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Novel β-Lactamase Genes from Two Environmental Isolates of *Vibrio harveyi*

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Two ampicillin-resistant (Amp^r) isolates of *Vibrio harveyi*, W3B and HB3, were obtained from the coastal waters of the Indonesian island of Java. Strain W3B was isolated from marine water near a shrimp farm in North Java while HB3 was from pristine seawater in South Java. In this study, novel β-lactamase genes from W3B (bla_{VHW-1}) and HB3 (bla_{VHH-1}) were cloned and their nucleotide sequences were determined. An open reading frame (ORF) of 870 bp encoding a deduced protein of 290 amino acids (VHW-1) was revealed for the *bla* gene of strain W3B while an ORF of 849 bp encoding a 283-amino-acid protein (VHH-1) was deduced for bla_{VHH-1}. At the DNA level, genes for VHW-1 and VHH-1 have a 97% homology, while at the protein level they have a 91% homology of amino acid sequences. Neither gene sequence showed homology to any other β-lactamases in the databases. The deduced proteins were found to be class A β-lactamases bearing low levels of homology (<50%) to other β-lactamases of the same class. The highest level of identity was obtained with β-lactamases from *Pseudomonas aeruginosa*, i.e., PSE-1, PSE-4, and CARB-3, and *Vibrio cholerae* CARB-6. Our study showed that both strains W3B and HB3 possess an endogenous plasmid of approximately 60 kb in size. However, Southern hybridization analysis employing bla_{VHW-1} as a gene probe demonstrated that the *bla* gene was not located in the plasmid. A total of nine ampicillin-resistant *V. harveyi* strains, including W3B and HB3, were examined by pulsed-field gel electrophoresis of *Not*I-digested genomic DNA. Despite a high level of intrastrain genetic diversity, the bla_{VHW-1} probe hybridized only to an 80- or 160-kb *Not*I genomic fragment in different isolates.

The farming of panaeid shrimp is a significant aquaculture activity in many Asian countries, like Thailand, Indonesia, and India (23). The industry is frequently plagued by bacterial infections, particularly vibriosis caused by luminous vibrios, such as *Vibrio harveyi* and *Vibrio splendidus*. *V. harveyi* is recognized as the main causative agent of luminous vibriosis (15, 16), which often results in mass mortality of the affected shrimp, hence leading to extensive farm losses (11). Consequently, antibiotics like streptomycin, erythromycin, and chloramphenicol are used to treat infections while oxytetracycline and penicillin are commonly used as prophylactic agents (32).

Luminous vibrios isolated from shrimp hatcheries on Java island, Indonesia, have demonstrated multiantibiotic resistance to antimicrobials like ampicillin, tetracycline, amoxicillin, and streptomycin (32). Therefore, it is likely that the excessive use of antibiotics has also contributed to increasing numbers of drug-resistant *V. harveyi* strains (1). On the other hand, antibiotic-resistant isolates of *V. harveyi* could also be isolated from pristine marine habitats, which might be an indication that the antibiotic-resistant determinants are already widely disseminated in nature. If this is the case, the use of antimicrobials in farming systems may not be responsible for the spread of bacterial resistance (35).

A number of mechanisms are known to operate in mediating bacterial resistance to β-lactam antibiotics (e.g., ampicillins and cephalosporins), but resistance predominantly results from the hydrolyzing activity of β-lactamases. Four molecular classes (classes A, B, C, and D) of β-lactamases are recognized, with classes A, C, and D having a serine residue at the active site of the enzyme (17). In many gram-negative bacteria, the structural gene for class A β-lactamases is frequently plasmid contained. However, chromosomal genes encoding class A β-lactamases have been described for *Yersinia* (26), *Klebsiella* (9), and *Serratia* (18) spp.

The genetic basis for β-lactam antibiotic resistance in *V. harveyi* has not been studied. This paper describes the cloning and sequence analysis of two novel chromosomally borne β-lactamase structural genes from two different environmental isolates of ampicillin-resistant *V. harveyi* cells. The deduced amino acid sequences of these β-lactamases were compared to other class A β-lactamases. The genomic locations and distribution of the β-lactamase genes in other *V. harveyi* isolates were also investigated.

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MATERIALS AND METHODS

Bacterial strains. Bacterial strains and plasmids used in this study are listed in Table 1. *V. harveyi* strains were isolated from shrimp farms and coastal seawaters of Java island, Indonesia. *Escherichia coli* strains were grown in Luria-Bertani (LB) media. Ampicillin-resistant (Amp^r) *V. harveyi* strains were grown routinely in LB media containing 100 μg of ampicillin/ml.

Antimicrobial agents and MIC determinations. Susceptibility to antimicrobial agents was determined by MICs. The antibiotics used were ampicillin, penicillin, carbenicillin, amoxicillin, cephalothin, cefotaxime, chloramphenicol, oxytetracycline, erythromycin, and streptomycin (Sigma Chemical Co., St. Louis, Mo.). MICs were determined by an agar dilution technique on Mueller-Hinton agar plates (Oxoid Ltd., Basingstoke, England) with an inoculum of 10^4 CFU/spot. All plates were read after an 18-h incubation at 37°C. The MIC for imipenem was determined by the E-test method (AB Biodisk, Solna, Sweden) following manufacturer's instructions.

Enzymes and chemicals. All chemicals used were of the highest grade commercially available. All restriction enzymes used were purchased from New England Biolabs, Inc. (Beverly, Mass.) and used according to manufacturer's recommendations.

DNA cloning and analysis of recombinant plasmids. Genomic DNA from *V. harveyi* HB3 and W3B was extracted by using phenol-chloroform (24). The DNA

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristic(s)	Source	Geographic location	Reference or source
Strains				
E. coli TOP10	F$^-$ *mcrA*(*mrr-hsdRMS-mcrBC*) ϕ80 *lac2* ΔM15 Δ*lac74 recA1 deoR araD139*Δ(*ara-leu*)*7697 galU galK rpsL endA1 nupG*)			Invitrogen
V. harveyi isolates				
W3B	Amp^r	Seawater near shrimp hatchery	Besuki, northern coast of East Java	
E_2	Amp^r	Shrimp egg	Besuki, East Java	
GCB	Amp^r	Shrimp gut	Besuki, East Java	
P1B	Amp^r	Shrimp larvae	Besuki, East Java	
M_1	Amp^r	Mysis (prawn larval stage)	Besuki, East Java	30
M3.4L	Amp^r	Mysis (prawn larval stage)	Labuhan, northern coast of West Java	
AP5	Amp^r	Seawater	Pacitan, southern coast of East Java	
AP6	Amp^r	Seawater	Pacitan, East Java	
HB3	Amp^r	Seawater	Pacitan, East Java	
Plasmids				
pCR 2.1-TOPO	PCR TOPO vector			Invitrogen
pAS900	Km^r Amp^s; derivative of pCR 2.1-TOPO (Invitrogen) cloning vector carrying an *Xmn*I-*Sca*I deletion in ampicillin resistance gene			A. Suwanto (unpublished)
pVHA1	pAS900 recombinant plasmid containing a 1.1-kb *Hin*dIII chromosomal fragment from W3B			This study
pVHA4	pAS900 recombinant plasmid containing a 1.1-kb *Hin*dIII chromosomal fragment from HB3			This study

was digested with *Hin*dIII, and the resulting fragments were ligated into the *Hin*dIII site of the pAS900 vector. The ligation mixture was transformed into *E. coli* TOP10 cells (Invitrogen Corp., Carlsbad, Calif.), and transformants were selected for ampicillin resistance. Recombinant plasmid DNA was prepared by alkaline lysis (24). T4 DNA ligase was purchased from New England Biolabs. Fragment sizes were estimated by comparison to the 1-kb DNA ladder (New England Biolabs) as the molecular size standard.

DNA sequencing. The 1.1-kb *Hin*dIII fragment from pVHA1 and pVHA4 was sequenced on both strands by using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit and ABI cycle sequencer A373 (Applied Biosystems/Perkin-Elmer, Foster City, Calif.). DNA sequencing was performed by using M13 forward and reverse primers. Later, internal sequencing primers were constructed from the available DNA sequences to complete the sequence walk. Sequencing primers were 18-mers chosen from the last 100 nucleotides read on the chromatograms. The oligonucleotides were synthesized by GENSET (Singapore Biotech. Pty., Ltd., Singapore).

DNA sequencing and protein analysis. DNA sequence analysis was performed with DNASIS (Hitachi Software Engineering Co. Ltd., San Bruno, Calif.). Database similarity searches for both the nucleotide sequences and deduced protein sequences were carried out at the National Center of Biotechnology Information website. Multiple sequence alignment of the deduced peptide sequence was carried out by using CLUSTALW over the Internet. A phylogenetic tree was also constructed by using the Treecon for Windows version 1.3b software package (33). Deduced amino acid sequences for pVHA1 and pVHA4 were compared to 13 other class A β-lactamases: PSE-1, PSE-4, CARB-3 from *Pseudomonas aeruginosa* (3, 13, 14), CTX-M-5, CTX-M-3 from *Salmonella enterica* serovar Typhimurium (4; M. Gazouli, unpublished data), AER-1 from *Aeromonas hydrophila* (25), CARB-6 from *Vibrio cholerae* (6), ROB-1 from *Actinobacillus pleuropneumoniae* (5), *Bacillus thuringiensis* β-lactamase (36), *Streptomyces fradiae* Y59 β-lactamase (20), OXY-2 from *Klebsiella oxytoca* (8), *Serratia marcescens* S5 β-lactamase (20), and β-lactamase from *Proteus mirabilis* N-29 (31). The identification of signal peptides was carried out with the program SignalP V1.1 at the Center for Biological Sequence Analysis over the Internet (http://www.cbs.dtu.dk/services/SignalP/) (19).

Preparation of genomic DNA gel inserts for PFGE. *V. harveyi* strains W3B and HB3 were grown overnight at 30°C in 10 ml of Luria-Bertani broth. Preparation of genomic DNA inserts in low-melting-point agarose Seaplaque (FMC Bioproducts) and restriction digestion of the inserts were performed as previously described (27). Restriction digestion of the inserts is briefly described as follows. Each gel slice was incubated with 200 μl of the appropriate 1× restriction enzyme buffer supplemented with 100 μg of bovine serum albumin per ml for at least 15 min on ice. The buffer was then removed, and fresh buffer was added together with 20 U of restriction enzyme. This was placed for another 15 min on ice before being left to incubate at 37°C for 4 h. The restriction enzyme *Not*I was used for digestion of inserts. The DNA fragments were electrophoresed on a 1% Seakem GTG (FMC Bioproducts) gel in a 0.5× Tris-borate-EDTA (TBE) buffer using a contour-clamped homogeneous electric field device (CHEF-DR III; Bio-Rad, Richmond, Calif.). Running conditions were 6 V cm^{-1} for 20 h with a ramping time of 20 to 60 s. *Ase*I-digested genomic DNA from *Rhodobacter sphaeroides* 2.4.1 was used as the pulsed-field gel electrophoresis (PFGE) molecular size marker (27).

Preparation of large endogenous plasmid for PFGE. Plasmids were extracted from two isolates, HB3 and W3B, by using a modification of the alkaline lysis method in which phenol extraction was performed with neutralized phenol equilibrated in 3% sodium chloride without chloroform and isoamyl alcohol (28). The dried plasmid pellet was resuspended in an appropriate volume of sterile water for restriction digestion. An equal volume of 1% low-melting-point agarose was added to the digested samples before loading. DNA fragments were electrophoresed in a 1.2% Seakem GTG agarose gel. The PFGE running conditions were 6 V cm^{-1} for 12 h with a ramping time of 1 to 8 s.

Southern blotting and hybridization for PFGE gels. The procedure for Southern blotting was according to the instructions given in the ECL Nonradioactive Detection Kit (Amersham Life Science, Little Chalfont, Buckinghamshire, England) except that the 15 min depurination was carried out twice. DNA fragments of PFGE gels were capillary blotted onto nylon hybridization membranes (Hybond-N$^+$; Amersham) and fixed by baking at 80°C for 2 h. The hybridization probe was the 1.1-kb *Hin*dIII-*Hin*dIII fragment from pVHA1. The fragment was excised from Seaplaque (FMC Bioproducts) low-melting-point gel and purified from the gel by using GFX PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech Inc., Uppsala, Sweden). Hybridization was performed overnight with high-stringency conditions as described by the manufacturer.

Nucleotide sequence accession numbers. The sequences for bla_{VHW-1} and bla_{VHH-1} have been deposited into the GenBank database under the accession numbers AF 217648 and AF 217649, respectively.

RESULTS

Sequence analyses of bla_{VHW-1}, bla_{VHH-1}, and their deduced amino acid sequences. The 1.1-kb DNA inserts present on pVHA1 and pVHA4 were sequenced on both strands. Analysis of the pVHA1 insert revealed the presence of an open reading frame (ORF) of 870 bp which encoded a putative 290-amino-acid (290-aa) preprotein (designated VHW-1). Similarly, the cloned insert in pVH4 was shown to have an ORF of 849 bp with a predicted 283-aa-long preprotein (designated VHH-1). A 19-aa signal peptide was deduced for both VHW-1 and VHH-1 (Fig. 1). Four important structural features found con-

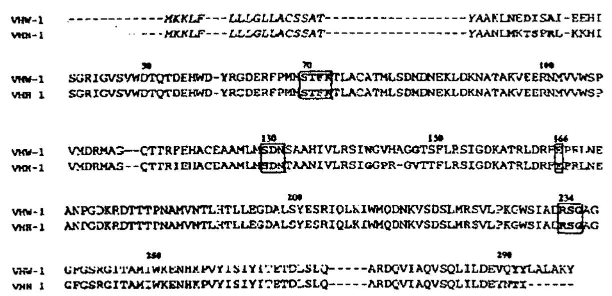

FIG. 1. Amino acid sequence alignment of VHH-1 and VHW-1. Ambler's standard numbering of β-lactamases was used (2). Dashes indicate gaps that were used to maximize the alignment. Unconserved residues between the two sequences are indicated in bold. Conserved amino acid regions important for catalytic function of β-lactamases are boxed. A 19-aa signal peptide is indicated in italics.

served in class A β-lactamases were present in the deduced amino acid sequences of VHW-1 and VHH-1, which included an STFK active site tetrad at position 70 to 73 according to Ambler's standard numbering of class A β-lactamases (2). This Ser-X-X-Lys tetrad is characteristic of penicillin binding proteins (PBPs) and serine β-lactamases (10). An SDN loop characteristic of class A β-lactamases (10) was located at position 130 to 132 on VHW-1 and VHH-1 as well as the unique Glu residue at position 166. Lastly, an RSG triad was established at position 234 to 236 on both β-lactamases (Fig. 1).

Sequence homology with other β-lactamases. Database searches of VHW-1 and VHH-1 β-lactamase genes generated no homology with any other class A β-lactamases in the databases, but they have a 97% homology between themselves. The deduced amino acid sequence of both VHW-1 and VHH-1 had less than 50% identity with other class A β-lactamases. VHW-1 had the highest level of identity (45%) with *P. aeruginosa* β-lactamases PSE-4 and CARB-3 and *V. cholerae* CARB-6. VHH-1 had the highest percentage identity with PSE-1, PSE-4, CARB-3, and CARB-6, at 46% (Table 2). The phylogenetic tree constructed for VHW-1 and VHH-1 shows that only these two enzymes clustered together and had a 91% amino acid sequence identity. Therefore, VHW-1 and VHH-1 β-lactamases are novel and distinctly different from the other known β-lactamases (Fig. 2).

TABLE 2. Amino acid identity of VHW-1 and VHH-1 with several related class A β-lactamases

β-Lactamase	% Identity					
	PSE-1	PSE-4	CARB-3	CARB-6	AER-1	VHW-1
PSE-4	99					
CARB-3	99	99				
CARB-6	94	94	43			
AER-1	41	43	43	42		
VHW-1	44	45	45	45	40	
VHH-1	46	46	46	46	41	91

Antibiotic susceptibility. *E. coli* TOP10 cells harboring recombinant plasmids pVHA1 and pVHA4 had elevated levels of resistance to penicillins compared to those of the host strain alone, indicating that the cloned insert did contain the *bla* gene which conferred β-lactam resistance (Table 3). MICs for imipenem, cephalothin, and cefotaxime were similar to those of TOP10 cells alone, thus revealing there was no resistance to these antibiotics. All the environmental isolates of *V. harveyi* showed a fairly high level of resistance to penicillins (Table 3). All the strains were, however, susceptible to streptomycin, erythromycin, oxytetracycline, and chloramphenicol with the exception of strain M3.4L, which had a significant level of resistance to oxytetracycline (Table 4).

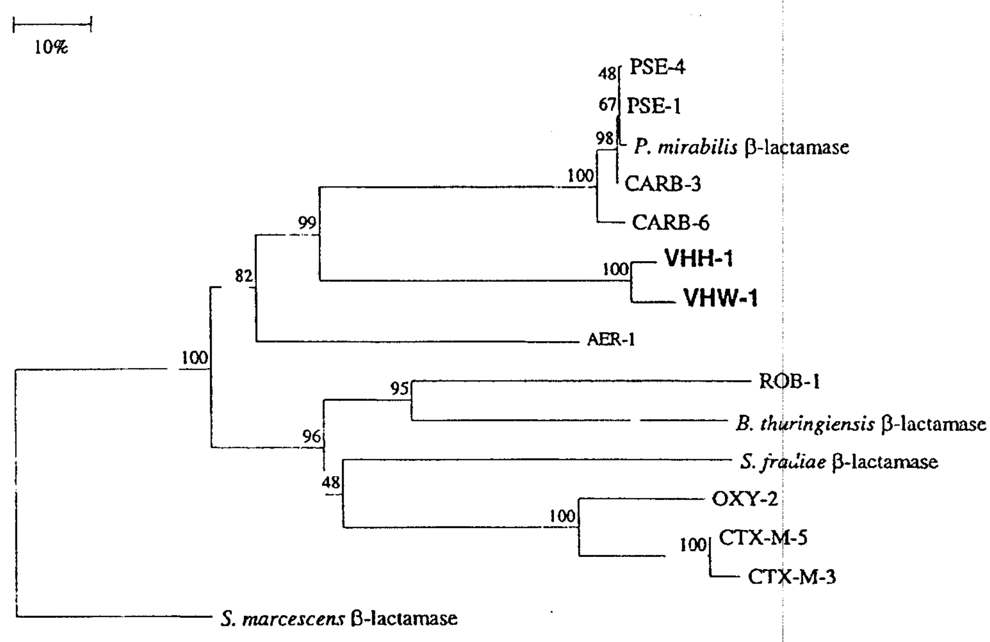

FIG. 2. Dendrogram of 15 class A β-lactamases. Branch lengths are drawn to scale and proportional to the number of amino acid changes. The bootstrap values are indicated at each node. The β-lactamases used for comparison were PSE-1, PSE-4, and CARB-3 from *P. aeruginosa* (3, 13, 14), CTX-M-5, CTX-M-3 from *S. enterica* serovar Typhimurium (4; Gazouli, unpublished), AER-1 from *A. hydrophila* (25), CARB-6 from *V. cholerae* (6), ROB-1 from *A. pleuropneumoniae* (5), *B. thuringiensis* β-lactamase (36), *S. fradiae* Y59 β-lactamase (20), OXY-2 from *K. oxytoca* (8), *S. marcescens* S5 β-lactamase (20), and β-lactamase from *P. mirabilis* N-29 (31).

TABLE 3. MICs of β-lactams for *E. coli* TOP10 cells harboring recombinant plasmids pVHA-1 and pVHA-4 and environmental isolates of *V. harveyi*

β-Lactam	MIC (μg/ml) of drug:												
	E. coli			*V. harveyi*									
	TOP10	TOP10(pVHA1)	TOP10(pVHA4)	W3B	HB3	M_1	E_2	GCB	P1B	AP5	AP6	M3.4L
Ampicillin	1	256	256	128	256	64	32	>512	256	>512	256	256
Penicillin	0.12	>512	256	128	128	128	64	>512	256	256	256	256
Carbenicillin	4	>512	>512	>512	>512	256	128	>512	>512	>512	>512	>512
Amoxicillin	8	>512	>512	128	128	128	128	128	128	128	128	128
Oxacillin	128	>512	>512	>512	>512	128	128	>512	>512	>512	>512	>512
Imipenem	0.125	0.125	0.125	0.25	0.125	—[a]	—	—	—	—	—	—
Cephalothin	8	8	8	16	16	—	—	—	—	—	—	—
Cefotaxime	<0.03	<0.03	<0.03	1	1	—	—	—	—	—	—	—

[a] —, not determined.

DNA profiling analysis employing PFGE. PFGE was employed to reveal the genetic diversity of *V. harveyi* isolated from various geographic locations. Six different restriction patterns or schizotypes (29) were obtained when digested with *Not*I (Fig. 3). This result indicated a high level of genetic diversity among the isolates. Strain W3B is genetically different from strain HB3, as shown from the PFGE data (Fig. 3, lanes 1 and 4). Strains AP5 and HB3, both isolated from the southern coast of East Java, demonstrated identical *Not*I schizotypes or distribution of restriction fragments (Fig. 3, lanes 3 and 4). The PFGE profile of strain W3B was found to be identical to that of strain GCB (both were isolated from the northern coast of East Java) (Fig. 3, lanes 1 and 2), and AP6 was identical to P1B (Fig. 3, lanes 6 and 7). The remaining strains M3.4L, E_2, and M_1 had unique *Not*I restriction patterns.

Distribution of bla_{VHW-1} gene in other *V. harveyi* isolates. The 1.1-kb *Hin*dIII fragment from the recombinant plasmid pVHA1 containing the bla_{VHW-1} gene was used to probe against *Not*I-digested DNA from eight other isolates (Fig. 3). Hybridization occurred at the 80-kb *Not*I chromosomal band in strains W3B, AP5, GCB, and HB3 and the 160-kb *Not*I chromosomal band in strains P1B, AP6, and M3.4L. No hybridization was detected for strains M_1 and E_2, indicating the absence of bla_{VHW-1} or a homologue in these strains. Using PFGE, a 60-kb plasmid with an identical plasmid profile was detected for strains HB3 and W3B (data not shown). Southern blot analysis employing bla_{VHW-1} as a probe demonstrated that the *bla* gene was not located in this large endogenous plasmid.

DISCUSSION

In this study, we have cloned novel *bla* genes from two environmental isolates of *V. harveyi*. The bla_{VHW-1} gene was derived from strain W3B isolated from seawater around a shrimp hatchery in the northern coast of East Java while bla_{VHH-1} was isolated from strain HB3, a pristine seawater isolate from the southern coast of East Java. Sequence analysis of the *bla* genes demonstrated that they did not have homology to any other β-lactamase genes. At the amino acid level, VHW-1 and VHH-1 possess low levels of homology to other class A β-lactamases, and regions which are strongly conserved are implicated in enzyme catalysis. These results reflect the extensive diversity of β-lactamase genes.

Southern blot analysis using bla_{VHW-1} as a probe revealed that the β-lactamase was chromosomally encoded and apparently present as a single copy. The gene bla_{VHW-1} and its highly homologous counterpart (bla_{VHH-1}) was also present in other ampicillin-resistant strains of *V. harveyi*, suggesting that this *bla* gene is widely disseminated. Since the gene is also present in isolates obtained from the pristine marine water environment (strains HB3, AP5, and AP6), this *bla* gene is likely not to have been a consequence of antibiotic selection pressure imposed by shrimp farming. In the marine habitat, cyanobacteria are known to naturally excrete antibiotics and possibly β-lactams (22). Hence, β-lactamase production in *V. harveyi* cells might have been maintained in response to natural environmental selection.

Southern hybridization and PFGE analysis of several other ampicillin-resistant *V. harveyi* isolates indicated that bla_{VHW-1}-like genes are located on either 80- or 160-kb *Not*I genomic fragments. These results suggests that the gene is present in a conserved segment surrounded by a variable genetic environment as demonstrated by the high genetic diversity of the isolates.

Resistance to β-lactams is often the result of β-lactamases that inactivate the antibiotics. In addition to class A β-lactamases, three other molecular classes of β-lactamases (B, C, and D) are recognized. The bla_{VHW-1} gene probe failed to hybridize to two ampicillin-resistant *V. harveyi* strains (M_1 and E_2); therefore, the ampicillin-resistant determinants of strains HB3 and W3B might not account entirely for β-lactam resistance in some other strains. It seems likely that β-lactamases belonging to the other molecular classes, such as B, C, or D, are also responsible for ampicillin resistance. Some gram-negative bacteria acquire resistance by changing the permeability of outer membrane porin channels, consequently leading to reduced drug influx into the bacterial cell. In addition, PBP-mediated resistance arises when PBPs failed to bind or exhibited reduced affinity to β-lactams (7). It is plausible that the latter two mechanisms are also contributing to β-lactam resistance in ampicillin-resistant strains.

A single, large 60-kb plasmid could be isolated from strain W3B or HB3. Although no hybridization to the bla_{VHW-1} probe could be detected, the plasmid might encode virulence factors, resistance determinants to other classes of antibiotics or factors required for survival and fitness in their natural

TABLE 4. MICs of antibiotics for environmental strains of *V. harveyi*

Antibiotic	MIC (μg/ml) for *V. harveyi* strains								
	HB3	W3B	M_1	E_2	GCB	P1B	AP5	AP6	M3.4L
Streptomycin	4	32	4	8	8	8	8	2	8
Erythromycin	2	4	4	4	4	4	2	2	2
Oxytetracycline	4	4	4	4	4	4	2	2	128
Chloramphenicol	0.25	0.25	0.5	0.25	0.25	0.25	0.25	0.5	0.5

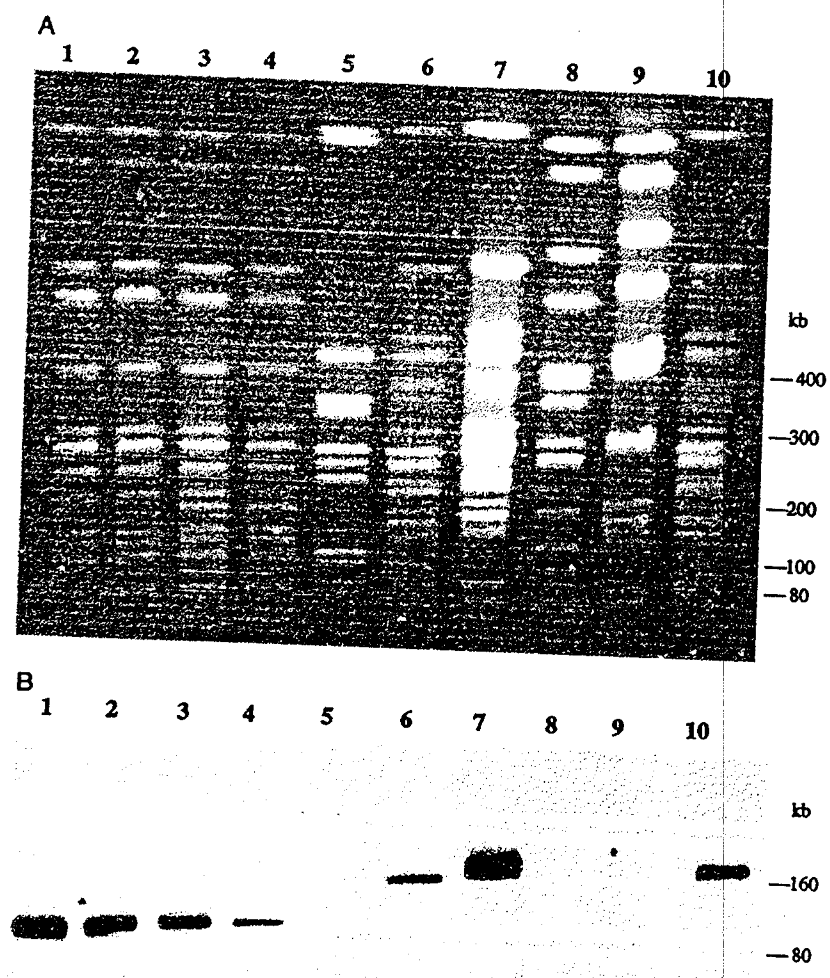

FIG. 3. (A) PFGE of *Not*I-digested DNA from nine environmental isolates of *V. harveyi*. (B) Southern hybridization analysis of DNA. The probe used was the 1.1-kb *Hin*dIII fragment containing bla_{VHW-1}. Lanes: 1, W3B; 2, GCB; 3, AP5; 4, HB3; 5, *R. sphaeroides* 2.4.1 DNA digested with *Ase*I (molecular size standard); 6, AP6; 7, P1B; 8, M_1; 9, E_2; 10, M3.4L.

environment. We are currently characterizing the plasmid and determining its ubiquity in *V. harveyi* isolates.

Class A β-lactamases of gram-negative bacteria can be divided into two subgroups (26). The first subgroup is the chromosomal branch and the second is the transposon branch. Members of each subgroup share distinctive residues. Nine out of 11 bases of the transposon branch are conserved in VHW-1 and VHH-1, suggesting that it is possible that *bla* genes might have been carried in a transposon or integron before being integrated into the chromosome. Often, antibiotic resistance genes encoding resistance to a variety of antibiotics, such as β-lactams, chloramphenicol, and aminoglycosides, are found integrated in a site-specific manner in a mobile gene cassette or integron (21). Both the 5′ and the 3′ ends of integrons are conserved. The 5′ conserved region encodes an integrase while the 3′ segment often carries *qac*EΔ1 and *sul*I genes, which determine resistance to ethidium bromide and quaternary ammonium compounds and to sulfonamides, respectively. Another conserved feature is the presence of an imperfect inverted repeat consensus of 59 bp located downstream of the inserted resistance genes. Integrons are found commonly in gram-negative pathogenic bacteria, especially from the *Enterobacteriaceae* and the pseudomonads. These mobile genetic elements are capable of interspecies transfer. The amino acid sequence homology of VHW-1 and VHH-1 indicates that the highest levels of homology are obtained with β-lactamases from *Pseudomonas*, i.e., PSE-1, PSE-4, and CARB-3, and a β-lactamase from *V. cholerae* CARB-6. These PSE- and CARB-type enzymes have structural genes that are part of a transposon or an integron (3). Currently, the lack of flanking upstream and downstream sequences of the bla_{VHW-1} and bla_{VHH-1} genes makes it difficult to ascertain their genetic context and whether the genes are integron or transposon borne.

A large, conjugative, chromosomally integrating transposon named the SXT element has been discovered in *V. cholerae* O139 (34). This 62-kb element is not only self-transmissible but can also be transferred into *E. coli* strains and its integration into the host genome is site specific. The transposon encodes multiantibiotic resistance against streptomycin, furazolidone, and trimethoprim. It can be envisaged that such conjugative elements might also exist in *V. harveyi* organisms, and the presence of the *bla* gene on either a transposon or an integron might help to explain the wide dissemination of the ampicillin resistance gene as well as the specific localization of the β-lactamase genes on either the 60- or 160-kb *NotI* fragment in various ampicillin-resistant isolates.

Future work will involve determining the flanking sequences of the β-lactamase gene, mechanisms of transfer, and distribution of ampicillin resistance, as well as the biochemical characterization of the protein.

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