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Prevalence of mobile genetic elements and transposase genes in *Vibrio alginolyticus* from the southern coastal region of China and their role in horizontal gene transfer

Peng Luo, Haiying Jiang, Yanhong Wang, Ting Su, Chaoqun Hu,* Chunhua Ren, Xiao Jiang

Key Laboratory of Marine Bio-resources Sustainable Utilization, Chinese Academy of Sciences, South China Sea Institute of Oceanology, Guangzhou, China

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Summary. *Vibrio alginolyticus* has high genetic diversity, but little is known about the means by which it has been acquired. In this study, the distributions of mobile genetic elements (MGEs), including integrating conjugative elements (ICEs), superintegron-like cassettes (SICs), insertion sequences (ISs), and two types of transposase genes (*valT1* and *valT2*), in 192 strains of *V. alginolyticus* were investigated. ICE, SIC, and IS elements, *valT1*, and *valT2* were detected in 8.9 %, 13.0 %, 4.7 %, 9.4 %, and 2.6 % of the strains, respectively. Blast searches and phylogenetic analysis of the acquired sequences of the ICE, SIC, IS elements and transposase genes showed that the corresponding homologues were bacterial and derived from extensive sources. The high prevalences of these MGEs in *V. alginolyticus* implied the extensive and frequent exchange of genes with environmental bacteria and that these elements strongly contribute to the genetic and phenotypic diversity of the bacterium. To our knowledge, this is the first report of *V. alginolyticus* harboring ICE and SIC elements. [**Int Microbiol** 2012; 15(4): 199-208]

Keywords: *Vibrio alginolyticus* · integrating conjugative elements · insertion sequences · superintegrons · transposases · horizontal gene transfer

Introduction

Vibrio spp. are members of the family Vibrionaceae and they are ubiquitous in marine and estuary environments [1,2,17]. *Vibrio alginolyticus* has acquired increasing importance as some strains are pathogenic to aquatic animals, resulting in huge economic losses, as well as to humans [2,8,16,36]. Several studies have sought to identify the virulence genes of *V. alginolyticus* and the molecualr basis of its pathogenic behavior. Others have been aimed at determining the dissemination among environmental *Vibrio* species of the virulence genes found in medically significant *V. cholerae* and *V. parahaemolyticus*. Together, these efforts have revealed that some *V. alginolyticus* strains carry virulence genes derived from pathogenetic *V. cholerae* and *V. parahaemolyticus* strains, such as *ace* [32], *zot* [24,30,32], *tdh* [6], and *trh* [12].

In addition to virulence genes acquired through horizontal gene transfer (HGT), there are putative genes, contained in a reported complete plasmid sequence of *V. alginolyticus*, that are apparently mosaics. These genes, largely of unknown function, appear to be spliced with multiple fragments of genes derived from different vibrios [34] and their presence suggests gene exchange and recombination between *V. alginolyticus* and other *Vibrio* species [34]. However, the vectors

***Corresponding author:** C.Q. Hu
Key Laboratory of Marine Bio-resources Sustainable Utilization, CAS
South China Sea Institute of Oceanology
Guangzhou 510301, China
Tel.+86-2089023216. Fax +86-2089023218
E-mail: hucq@scsio.ac.cn

or mobile elements containing these genes in *V. alginolyticus* are as yet unknown.

In the process of searching for virulence genes of *V. alginolyticus*, we detected several mobile genetic elements (MGEs), including integrating conjugative elements (ICEs), insertion sequences (ISs), superintegron-like cassettes (SICs), and heterogenous transposase genes. As reported herein, further investigation of their distribution in environmental *V. alginolyticus* strains showed that they were highly prevalent in this species.

Materials and methods

***Vibrio alginolyticus* strains and DNA extraction**. In this study of the distribution of ICEs, ISs, SICs, and heterogenous transposase genes, 192 *V. alginolyticus* strains, isolated from seawater and from marine animals (healthy or sick) in the southern coastal region of China in 2006–2009 were investigated. All of the strains were isolated with thiosulfate-citrate-bile salt-sucrose (TCBS) agar, cultured in Broth 2216E (2 % NaCl; Oxoid), and identified by PCR [17] as well as by the standard biochemical tests listed in *Bergey's Manual of Systematic Bacteriology* [5]. Genomic DNA for PCR assays was extracted from the strains using a bacterial DNA extraction kit (Tiangen, China) according to the manufacturer's instructions.

PCR assays of the distribution of ICEs, ISs, SICs, and transposase genes in *Vibrio alginolyticus*. The sequences of ICEs, ISs, and the transposase gene *ValT1* from multiple bacterial species were downloaded from the GenBank database and aligned with Clustal-W in BioEdit software. Repeat sequences in *Vibrio cholerae* (VCRs) strains were also adopted for primer design aimed at SIC amplification in *V. alginolyticus*. A correlation between SIC and integrase genes (*int*) was tested by collecting *int* genes derived from the integrons or superintegrons of *Vibrio* species (Table 1) for use in primer design. The respective consensus sequences were established and used to design primers pairs, which were theoretically tested by BLAST searches against sequences in the GenBank database. All PCRs were performed in a 25-μl reaction containing 1 μl of genomic DNA, 0.4 μM of each primer, 2.5 μl of 10 × PCR buffer , 0.2 mM dNTP, and 1 U of Taq DNA polymerase (Takara, China). The amplification program consisted of an initial denaturation at 94 °C for 4 min, 32 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 8 min.

In addition, a PCR assay for the transposase gene *valT2*, which is highly similar to the gene *vpiT* harbored in the *V. cholerae* pathogenecity island (VPI), was carried out using a previously reported method [30], in order to test the gene's distribution in *V. alginolyticus*. After amplification, 4 μl of each product was electrophoresed in a 1.0 % agarose gel. The resulting bands were visualized under UV light. The predicted lengths of the amplification products are listed in Table 1, as are the primers used in the PCR detection in *V. alginolyticus* of ICE, IS, and SIC elements, and the two transposase genes.

Sequence determination and phylogenetic analysis. To confirm that the PCR products were indeed derived from the ICE, IS, and SIC

Table 1. Primers used in this study and the PCR results for the different genetic elements

Genetic elements	Primers and their sequences (5′–3′)	Product size (bp)	No. of positive strains§	Reference strains for primer design or primer origin
ICE	Ice-F: TGCGGCTCATTTCGACGATCT Ice-R: ACTCGGCCAATATGTACCTGCT	1285	17	*Vibrio fluvialis* Ind1 (GQ463144) *Vibrio cholerae* MJ-1236 (CP001485) *Providencia alcalifaciens* (GQ463139)
SIC	SIC-F: ACTGTCAACGCGCGGCGTTT SIC-R: CAGTCCCTCTTGAGGCGTTTG	N¶	25	*Vibrio cholerae* LMA3894-4 (CP002556) *Vibrio cholerae* O395 (CP001236) *Vibrio cholerae* MJ-1236(CP001486)
int	int-F1: WRGYGTHMAAGAKCAYATG int-R1: GATGGRAABARAWAGTGCCA	655	25	*Vibrio vulnificus* YJ016 (BA000037.2) *Vibrio natriegens* CIP 103193 (AY181034.1) *Vibrio harveyi* ATCC BAA-1116 (CP000789.1) *Vibrio parahaemolyticus* RIMD 2210633 (BA000031.2
IS	Is-F: TCAACCCGGTACGCACCAGAAA Is-R: AGCGGCCAGCCATCCGTCAT	365	9	*Enterobacter cloacae* (AJ539161) *Escherichia coli* BL21 (CP001509) *Escherichia coli* ED1a (CU928162)
valT1	valT-F1: CTCGGCGCACAGCAGCAAATACAG valT-R1: CGCTGAATCGGCGAGGTCTACCAC	414	18	*Shewanella baltica* OS195 (NC_009997) *Vibrio furnissii* CIP 102972 (NZ_ACZP01000023) *Vibrio cholerae* PL107b (AY961483)
valT2	vpiT-F: GCAATTTAGGGGCGCGACGT vpiT-R: CCGCTCTTTCTTGATCTGGTAG	680	5	Sechi et al. [Ref. 30]

§In total, 192 *Vibrio alginolyticus* strains were tested.

¶The length of the amplicons depends on the number and size of the cassettes.

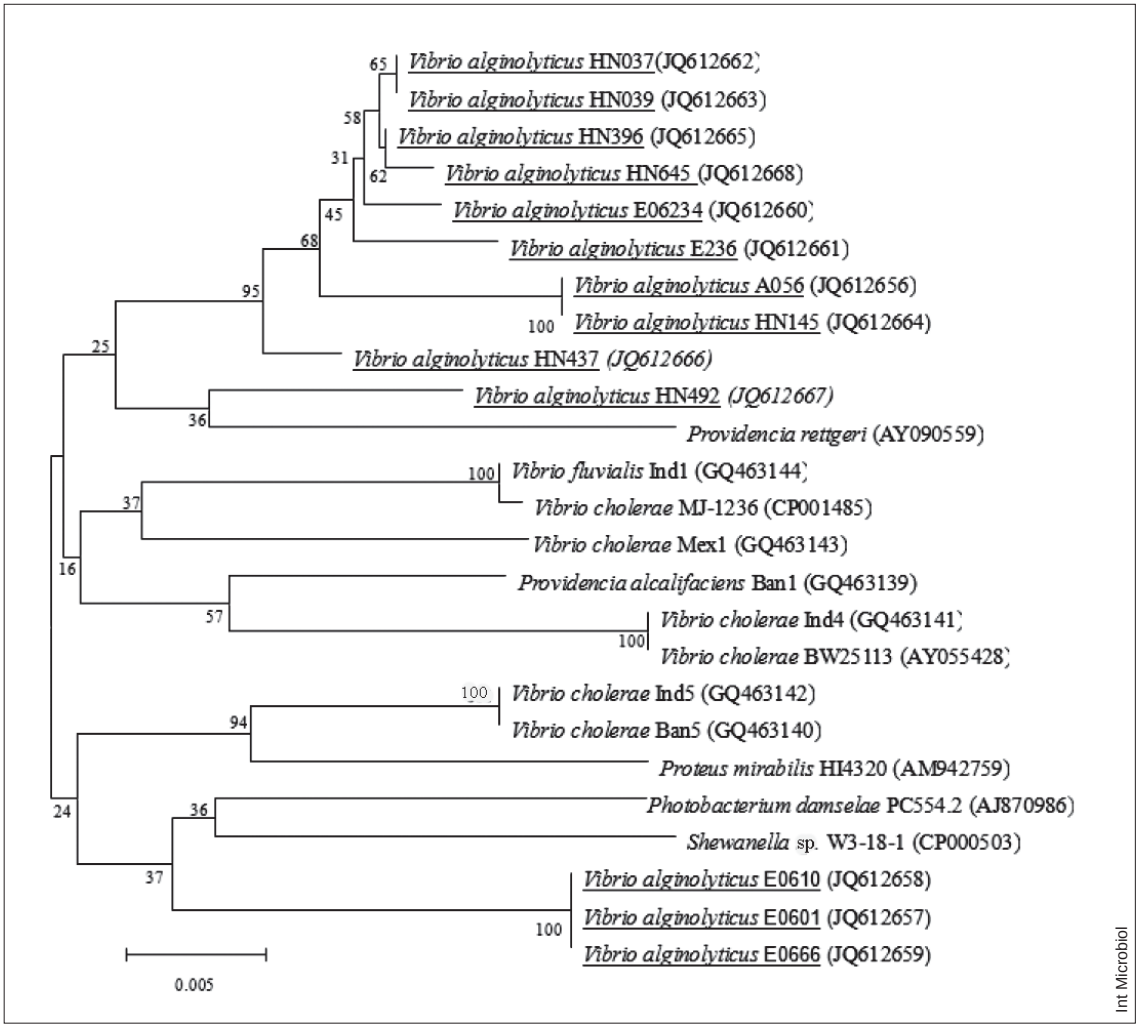

Fig. 1. ICE sequence-based phylogenetic tree constructed using the neighbor-joining method. Bootstrap values were obtained after 1000 repetitions. Scale bar indicates 0.5 % sequence dissimilarity. Underlined strains are those sequenced for this work.

elements, the transposase genes, and the integrase gene and to determine the phylogenetic relationship of these elements with related genetic elements, randomly selected positive PCR products were purified and then directly sequenced using an Applied Biosystems 3730 Automatic Sequencer. The retrieved sequences and related sequences obtained by Blast searches or the IS Finder database [http://www-is.biotoul.fr/is.html] were aligned and then used for similarity comparisons as well as the construction of a phylogenetic tree using Mega 4.0. All of the sequences retrieved were deposited in GenBank (accession numbers: JQ612656–JQ612700, JQ928706–JQ928709, and EU787499).

Results

Distribution and features of ICE elements in *Vibrio alginolyticus*. PCR assays of the ICE elements were positive for 17 of the 192 *V. alginolyticus* strains (8.9 %). Among them, 13 ICE-positive PCR products were randomly selected for direct sequencing and phylogenetic analysis. The results showed that all of the sequences included three genes, *TraC* (encoding a type-IV secretion system protein), *hpoA* (encoding a hypothetical protein), and *pcs* (encoding a plasmid conjugation signal peptidase). The 12 similar sequences acquired by Blast searches, together with our query sequences, were used in the construction of a phylogenetic tree (Fig. 1). The ICE sequences of *V. alginolyticus* did not form a single clade, and closely related homologues were widely attributed, including seven species from five genera, *Vibrio*, *Providencia*, *Proteus*, *Photobacterium*, and *Shewanella* (Fig. 1). Despite the relatively low identity between the ICE of *V. alginolyticus* HN492 (95.6 %) and that of *Proteus mirabilis* HI4320, the percentage was still high enough to suggest the rather high

Table 2. The features of Vibrio alginolyticus repeats (VARs) and predicted genes (ORFs) in cassettes of *Vibrio alginolyticus*

Strains	ORF	Length (bp)	GenBank no.	Closely related encoding gene	Closely related species (accession number)	% Identity	Related to integron or SI	VARs Position	Complementary core sites in VARs
HN045	1	636	JQ612674	Hypothetical protein	*Geobacter metallireducens* GS-15 (NC_007517)	49.5	Y	<1–111 758–787>	GTTAGCC GGCTAAC
HN261[a]	1	201	JQ612676	Hypothetical protein	*Vibrio parahaemolyticus* 10329 (AFBW01000029)	92.5	N	<1–28 515–605>	GTTAGTT AACTAAC
	2	216		Hypothetical protein	*Vibrio sp.* DAT722 (DQ139261)	68.8	Y		
	3	180		Hypothetical protein	*Vibrio vulnificus* CMCP6 (AE016795)	51.8	Y		
HN076	1	174	JQ612675	Hypothetical protein	*Vibrio cholerae* MZO-3 (AAUU01000163)	80.7	Y	430–555	GCATAAC GTTAACT[c]
	2	156		Hypothetical protein	*Vibrio vulnificus* YJ016 (BA000037)	88.4	Y		
HN266[b]	1	252	JQ612677	Ethylenetetrahydrofolate dehydrogenase	*Vibrio cholerae* TMA 21 (NZ_ ACHY01000017)	78.5	N	415–533	TTCTAAC GTTACCA[c]
HN401	1	372	JQ612678	NADPH-P-450 reductase	*Vibrio* sp. Ex25 (NC_013456)	98.4	Y	<1–70 460–486>	GTTATGC GCATAAC
E06333	1	405	JQ612679	Hypothetical protein	*Vibrio alginolyticus* 12G01 (NZ_AAPS01000005)	99.7	N	<1–65 492–519>	GTTAGCT AGCTAAC
E06381	1	255	JQ612680	Hypothetical protein	*Vibrio vulnificus* CMCP6 (NC_004459)	91.8	Y	<1–27 514–594>	GTTAGTT AACTAAC

[a]ORF1, ORF2, and ORF3 in HN261 have overlapping reading frames.
[b]ORF1 in HN266 was not intact.
[c]Core sites are an imperfect repeat.

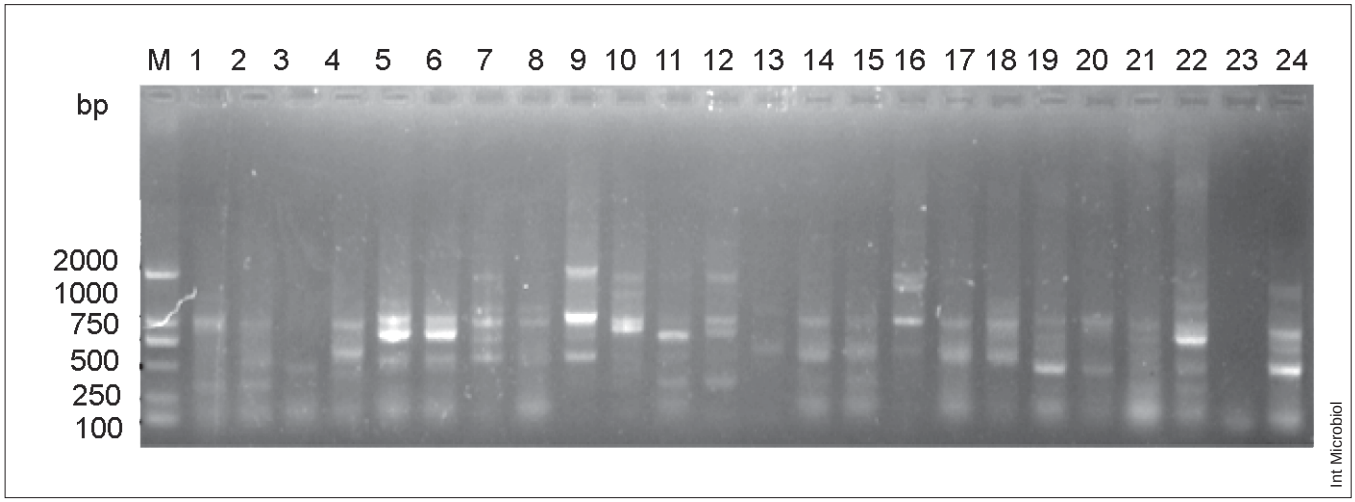

Fig. 2. Typical amplification results of *Vibrio alginolyticus* repeats (VARs) in 25 strains. M: DNA Marker DL2000. 1: A056. 2: HN017. 3: HN029. 4: HN034. 5: HN045. 6: HN063. 7: HN 066. 8: HN072. 9: HN076. 10: HN179. 11: HN261. 12: HN 269.13:HN 271. 14: HN275. 15: HN 283. 16: HN296. 17: HN 318. 18: HN 332. 19: HN401. 20: HN 441. 21: HN 445. 22: E06333. 23: E06346. 24: E06381.

identity between ICEs of *V. alginolyticus* and these elements of other bacterial species. However, Blast searches showed that counterparts to the ICE elements are not contained in any reported *V. alginolyticus* sequences.

Distribution and features of SIC elements in *Vibrio alginolyticus*. Primers used in the amplification of the SIC elements were designed to match the *V. alginolyticus* repeats (VARs) corresponding to the repeated sequences (VCR) in the superintegron of *V. cholerae*. Thus, the amplified region should theoretically contain gene cassettes and partly repeated sequences. PCR assays showed that 25 strains (13.0 %) were clearly positive for VAR and that they gave rise to multiple bands (Fig. 2). Among the bands excised for direct sequencing, each of the seven acquired sequences contained one gene cassette and complete or partial VAR sequence. Genes closely related to those in the cassettes were from a wide range of sources, i.e., four *Vibrio* species (*V. cholerae*, *V. vulnificus*, *V. parahaemolyticus*, and *V. alginolyticus*), two unnamed *Vibrio* species (*Vibrio* sp. DAT722 and *Vibrio* sp. Ex25), and one *Geobacter* species (*G. metallireducens*). Of the ten predicted genes (ORFs), eight encoded hypothetical proteins with unknown function, while the other two genes encoded ethylenetetrahydrofolate dehydrogenase and NADPH-P-450 reductase, respectively.

Further analysis of the flanking regions of these related genes in GenBank showed that seven of the ten genes were derived from superintegrons (4 genes) or integrons (3 genes) (Table 2). Through Blast searches and Clustal alignments, complete or partial VAR sequences of these cassettes were identified that had perfect or imperfect complementary core sequences featuring conservative inverse core sites (RYYTA-AC) and conservative core sites (GTTARRY) (Table 2). The subsequent PCR of the *int* gene indicated that all 25 SIC-positive strains were positively amplified while the SIC-negative strains were not. Four positive PCR products were randomly selected for direct sequencing, and the acquired sequences (JQ928706–JQ928709) confirmed that they derived from *int* genes.

Distribution and features of IS elements in *Vibrio alginolyticus*. The primers used in the IS amplification were designed to match similar transposase genes (*traIS*) in terms of the IS1 elements of *Escherichia coli* and *Shigella sonnei*. Nine of the 192 *V. alginolyticus* strains were positive (4.7 %) for the amplification, and five sequences were acquired by direct sequencing. A comparison and phylogeny determination of those sequences with similar sequences acquired using the IS Finder database revealed the 100 % identity of sequences from *V. alginolyticus* strains E06235, E06236, E06242, and HN381 with the *traIS* sequences of IS elements belonging to the IS1 family in *E. coli* strains ED1a and BL21(DE3), *Salmonella enterica* AKU 12601, and *Enterobacter cloacae* Z-2376 (Fig. 3). IS elements from *V. alginolyticus* strains E0601 had 100 % sequence identity with the IS element belonging to the IS1 family in *E. coli* MS2027. The lowest identity (97.7 %) was between *E. coli* MS2027 and *Klebsiella pneumoniae* NTUH-K2044, but the value was still high enough to show a close phylogenetic relationship. IS sequences of *V. alginolyticus* strains were clustered into two

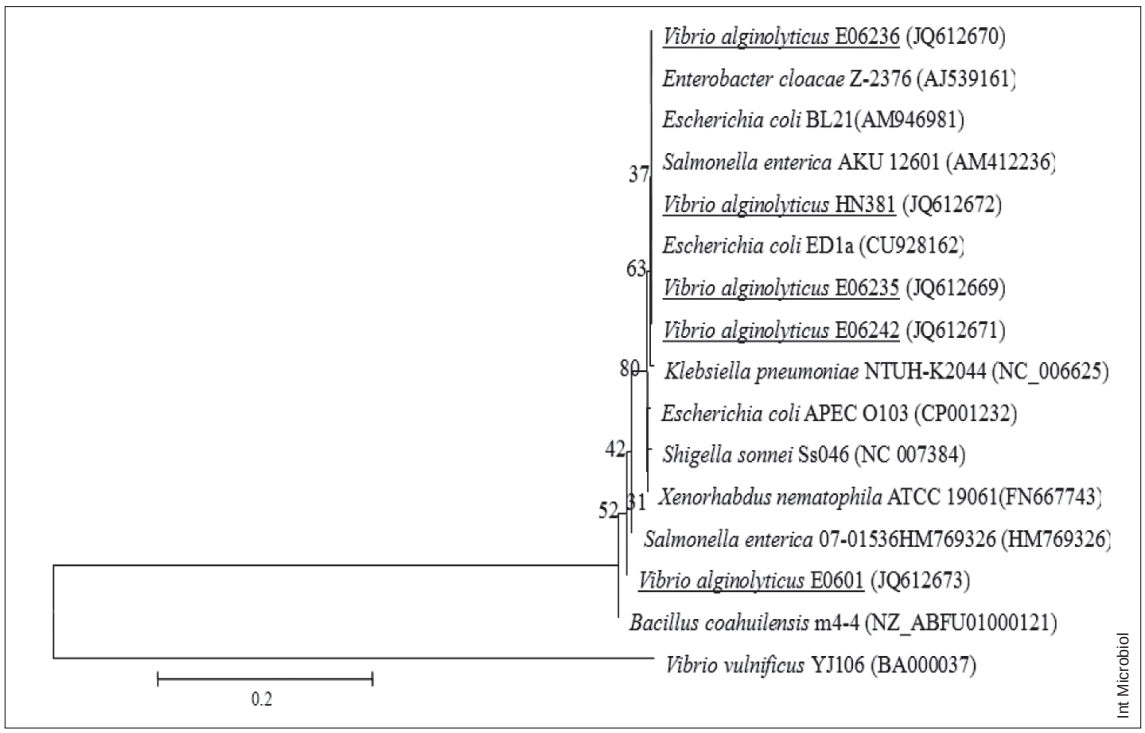

Fig. 3. IS-based phylogenetic tree constructed using the neighbor-joining method. Bootstrap values were obtained after 1000 repetitions. Scale bar indicates 20 % sequence dissimilarity. Underlined strains are those sequenced for this work.

clades. All related bacteria in both were from Enterobacteriaceae and they formed a large branch that was clearly distinct from the stand-alone branch of another IS1 sequence of *V. vulnificus* YJ106, although both species are members of *Vibrio*. Blast searches and IS searches failed to detect highly similar IS sequences in any other Vibrionaceae species.

Distribution and features of transposase genes in *Vibrio alginolyticus*. PCR results indicated that 18 of the 192 *V. alginolyticus* strains (9.4 %) were positive for the transposase gene *valT1*. Sixteen sequences were retrieved by direct sequencing. The acquired and the related sequences were used in a phylogenetic analysis and to construct a phylogenetic tree (Fig. 4). The results showed that *valT1* from *V. alginolyticus* strains A056, HN318, and HN303 had 100 % sequence identity with the transposase gene from *V. parahaemolyticus* K5030, and the *valT1* sequence from *V. alginolyticus* HN145 had 100 % identity with that from *V. furnissii* CIP 102972. The *valT1* sequences from *V. alginolyticus* clustered in different clades with bacteria belonging to distinct genera. Blast searches (Blastn and Blastx) did not identify any similar sequences from *V. alginolyticus* that had been deposited in GenBank.

A PCR assay for *valT2* was also carried out, with five of the 192 *V. alginolyticus* strains found to be positive (2.6 %). The PCR products were subsequently purified for direct sequencing. Blast searches and a phylogenetic analysis (Fig. 5) showed that all *valT2* genes had ≥ 92 % sequence identity with transposase genes from multiple *Vibrio* species (*V. cholerae*, *V. vulnificus*, *V. alginolyticus*, and *V. parahaemolyticus*). The most similar sequences, obtained from five *valT2* genes, were all from *V. vulnificus* or *V. cholerae* strains, including the transposase gene (*vpiT*) of pathogenecity island (VPI) of *V. cholerae* N16961. Except for the *vpiT*-like transposase gene sequence (AY825359) of *V. alginolyticus* (highly similar to the above-mentioned *vpiT* of *V. cholerae*), previously submitted by our laboratory, none of the other similar sequences in *V. alginolyticus* have been reported.

Discussion

Previous work showed that *V. alginolyticus* is ubiquitous in marine and estuary environments [2,17] and that it exhibits high genetic and phenotypic diversity [23,25,32]. To our knowledge, for *V. alginolyticus* neither the distribution of mo-

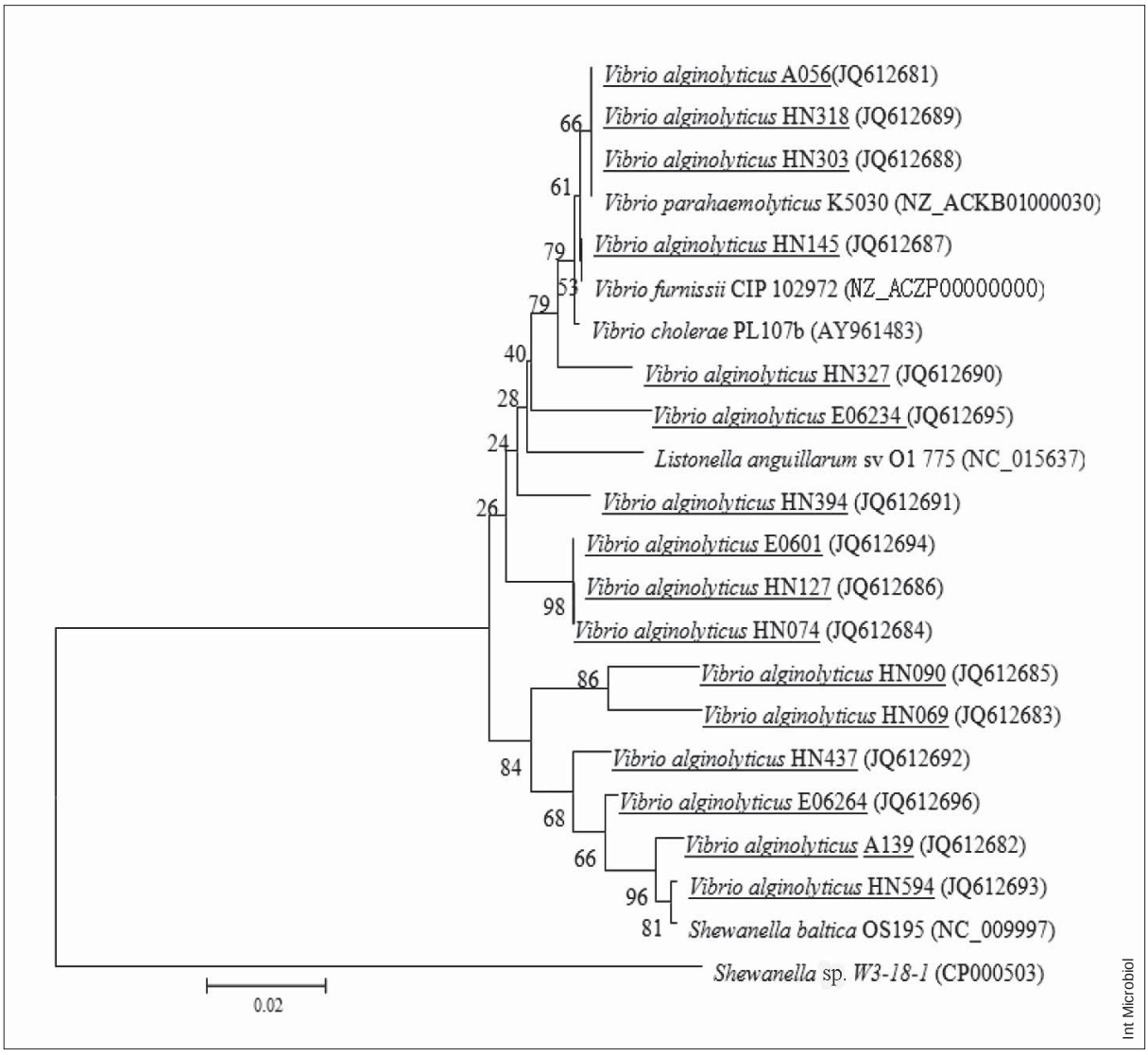

Fig. 4. Phylogenetic tree constructed from the *valT1* sequences of *Vibrio alginolyticus* and from closely related sequences using the neighbor-joining method. Bootstrap values were obtained after 1000 repetitions. Scale bar indicates 2 % sequence dissimilarity. Underlined strains are those sequenced for this work.

bile genetic elements (especially those mainly found in other bacteria) nor the relationship between the genetic diversity of this species and the various MGEs has been studied. Furthermore, few articles have focused on the contribution of MGEs from *V. alginolyticus* to the transmission of genes involved in virulence, antibiotic resistance, or host adaptation in marine environments. Our results confirmed the wide distribution of ICEs, ISs, SICs, and transposase genes in the environmental *V. alginolyticus* isolates analyzed herein.

ICEs can be transferred from a donor to a recipient cell, integrating into the host's chromosome [15]. These elements contain conserved as well as variable regions, with the latter allowing the capture of foreign genes, such as those encoding antibiotic or heavy metal resistance [18,38]. Since the ICEs SXT and R391 were first reported, in isolates of *V. cholerae* and *Providencia rettgeri*, more than 30 elements belonging to the SXT/R391-like family have been described [18]. In the *V. alginolyticus* strains analyzed in this study, ICEs were determined with 8.9 % of the occurence rate, indicating their wide distribution in this bacterium. The fact that the ICEs in *V. alginolyticus* did not not form a single clade in the phylogenetic tree and their homologues had distinct sources, including seven species from five genera, strongly suggests that these elements do not derive from a single lineage and that their acquisition by *V. alginolyticus* strains was from different sources. Moreover, these strains may further act as ICE do-

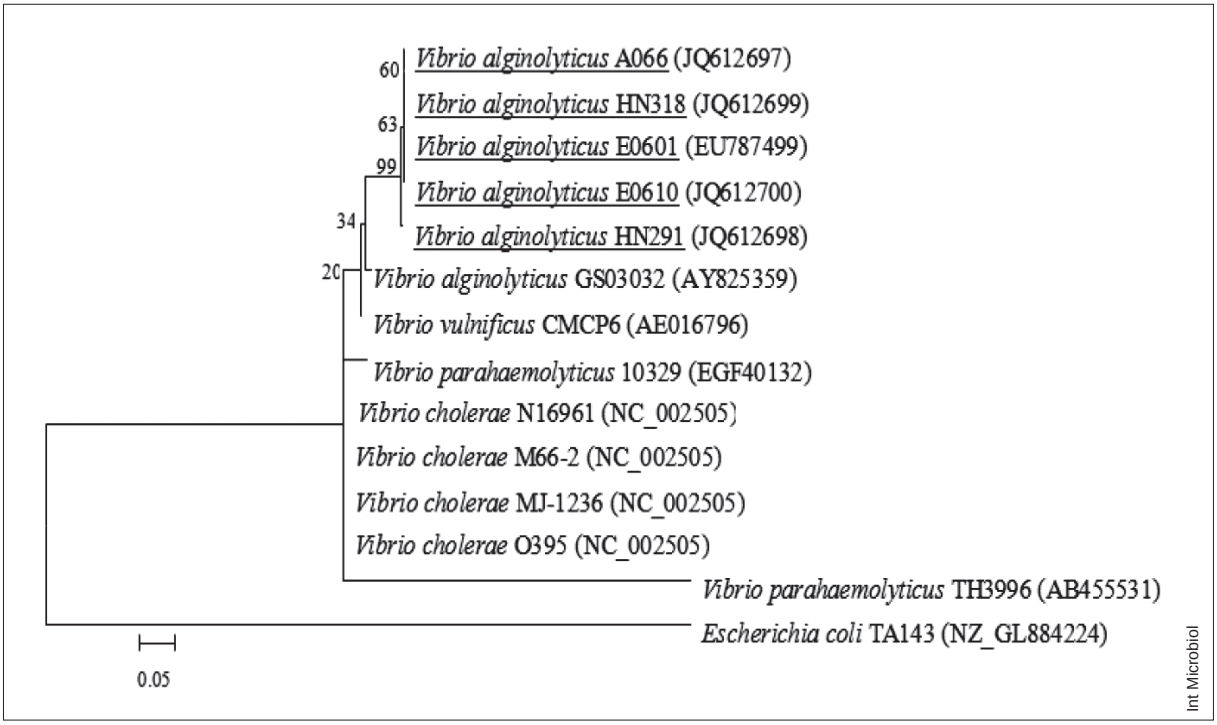

Fig. 5. Phylogenetic tree constructed from the *valT2* sequences of *Vibrio alginolyticus* and closely related sequences using the neighbor-joining method. Bootstrap values were obtained after 1000 repetitions. Scale bar indicates 5 % sequence dissimilarity. Underlined strains are those sequenced for this work.

nors, since transmission of these elements is not solely unidirectional. To our knowledge, this is the first report of ICE elements in *V. alginolyticus*. Previously they have been described only in *V. cholerae* [18] and *V. fluvialis* [38] but not in other *Vibrio* species.

The simplest forms of transposable elements in bacteria are ISs [11]. In fact, most of them encode only a single gene, for transposase (Tnp), bordered by inverted repeats (IRs), the sites for Tnp binding and action [7]. While ISs are known to alter the expression of adjacent genes, through insertion or deletion, there is also evidence that they can efficiently enrich the pool of mobile DNA, which could strongly impact lateral gene transfer and the evolution of bacterial genomes [3,21]. Thus, ISs may well have importantly contributed to genetic diversity within a single species. Although we could not obtain more recent data on the number of discovered ISs, by 2006 over 1500 IS sequences had been identified [31]. IS searches using the IS Finder database showed that no more than 60 ISs have been reported from *Vibrio* species. All of the IS highly similar to those of *V. alginolyticus* were from the IS1 family. Likewise, we inferred that the ISs detected in the *V. alginolyticus* strains analyzed in this study belonged to the IS1 family. The IS1 of *V. vulnificus* YJ106 formed a stand-alone clade, distinct from clades containing all *V. alginolyticus* strains and Enterobacteriaceae strains. No highly similar ISs were found in any Vibrionaceae species by either Blast or IS Finder searches when using the above-mentioned *Vibrio* ISs as queries, consistent with the infrequency of this type of IS1 element in *V. alginolyticus*. By contrast, all 100 % identical IS elements were from Enterobacteriaceae strains, which strongly supported the hypothesis that they were obtained through HGT from distantly related sources.

We recently reported the detection of ISs, belonging to the IS5 family, which were highly similar to those from *V. parahaemolyticus* and detected in *V. alginolyticus* strains [26]. In this study, ISs in several *V. alginolyticus* strains were determined to be highly similar to those from Enterobacteriaceae. Previous reports have shown the IS-mediated spread of the thermostable direct hemolysin gene among *Vibrio* species, including *V. alginolyticus* [12,35]. This finding supports the idea that *V. alginolyticus* extensively exchanges genes with other bacteria in the environment. To our knowledge, ours is the first report showing that *Vibrio* species have IS1 sequences sharing high identity with those of Enterobacteriaceae.

Our attempts to amplify the regions between VARs yielded sequences showing that these regions include the gene cas-

settes and complete or partial VARs. Most of the acquired genes in the cassettes were superintegron- or integron-related and the VARs contained perfect or imperfect inverted core sites and core sites identical to those in the VXRs of the *Vibrio* superintegron [27]. Electrophoretic analysis of the PCR products revealed multiple bands with different lengths in these VAR-positive strains, which could be explained by the fact that VAR primers can, at least theoretically, anchor repeat regions located at both sides of every cassette. Similar PCR profiles evidencing superintegron detection were reported in other *Vibrio* species [20], providing indirect support for the presence of a superintegron in the *V. alginolyticus* strains analyzed. In order to obtain additional evidence for the presence of a superintegron in *V. alginolyticus*, in addition to multiple gene cassettes, we specifically amplified and then sequenced the integrase gene (*int*) of this superintegron. The results showed that all VAR-positive strains simultaneously had an *int* gene highly similar to the integrase gene from the superintegron of *V. cholerae* or other *Vibrio* species.

Further sequence analysis was performed through PCR walking and other methods using the VAR- and *int*-positive strain E06333. The acquired sequence contained more than 18 cassettes (data not shown). Moreover, the results strongly suggested that *V. alginolyticus* had a complete superintegron. Since the initial discovery of a superintegron in *V. cholerae* [20], these elements have been found in the genomes of at least 45 bacteria, including *V. parahaemolyticus*, *V. metschnikovii*, *V. mimicus*, and *V. vulnificus* [19]. Among them, the superintegron of *V. cholerae* has been explored in the greatest detail; however, the potential functions of its gene cassettes are not yet known. There is much speculation about superintegrons as ancestors or reservoirs of various integrons, based on the fact that, in some bacteria, gene cassettes recruited from superintegrons form multiple resistance integrons [26]. *V. alginolyticus* is more common than *V. cholerae* and other *Vibrio* species, and it is more widely distributed. The genes in the cassettes are closely related to those found in other bacteria from extensive sources. Therefore, potential superintegrons in *V. alginolyticus* might carry out extensive gene exchange with environmental bacteria and serve as the reservoirs of gene cassettes. To our knowledge, this is the first report of SICs in *V. alginolyticus*.

The two *V. alginolyticus* transposase genes investigated in this study occurred with a frequency of <10 %. They were highly similar to those found in other *Vibrio* species but not in any reported sequences from *V. alginolyticus* (except one previously reported by our laboratory). The fact that *valT1* sequences from *V. alginolyticus* did not form a clade suggests their different origins. Further sequence analysis showed that some transposase genes were parts of a transposon. Thus, either transposase genes carry other transferable genes for transfer or the latter were acquired from other bacteria through HGT. A transposon is one type of bacterial MGE [13] and it plays a major role in bacterial adaptation and genomic evolution, together with other MGTs, through HGT [4,20,33]. Further work is needed to verify the presence of complete transposons in *V. alginolyticus* and to analyze their structure and function.

Nowadays, it is well recognized that MGEs are of great importance in the evolution of bacterial pathogenesis, antibiotic resistance, and host adaptation [9,10,29,33]. The prevalence of MGEs and the wide distribution of *V. alginolyticus* not only suggest that these elements account for the high genetic diversity and phenotypic differences of this bacterium (including pathogenic and nonpathogenic strains) but also that the bacterium is an important donor of MGEs to other environmental bacteria. The abundance of *V. alginolyticus* MGEs provides a precondition for the HGT of virulence genes and the development of new pathogenetic strains. Other authors have already pointed out that *V. alginolyticus* is a major reservoir for virulence factors in marine environments [14,40]. Our report of the strong prevalence of MGEs in *V. alginolyticus* provides a mechanism explaining this observation.

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Competing interests. None declared.

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