Conserved domains	*Clostridium leptum* Na^+/H^+ antiporter (ZP_02079635); $E = 8 \times 10^{-101}$
12	01435[c]	Unknown	Conserved domains	*Ruminococcus gnavus* unknown function protein (ZP_02040322); $E = 3 \times 10^{-58}$
13	01433[c]	Adh	Conserved domains	*B. hyodysenteriae* WA-1 alcohol dehydrogenase (ABS12704); E = 0.0

[a] Described previously by Matson et al. (15). ORF number designations in column 2 refer to positions in the *B. hyodysenteriae* B204 draft genome. Closest protein matches in column 5 are all hypothetical proteins.

[b] *B. hyodysenteriae* B204 genes (bacterial gene homologs) are labeled, for convenience, according to their positions in Fig. 1.

[c] ORFs 01433 and 01435 have adjacent gene homologs on the *Ruminococcus gnavus* genome map.

VSH-1 particles contain 7.5-kb fragments of DNA (11). The newly discovered 3.6-kb cluster of VSH-1 tail genes together with the previously identified 16.3-kb VSH-1 gene cluster spans 36.6 kb of DNA in the *B. hyodysenteriae* B204 genome. It is highly unlikely, therefore, that a single VSH-1 particle carries sufficient DNA (i.e., five 7.5-kb fragments) to encode proteins involved in structural and escape-from-host functions. This genome arrangement strongly suggests that it is impossible for VSH-1 to have an independent, self-propagating lifestyle and explains the avirulence of VSH-1 particles.

The discovery of noncontiguous genes, i.e., a "divided" genome for VSH-1, has several implications for GTAs and prophage-like gene clusters in *Brachyspira* and other bacterial species. First, the contiguity or dispersion patterns of VSH-1 genes in other *Brachyspira* genomes could provide insight into the origin, evolution, and spread of VSH-1-like elements among *Brachyspira* species. Second, gene proximity is not essential for VSH-1 production. A contiguous genome, therefore, may not be a requirement for the production of GTAs by other bacteria. No recognizable lysis genes are associated with the gene cluster of RcGTA, GTA of *Rhodobacter capsulatus*, and these and other essential genes could be positioned at other undiscovered locations in the *R. capsulatus* genome (12). Additionally, certain *Rickettsiales* species contain multiple gene homologs of RcGTA dispersed within their genomes (13). Techniques for identifying GTAs (19) might be applied to detect functional GTAs in these bacteria. Finally, gene clusters for incomplete prophages have been found in bacterial chromosomes (6, 10, 18, 25). Although clearly defective as self-propagating prophages, perhaps some of these gene clusters can interact with distal genes to form effective GTAs. While there is no direct evidence to support this speculation, it is worth considering in view of the widespread distribution of GTA genes among alphaproteobacteria (13). Indeed, the possibility that some "defective" prophages serve as GTAs may provide an alternative explanation for the occasional failure (9) to demonstrate that mitomycin C-induced "bacteriophages" are capable of lytic (plaque-forming) growth.

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