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Sequence Characterization and Comparative Analysis of Three Plasmids Isolated from Environmental *Vibrio* spp.▽†

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The horizontal transfer of genes by mobile genetic elements such as plasmids and phages can accelerate genome diversification of *Vibrio* spp., affecting their physiology, pathogenicity, and ecological character. In this study, sequence analysis of three plasmids from *Vibrio* spp. previously isolated from salt marsh sediment revealed the remarkable diversity of these elements. Plasmids p0908 (81.4 kb), p23023 (52.5 kb), and p09022 (31.0 kb) had a predicted 99, 64, and 32 protein-coding sequences and G+C contents of 49.2%, 44.7%, and 42.4%, respectively. A phylogenetic tree based on concatenation of the host 16S rRNA and *rpoA* nucleotide sequences indicated p23023 and p09022 were isolated from strains most closely related to *V. mediterranei* and *V. campbellii*, respectively, while the host of p0908 forms a clade with *V. fluvialis* and *V. furnissii*. Many predicted proteins had amino acid identities to proteins of previously characterized phages and plasmids (24 to 94%). Predicted proteins with similarity to chromosomally encoded proteins included RecA, a nucleoid-associated protein (NdpA), a type IV helicase (UvrD), and multiple hypothetical proteins. Plasmid p0908 had striking similarity to enterobacteria phage P1, sharing genetic organization and amino acid identity for 23 predicted proteins. This study provides evidence of genetic exchange between *Vibrio* plasmids, phages, and chromosomes among diverse *Vibrio* spp.

The *Vibrionaceae* are gram-negative *Gammaproteobacteria* that occur in temperate to tropical, coastal, and estuarine marine systems (62). *Vibrio* spp. occupy a diverse range of ecological niches, including sediments, the water column, and in association with organisms either as symbionts (48) or pathogens (26, 37). Phages contribute to *Vibrio* evolution and ecology by regulating host abundance (29) and transferring virulence genes, such as the cholera toxin encoded by *ctxAB* of the CTXϕ phage of *V. cholerae* (64). Plasmids such as pJM1 of *V. anguillarum* (20) have also been shown to play a role in *Vibrio* pathogenicity. In recent years, sequencing has revealed the vast diversity of phage genomes (10) and their globally significant contributions to horizontal gene transfer within marine environments (35). In contrast to the demonstrated genetic diversity of vibriophages (16, 66), much less is known of *Vibrio* plasmid diversity and the role of plasmids in gene transfer. A few studies have reported the occurrence of plasmids among *Vibrio* populations (19–21, 44, 63), and several have reported complete sequences of *Vibrio* plasmids associated with pathogenic vibrios; however, the distribution and sequence diversity of *Vibrio* plasmids has not been studied as extensively as vibriophages.

As of September 2007, there are 16 plasmid and 20 phage sequences in GenBank that were isolated from vibrios (12–14, 20, 21, 23, 25, 27, 28, 31, 38, 41, 43, 45–48, 51, 67). These sequences are biased toward small elements (i.e., nine plasmids of <8 kb and 10 phages of <9 kb) and are primarily associated with well-characterized human and fish pathogens. Among these are plasmids isolated from *V. anguillarum* (20, 67), *V. cholerae* (46, 47), *V. vulnificus* (14), *V. parahaemolyticus* (41), and *V. salmonicida*. The lack of plasmid sequence data, particularly of plasmids from *Vibrio* hosts isolated from coastal water and sediment, limits our understanding of *Vibrio* plasmid evolution and diversity.

In the present study we provide a comparative assessment of plasmids with diverse sizes and gene contents isolated from vibrios. Similarities of replication initiation and hypothetical proteins revealed relatedness of plasmids from vibrios occupying diverse niches. In addition, these elements contained numerous phage-like proteins, including proteins with considerable similarity and conserved gene order to enterobacteria phage P1. To our knowledge, this is the first report of P1-like phage sequences isolated from a marine bacterium. A previous study identified two P1-like genes as part of a marine viral metagenome (10); however, no additional P1 genes or nearly complete P1 genomes have been characterized from the marine environment.

MATERIALS AND METHODS

Bacterial strains, media, and plasmid isolation. *Vibrio* sp. strains 0908, 23023, and 09022 were isolated from salt marsh sediment of Charleston, SC, in December 1998 (17). DNA for sequencing was obtained by purification of supercoiled plasmid DNA by cesium chloride density gradient centrifugation as previously described (52).

Plasmid sequencing and sequence analysis. Plasmids were sequenced using whole-genome shotgun sequencing and finishing methods (26). Initial open reading frame designations and annotation of select open reading frames was done using an automated annotation system (26). Protein-coding sequences (CDSs) were confirmed by independent analysis using GeneMark software (7). Putative similarity to known proteins was determined by amino acid sequence comparison and identification of common motif and domain structure using a combination of PSI-BLAST (3) from the National Center for Biotechnology Information, SMART (50), COG (57), and Pfam (6) Web-based software. PSI-BLAST anal-

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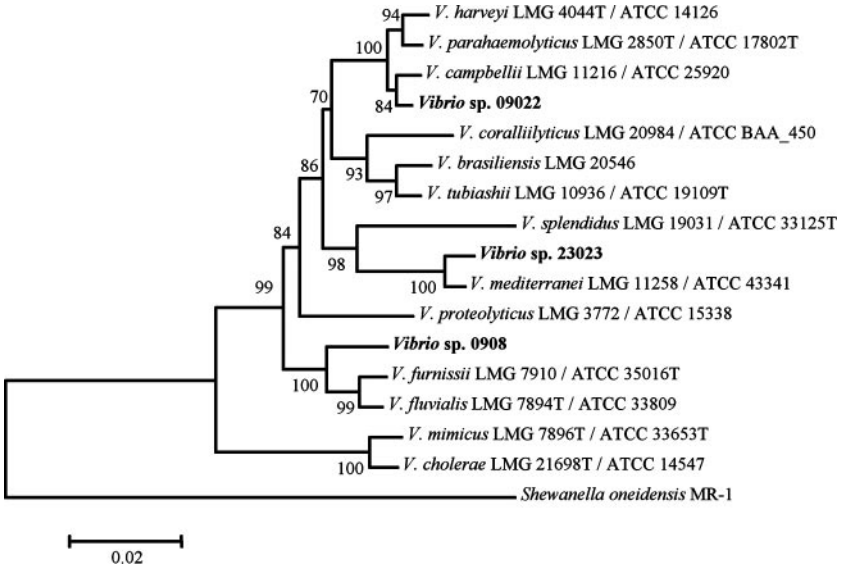


FIG. 1. A concatenation of 16S rRNA and *rpoA* nucleotide sequences of the plasmid hosts, *Vibrio* sp. strains 0908, 23023, and 09022, was used to determine relatedness of the hosts to other *Vibrio* spp. as examined in a previous study (60). The neighbor-joining method with the Jukes-Cantor model of distance estimation (30) was used to generate the tree with a concatenation of 16S rRNA (1,452 nucleotides) and *rpoA* (772 nucleotides) sequences. Bootstrap values represent 1,000 replications, and only those with values of ≥50 are shown.

ysis was performed with the default threshold E-value of 0.005 and a maximum threshold of 1.0 over one to two iterations. ClustalW was used to generate all alignments (61).

Phylogenetic analyses and sequence alignments. Host strains were identified by a concatenated phylogenetic analysis of 16S rRNA and *rpoA* nucleotide sequences as previously described (18). The neighbor-joining tree was generated using MEGA with the Jukes-Cantor (30) distance estimation model with 1,000 replications for the nucleotide concatenation or the Poisson correction for the amino acid RecA tree (42). Percent identities of the nucleotide sequences to the most related organism were determined using BLASTN (3) and BLAST2 (58) sequences. Sequencing was performed by the University of Nevada, Reno, Genomics Center and the Core Genomics Facility at the Georgia Institute of Technology.

Identification of phage-like proteins. Prophage Finder (9) was used with BLAST analysis (3, 49) of a phage sequence database to identify prophages and proteins with similarities to phage-associated proteins for all sequenced *Vibrio* plasmids available in GenBank as of July 2007. An E value of 0.001 with 10 hits/prophage and a hit spacing of 3,500 were used as parameters for all plasmids examined.

Nucleotide sequence accession numbers. The plasmid sequences have been submitted to the GenBank database under accession numbers CP000755 to CP000757. All additional sequences have been submitted to the GenBank database under accession numbers EU022567 to EU022572.

RESULTS AND DISCUSSION

Host phylogeny and plasmid features. In this study we examined the sequence diversity of plasmids previously isolated from three *Vibrio* hosts (17). A concatenation of 16S rRNA sequences and *rpoA* nucleotide sequences was used for greater resolution of related *Vibrio* spp. (60). The 16S rRNA and *rpoA* nucleotide sequences of *Vibrio* sp. strains 0908, 23023, and 09022 were 98 and 97%, 98 and 99%, and 99 and 98% identical to those of *V. fluvialis*, *V. mediterranei*, and *V. campbellii*, respectively. Phylogenetic analysis of concatenated 16S rRNA and *rpoA* nucleotide sequences of *Vibrio* sp. strains 23023 and 09022 indicated they were most related to *V. mediterranei* and *V. campbellii*, respectively (Fig. 1). *Vibrio* sp. strain 0908 forms a clade with the closely related *V. furnissii* and *V. fluvialis* group (11). To date, the only report of mobile genetic elements (MGEs) associated with any of these *Vibrio* species is an SXT-like element of *V. fluvialis* with similarity to the multiple antibiotic resistance element SXT previously characterized from *V. cholerae* (2). This previous study indicated there may be transfer of MGEs among well-characterized pathogens such as *V. cholerae* and emerging marine pathogens such as *V. fluvialis* (11, 34, 55).

The nucleotide sequences of the *Vibrio* plasmids p0908, p23023, and p09022 were 81,413 bp, 52,527 bp, and 31,036 bp in length with overall G+C contents of 49.2%, 44.7%, and 42.4%, respectively (see Table S1 in the supplemental material). With the exception of p0908, the G+C contents of the plasmids were within the range of percentages reported for *Vibrio* genomes (38 to 47%) (14, 26, 37, 48). The plasmids p0908, p23023, and p09022 encoded 99, 64, and 32 predicted CDSs, respectively (see Table S1 in the supplemental material). The predicted proteins were assigned primarily to the following functional categories: replication, stable maintenance, partitioning, and recombination. Additional predicted proteins identified on one or more of the plasmids may be involved in mobilization, restriction modification, or transcriptional regulation (see Tables S1 to S3 in the supplemental material). The only genes common to at least two of the three plasmids were the putative replication initiation and partitioning proteins. The predicted replication initiation protein of p09022 encoded by CDS19 was 94% identical to the replication initiation protein of plasmid pKA1 from *V. cholerae* and 39% identical to the replication initiation protein of p0908. The predicted protein of p23023 most closely resembling a replication initiation protein was that encoded by CDS11, although it had little similarity to predicted replication proteins from characterized *Vibrio* or other marine plasmids.

Plasmids encoding putative proteins for self-mobilization,

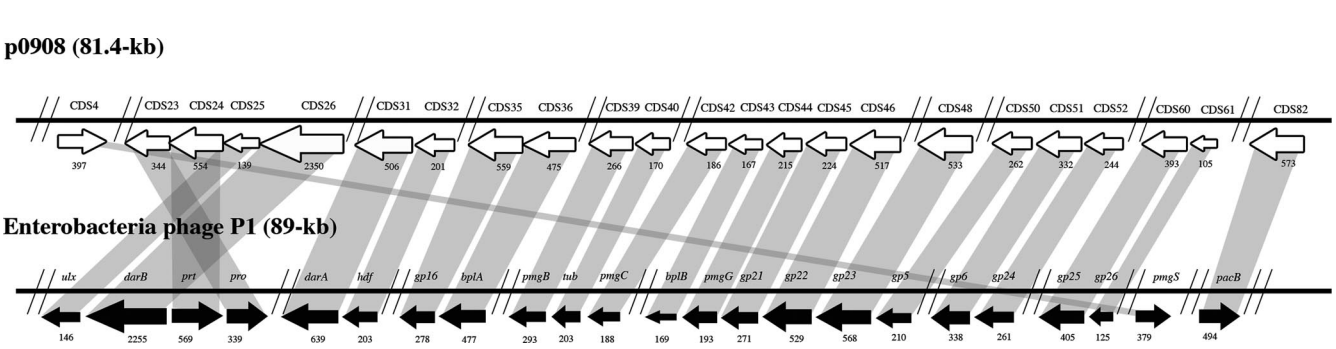


FIG. 2. Genetic organization and amino acid conservation of predicted CDSs of p0908 compared to CDSs of enterobacteria phage P1. Shading indicates regions with amino acid similarity, while the protein lengths (number of amino acids) are designated under each arrow and approximated by the arrow size. The orientation of each arrow indicates the direction of transcription. Vertical lines indicate the presence of additional genes that are not shown.

such as p23023, may be frequently transferred between *Vibrio* hosts. In contrast, plasmids such as p0908 and p09022, without identifiable proteins aiding transfer, may rely on transmission by phages or other mechanisms. *V. cholerae* was recently shown to naturally transform 22-kb segments of genomic DNA, suggesting mechanisms of DNA uptake may facilitate incorporation of large DNA molecules (39). Additional studies would be required to determine mechanisms promoting transmission of the plasmids described in this study.

The significant amino acid identity (≥83%) (see Table S3 in the supplemental material) and conserved gene order of six predicted proteins encoded by p09022 compared to those of *V. cholerae* plasmid pKA1 suggest *Vibrio* plasmids from diverse hosts may undergo frequent gene exchange. Alternately, this may indicate a common *rep* family exists among diverse *Vibrio* hosts, as the conserved genes included three proteins likely to be involved in replication initiation and partitioning. The remaining three predicted proteins were hypothetical (see Table S3 in the supplemental material). An additional protein encoded on p09022 had 94% amino acid identity to a hypothetical protein of plasmid p0471 from an uncharacterized marine bacterial host (1).

Identification of P1-like proteins on *Vibrio* plasmid p0908. The phage P1 of enterobacteria has been isolated from enteric bacteria (40) and has been shown to infect diverse bacteria under certain laboratory conditions (40). P1-like proteins and evidence of intact P1 phage have been identified in freshwater (5); however, to date none has been identified in marine systems. We identified 23 CDSs on p0908 with similarity to P1-encoded proteins and 20 additional proteins with similarity to other phage-encoded proteins. The remaining 16 predicted proteins were similar to chromosomally or plasmid-encoded proteins, and 41 had no similarity to previously characterized proteins. The 23 CDSs of p0908 encoding P1-like proteins also occur in the same genomic arrangement as reported for the P1 genome, with a few differences, possibly due to rearrangements (Fig. 2) (36). The majority of these P1-like proteins (20 of 23) exhibited the same direction of transcription (Fig. 2). The G+C contents of CDSs encoding the P1-like proteins (47 to 54%) were more similar to the overall G+C contents of p0908 (49.2%), P1 (49%) (36), and the *Escherichia coli* host (50%) (8) than *Vibrio* chromosomes (39 to 47%) (14, 26, 37, 48).

Of the proteins encoded on p0908 with similarity to P1 proteins, there were 16 structural proteins, 6 antirestriction and head-processing proteins, and 1 involved in DNA packaging (see Table S1 in the supplemental material). The structural proteins included those described as base plate and tail tube (gp16, BplA, PmgB, Tub, PmgC, BplB, PmgG, gp5, gp6, gp24, gp25, and gp26), sheath (gp21 and gp22), and head (PmgS and gp23) components. The P1-like proteins involved in antirestriction and head processing include DarA and DarB (36). Antirestriction proteins such as DarA and DarB prevent damage of phage DNA by host restriction enzymes (36). Identification of a protein encoded on p0908 with similarity to DarA strongly suggests p0908 may have acquired the P1-like genes from a P1 phage, since DarA was shown to be unique to P1 (36). An additional indication of gene exchange between P1 and p0908 is the presence of a CDS encoding a protein similar to DarB. The predicted protein of CDS26 (2,350 amino acids) is comparable to DarB, which is the largest P1-encoded protein (2,255 amino acids) (36) (see Table S1 in the supplemental material). Most of the P1-like genes of p0908 encode proteins for phage structures, such as *tub*, encoding a tail protein, and *pro*, involved in cleavage of head proteins during phage formation (36). P1 genes involved in prophage addiction, *phd* and *doc* (26), were noticeably absent from p0908. Although there were many phage structural proteins encoded on p0908, it is unlikely this is a functional phage, as critical proteins for packaging and dispersal were absent. These included *lydA* and *lydB*, encoding a holin and antiholin for host cell lysis (36). Of the proteins known to be required for functional packaging, PacB was identified; however, PacA was absent. The gene encoding PacA includes the *pac* cleavage site, which is cleaved by the pacase enzyme, which is composed of PacA and PacB proteins (36). A few proteins of KVP40, a T4-like phage, were similar to proteins of P1; however, this similarity was attributed to the relatedness of P1 and T4, both of which are in the viral family *Myoviridae* (38). Two of these shared proteins were identified on p0908, BplA and Tub (38); however, numerous additional proteins similar to those encoded in the P1 genome were identified on p0908 that were not present on KVP40. Also encoded on p0908 are integrase-like proteins, indicating the potential for integration of this element into a host chromosome (see Table S1 in the supplemental material). Although *Vibrio* phages have been characterized with similarity to T4 (38), T7 (25), and P2 (43) phages, none have been character-

ized with similarity to P1 (36). The P1 integrase (*cre*) was identified in bacterial lysogens from a freshwater pond, indicating the presence of P1 in a freshwater environment (5). A viral metagenomic study produced two sequences with similarity to P1 PacA and PacB in estuarine waters of southern California (10). To our knowledge, no additional P1-like sequences or nearly complete P1 genomes have been identified from marine environments. The complete P1 sequence was finished after the viral metagenome was performed and, therefore, some of the P1 genes may have not been identified in the viral metagenome; however, a recent comparison of the P1 nucleotide sequence to the viral metagenome database primarily yielded hits to prophage from fish ponds (F. Rohwer, personal communication). This indicates that additional P1 genes were not present in the marine viral metagenome. The pathogenic nature of some *Vibrio* spp. and possible residence in the gut may have facilitated a *Vibrio* MGE to exchange genes with P1 of an enteric bacterium, resulting in an element such as p0908.

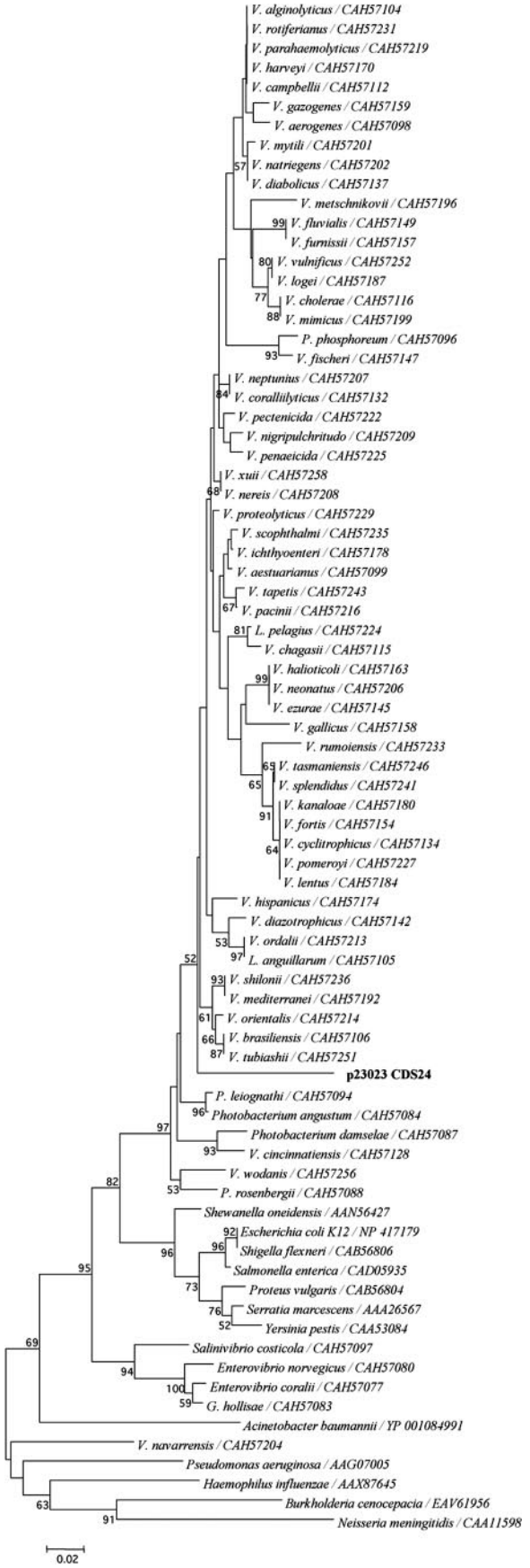
Identification of additional phage-like proteins on *Vibrio* plasmids. The prevalence of P1-like proteins on p0908 led us to examine the occurrence of additional proteins typical of phage on all available *Vibrio* plasmids. Several non-P1 phage-like proteins were identified on the plasmids and in some cases had a conserved gene order as well as amino acid identity. BLAST searches (3, 49) of the plasmid genomes to a phage-only sequence database using Prophage Finder (9) identified plasmid CDSs encoding proteins similar to phage proteins. There were 43, 5, and 6 predicted proteins with similarity to phage proteins encoded by CDSs of p0908, p23023, and p09022, respectively (see Tables S1 to S3 in the supplemental material). Functions assigned to these proteins included replication, partitioning, transcriptional regulation, methylation, and recombination.

Of the 16 complete *Vibrio* plasmid sequences currently available (pES100, pYJ106, pJM1, pEIB1, pPS41, pSA19, pSIO1, pTC68, pVS43/pVS54, pES213, pTLC, pC4602-1, pC4602-2, pMP-1, and pR99), we detected the highest frequency of phage proteins on plasmids described in this study. These proteins encoded by CDSs of p0908, p23023, and p09022 represented 43, 8, and 20% of the total predicted CDSs, respectively. In contrast, the other large plasmid sequences available, pES100 (45.8 kb) (48), pJM1 (65 kb) (20), pEIB1 (66.1 kb) (67), and pC4602-1 (56.6 kb), pC4602-2 (66.9 kb), pR99 (68.4 kb), and pYJ106 (48.5 kb) (14), isolated from *V. fischeri*, *V. anguillarum*, and *V. vulnificus*, respectively, encoded proteins with similarity to phage proteins that comprised between 4 and 14% of the predicted CDSs. Of the remaining nine plasmids, all less than 8 kb in size, pMP-1 (7.6 kb) had three proteins and pTLC (4.7 kb) (47), pVS43 (4.3 kb), and pVS54 (5.4 kb), isolated from a single strain of *V. salmonicida*, encoded a protein with similarity to a protein associated with a phage.

The additional non-P1 phage proteins identified by BLAST analysis included recombinases, transcriptional regulators, transposases, and hypothetical proteins. Specifically, CDSs 53 to 56 of p0908 encoded proteins with 49 to 72% amino acid identity to CDSs 35 to 38 of phage VHML of *V. harveyi* (see Table S1 of the supplemental material) (43). The comparable predicted amino acid sizes, identical gene order, and high amino acid identities of these proteins suggest recombination between *Vibrio* phage and plasmid elements. Additional proteins identified on phages and other plasmids include hypothetical proteins with a helix-turn-helix (H-T-H) motif. The H-T-H motif is typical of transcriptional regulators and other proteins with DNA-binding activity (6, 56). The amino acid sequence of CDS27 of p0908 is one example with 30% amino acid identity to a hypothetical protein of *Photorhabdus luminescens* and 25% identity to the *luxR* of *V. parahaemolyticus*. CDSs 10 and 21 of p23023 were shown to have similar H-T-H motifs. Hypothetical proteins with H-T-H motifs were also reported for predicted proteins of vibriophages VP16C and VP16T (51). To our knowledge, the function and role of these putative transcriptional regulators for plasmid or phage stability have not been characterized.

Identification of conserved *Vibrio* chromosomal genes on *Vibrio* plasmids. The three plasmids examined in this study encoded numerous CDSs with significant amino acid identity (33 to 81%) to chromosomally encoded genes of vibrios (see Tables S1 to S3 in the supplemental material). To our knowledge, these chromosomally encoded genes have exclusively been identified on chromosomes and not on MGEs. Among those with significant amino acid identity were RecA (81%), a nucleoid-associated protein, NdpA (65%), a type IV helicase, UvrD (65%), and a number of hypothetical proteins (50 to 80%). A RecA protein was previously reported on plasmid pNP40 (65 kb) from *Lactococcus lactis* (24); however, none has been identified to date on *Vibrio* plasmids. Also, the plasmid-encoded RecA described in this study has greater protein identity (81%) to *Vibrio* RecAs than the lactococcal plasmid RecA had to other characterized lactococcal RecAs (45% amino acid identity) (24). The RecA (CDS24) encoded on p23023 (see Table S2 in the supplemental material) was more similar to RecA of other vibrios (81% amino acid identity) than to that of related *Gammaproteobacteria*, such as *Photobacterium* spp. (Fig. 3). This indicates the plasmid-encoded *recA* was likely from a *Vibrio* host. Sequence alignment of the predicted amino acid sequence of CDS24 to RecA sequences of *V. mediterranei*, *V. splendidus*, *V. parahaemolyticus*, and *E. coli* shows the extent of conservation of CDS24 to *Vibrio* RecAs (Fig. 4). The RecA signature motif characteristic of RecA proteins is present in all the aligned sequences (Fig. 4) (6). Also, the P-loop motif for ATP binding, which is characteristic of ATPase-like proteins, is present in CDS24 (4, 65) (Fig. 4). The DNA-binding loops L1 and L2, which are involved in double-stranded and single-stranded DNA binding, respectively (24, 54), are also present in CDS24. The DNA-binding loop L1 of CDS24 is identical to the same motif found in other RecAs (24, 54). In contrast, loop L2 contains a gap and two other amino acid changes that may alter the single-stranded DNA-binding activity of the protein encoded by CDS24. Based on sequence analysis of CDS24, the predicted protein likely has the recombinase (15) and proteolytic cleavage activities that have been characterized to date for other RecAs (22, 32). Future experimental studies are required to confirm these predicted functions of CDS24.

RecA protein sequences have been shown for some bacterial species to provide greater resolution than phylogenetic analyses of an equal number of 16S rRNA sequences (22). Phylogenetic assessments of vibrios have previously demonstrated that *recA* nucleotide sequences can be used as an alternate



phylogenetic marker to 16S rRNA (53, 59, 60). Several studies revealed considerable sequence variation (0 to 6%) of *recA* for certain *Vibrio* spp. (60), with as low as 94% *recA* nucleotide identity within a species. In contrast, *Photobacterium* spp., also within the *Vibrionaceae*, had less than 94% *recA* identity to the closest-related *Vibrio recA.* Overall, CDS24 is highly conserved compared to other RecA sequences; however, the N- and C-terminal regions have significantly diverged (Fig. 4). Sequence analyses of RecAs from diverse bacteria revealed the majority of the protein to be highly conserved while the N and C termini were significantly variable (22). The observed sequence divergence of the termini of the predicted protein sequence of CDS24 may have occurred by recombination with alleles having greater sequence divergence after the sequence was acquired by the plasmid. Alternately, selection pressure for a specialized role of the plasmid-encoded RecA for plasmid stability or uncharacterized functions may have led to the sequence divergence in the terminal regions.

This study is the first report of a *recA* encoded on a plasmid isolated from a *Vibrio* host. The potential for horizontal transmission of *recA* by *Vibrio* plasmids raises questions of whether *recA* provides reliable resolution for discriminating between related *Vibrio* spp. (59) or determining the extent of O-antigen gene exchange (53). Additional plasmid-encoded proteins with similarity to conserved chromosomal genes include the nucleoid-associated protein NdpA and a UvrD-like helicase. The plasmid-encoded NdpA reported here, CDS97 of p0908, had 65% amino acid identity (79% similarity) to NdpA of *V. vulnificus* (see Table S2 in the supplemental material). Also, CDS48 of p23023 had 65% amino acid identity (79% similarity) to a UvrD-like helicase of *Vibrio splendidus* (see Table S2 in the supplemental material). Recombination of host and plasmid-encoded *uvrD*-like genes may increase diversity of *uvrD*, potentially disrupting function of the host protein. To our knowledge this is the first description of an NdpA-like protein and a UvrD-like helicase encoded on a plasmid. These results indicate *Vibrio* plasmids may be involved in horizontal dissemination of conserved genes, such as *recA* and *uvrD*, both involved in host adaptive responses. Further investigation of the diversity encoded by *Vibrio* plasmids would be necessary to determine the extent that these elements transfer conserved genomic regions among diverse *Vibrio* spp.

In addition to high amino acid identity, several proteins had an identical gene order in the plasmid as that found in the *Vibrio* chromosomes. Specifically, CDSs 51 to 54 and 59 to 60 of p23023 encoded predicted proteins with amino acid identities and a conserved gene order to those reported for hypothetical proteins of *V. fischeri* (see Table S2 in the supplemental material).

FIG. 3. Phylogenetic comparison of the predicted amino acid sequence encoded by CDS24 of p23023 with RecA amino acid sequences representing most *Vibrio* spp. and additional members of the *Vibrionaceae* available in GenBank as of March 2007. Distantly related proteobacteria were included as outgroups. A neighbor-joining tree was constructed in MEGA (33) using the Poisson correction (42). Bootstrap values were generated over 1,000 replications and are indicated where the value was ≥50.

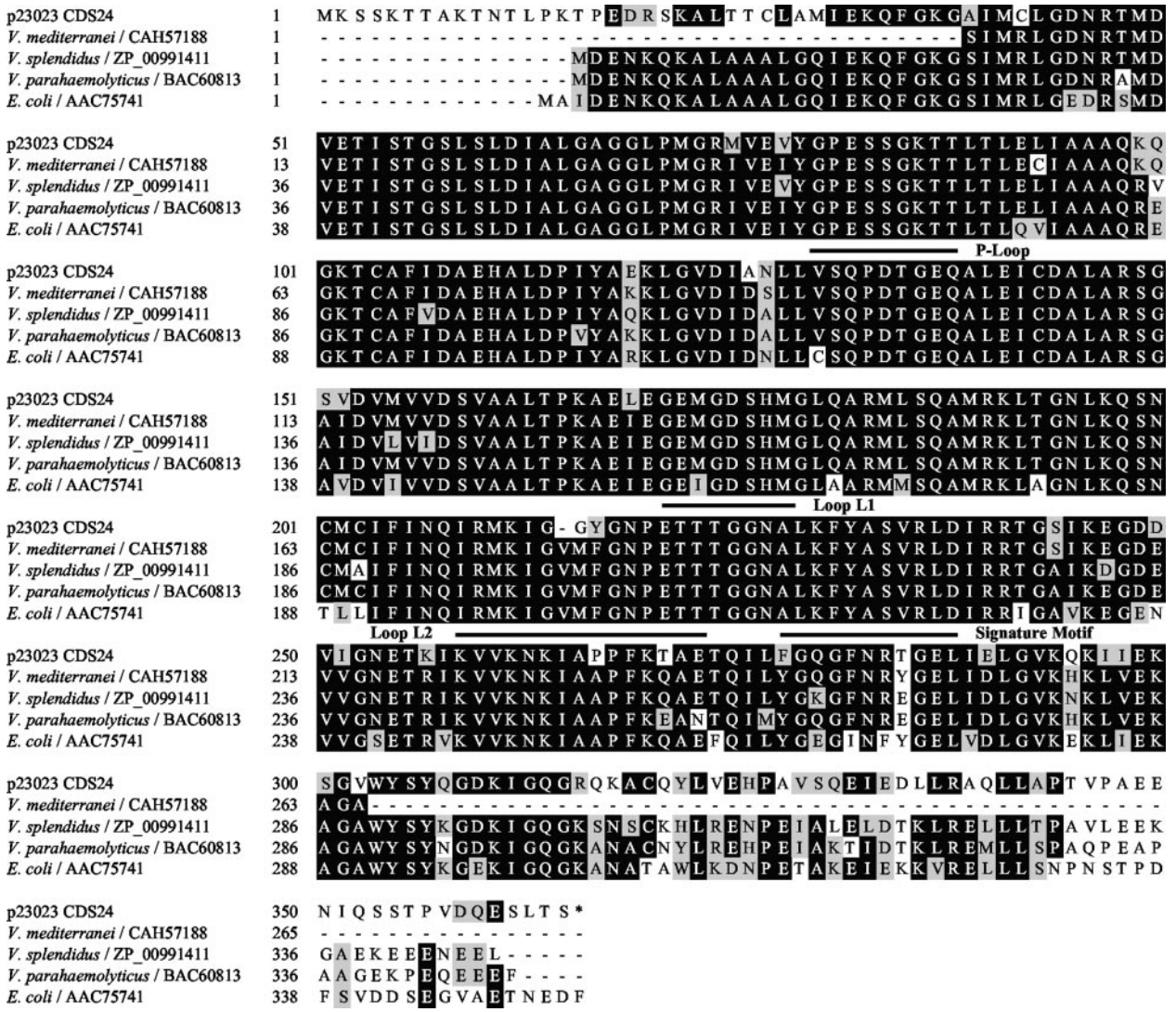


FIG. 4. Sequence alignment of RecA amino acid sequences of CDS24 from p23023 with those sequences encoded on the genomes of *V. splendidus*, *V. parahaemolyticus*, and *E. coli*. The *V. mediterranei* RecA sequence was a partial sequence obtained from a multilocus sequence analysis of *Vibrio* spp. (60). The RecA signature motif (4), P-loop motif (Walker A motif) involved in ATP binding (6), loop L1 motif involved in binding of double-stranded DNA (24, 54), and the loop L2 motif for binding single-stranded DNA (24, 54) are all indicated.

Conclusion. This study provides evidence for a role of *Vibrio* plasmids in gene exchange among diverse *Vibrio* spp., as evidenced by the gene content and unique genomic signatures of *Vibrio* plasmids relative to *Vibrio* chromosomes. Identification of P1-like proteins and other phage-like proteins on *Vibrio* plasmids supports the mosaicism of *Vibrio* MGEs and the potential for recombination between *Vibrio* plasmids and phages. The considerable diversity of *recA* among strains of certain *Vibrio* spp. may be facilitated by recombination of plasmid-encoded genes, such as the p23023 *recA*. Further studies into the genetic diversity of *Vibrio* plasmids as well as their potential host range are needed to better understand the evolution of MGEs and their role in diversification of *Vibrio* spp. This will serve as the basis for future molecular investigations into the role of plasmids for unique phenotypes promoting adaptation to fluctuating environmental conditions and the potential emergence of pathogens.

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