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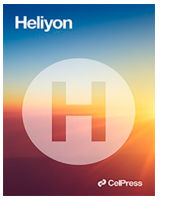

Research article

Genomic analysis of *Vibrio harveyi* strain PH1009, a potential multi-drug resistant pathogen due to acquisition of toxin genes

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ABSTRACT

In has increasingly been observed that viral and bacterial coinfection frequently occurs among cultured shrimp and this coinfection could exacerbate the disease phenotype. Here, we describe a newly discovered bacterial strain, *Vibrio harveyi* PH1009 collected from Masbate Island, Philippines that was found to be co-infecting with the White Spot Syndrome virus in a sample of black tiger prawn, *Penaeus monodon*. The genome of *V. harveyi* PH1009 was sequenced, assembled, and annotated. Average Nucleotide identity calculation with *Vibrio harveyi* strains confirmed its taxonomic identity. It is a potential multi-drug and multi-heavy metal resistant strain based on the multiple antibiotic and heavy metal resistance determinants annotated on its genome. Two prophage regions were identified in its genome. One contained genes for Zona occludens toxin (Zot) and Accessory cholera toxin (Ace), essential toxins of toxigenic *V. cholerae* strains apart from CTX toxins. Pan-genome analysis of *V. harveyi* strains, including PH1009, revealed an "open" pan-genome for *V. harveyi* and a core genome mainly composed of genes necessary for growth and metabolism. Phylogenetic tree based on the core genome alignment revealed that PH1009 was closest to strains QT520, CAIM 1754, and 823tez1. Published virulence factors present on the strain QT520 suggest similar pathogenicity with PH1009. However, PH1009 Zot was not found on related strains but was present in strains HENC-01 and CAIM 148. Most unique genes found in the PH1009 strain were identified as hypothetical proteins. Further annotation showed that several of these hypothetical proteins were phage transposases, integrases, and transcription regulators, implying the role of bacteriophages in the distinct genomic features of the PH1009 genome. The PH1009 genome will serve as a valuable genomic resource for comparative genomic studies and in understanding the disease mechanism of the *Vibrio harveyi* species.

1. Introduction

Vibriosis is one of the most serious diseases in the aquaculture industry [12]. One of the most prominent pathogens that cause

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Vibriosis is *Vibrio harveyi*, a Gram-negative, halophilic bacterium that infects various fish and invertebrates. Disease manifestation of *V. harveyi* varies from host to host. *V. harveyi* infections in fish manifest as eye disease, gastroenteritis, tail-rot disease, Scale drop, and muscle necrosis [20,21,26,63]. In shrimp, *V. harveyi* was identified as the primary causative agent of luminous vibriosis that afflicted shrimp farms in the Philippines during the 1990s [17,36]. It was also known to cause other shrimp diseases such as bacterial white tail disease, black shell disease, and bolitas nigricans [28,52,62], and was reported to cause Acute Hepatopancreatic Necrosis Disease (AHPND) due to acquisition of plasmid-borne pirAB toxin genes [42].

The broad range of *V. harveyi* hosts requires further understanding of its pathogenicity and associated virulence factors. Genomes of *V. harveyi* strains are valuable resources that can advance our knowledge of its disease mechanisms. This paper describes the genome of *V. harveyi* PH1009, an isolate from *Penaeus monodon* from Masbate Island, Philippines.

2. Materials and methods

2.1. Isolation of Vibrio harveyi strains

The bacterial collection from the Philippine Shrimp Pathogenomics Program of the Philippine Genome Center was completed in 2014–2016. The shrimp hepatopancreas was aseptically removed then homogenized in sterile normal saline solution. Serial dilutions from the mixture were spread plated on Nutrient Agar (Pronadisa) with 1.5% NaCl, Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar (Pronadisa), and Vibrio chromogenic agar (Pronadisa) in duplicates. Dominant colonies were purified, prepared as glycerol stock, and stored at −80 C.

2.1.1. DNA isolation, library preparation, and sequencing

The Genomic DNA was isolated from 24hr culture of PH1009 using the KingFisher cell and tissue DNA isolation kit (Thermo Fisher Scientific). A paired-end library from the genomic DNA was prepared using the Nextera XT DNA library prep kit (Illumina). The generated paired-end library was sequenced on the Illumina MiSeq (2 × 300-bp) platform (with the MiSeq reagent kit v. 3) at the DNA Sequencing Core Facility, Philippine Genome Center.

2.1.2. Genome assembly

The quality of raw reads was assessed using FastQC v. 0.11.9 [2] and preprocessed using fastp v. 0.20.1 [14]. Trimmed and filtered paired-end reads were assembled de novo using SPAdes v.3.14 [7] implemented in Shovill v.1.0.4 [54]. QUAST v.5.02 [41] generated the assembly statistics while genome completeness was assessed using CheckM [46].

2.1.3. Taxonomic identification

Tetra correlation search (TCS) in JspeciesWS [51] initially identified the taxonomic classification of PH1009. Further verification by Average Nucleotide Identity using MUMmer [35] (ANIm) and BLAST [1] (ANIb) was implemented in pyani v0.2.9 [50].

2.1.3.1. Genome annotation and identification of virulence and antimicrobial resistance determinants. Prokka [53] and the RAST web-server [6] were used to annotate the assembled genome, and ARIBA v.2.14.6 [25] employing the VFDB [13] and CARD [40] databases were used to determine the virulence and antimicrobial resistance determinants from the trimmed and filtered reads. The presence of prophages was detected through PHASTER [5]. Zonula occludens toxin (Zot) identified from a prophage from PH1009 was compared with Zot from Zot-containing phages from *Vibrio cholerae* and *Vibrio parahaemolyticus* [47]. The amino acid sequences of Zot were aligned using Prank [39], and a maximum likelihood tree with 500 bootstraps was generated using MEGA X [34] with the Whelan and Goldman model.

2.2. Pan-genome analysis

Vibrio harveyi genomes were obtained from NCBI Refseq and annotated using Prokka [53]. Pan-genome analysis of *V. harveyi* genomes, including PH1009, was done using Roary [45]. FastTree [49] was used to construct a phylogenetic tree based on the MAFFT [30] core genome alignment from Roary. The final tree was visualized using iTOL. Unique genes from PH1009 were re-annotated with eggNOG mapper [24] to identify the hypothetical proteins from the Prokka annotation.

Default settings were used in all analyses unless otherwise stated.

3. Results and discussion

3.1. PH1009 is a novel strain of Vibrio harveyi

The genome assembly of PH1009 resulted in 34 contigs, all of which have a size of at least 1000 bp. The total length of the genome is 5708170 bp with a GC content of 44.99%. CheckM assessment also revealed that the assembled genome is 100% complete. Initial identification using TCS showed that PH1009 was highly probable to be *Vibrio harveyi* with z scores of above 0.989 with the genomes of several *Vibrio harveyi* strains in the database (Table 1). This taxonomic identification was supported by average nucleotide identity calculations (ANIm and ANIb). The percentage identity of the genome PH1009 with the reference genome of *Vibrio harveyi* ATCC

33843 was 98.6% for ANIm and 98.48% for ANIb, more than the threshold of 95% for both methods [4]. However, *V. harveyi* strains 1DA3 (90.26%, 88.76%), CUB2 (90.23%, 88.78%), MOR3 (88.70%, 86.73%), BSW5(88.72%, 86.73%), BSW7 (88.52%, 86.5%), and KC13.17.5 (89.79%, 88.31%) had less than 95% percentage identity (ANIm, ANIb) with PH1009 and other *Vibrio harveyi* strains from Refseq (Fig. 1), indicating they were not the same species as PH1009.

Among the harveyi clade, *V. harveyi* was closely related to *V. campbellii, Vibrio owensii, and Vibrio jasicida* [57]. This may have been the reason for the identification of members of these species as *Vibrio harveyi* [10]. Cano-Gomez [10] reassigned *V. harveyi* 1DA3 as a probable *V. owensii* based on the sequences of genes *topA* and *mreB* while strains MOR3, BSW5, BSW7 were reassigned as *Vibrio jasicida*, CUB2 as *V. owensii*, and KC13.17.5 as *V. campbellii* by Ke et al. [32,33] based on the concatenated alignment of single-copy orthologous genes. ANI calculations for species verification were necessary for the harveyi clade due to the close relationship and frequent recombination of its members resulting in similar biochemical profiles in conventional biochemical identification methods [11]. The 16s rRNA gene sequences of the harveyi clade were also highly similar, rendering them ineffective in species identification and evolutionary relationship resolution [22,57]. This illustrates the need for whole genomes as basis for identification. While traditional methods offer a more rapid approach in identifying strains, genome analysis provides greater classifying power especially with the availability of more complete genome sequences.

3.2. PH1009 is a potential multi-drug resistant and multiple heavy metal resistant strain

RAST annotation of the assembled genome resulted in 5285 coding sequences (CDS) and 103 RNAs. There are 2298 CDS found in 389 RAST subsystems in 26 categories (Fig. 2). Based on its annotated genome (Table 2), the PH1009 was a potential multi-drug resistant (MDR) and multiple heavy metal resistant (MHMR) strain. Since it was an aquaculture isolate, it was likely exposed to various antibiotics and heavy metal concentrations and developed resistance mechanisms against these toxic substances. Seiler and Berendonk [55] hypothesized that significant concentrations of heavy metals in the aquatic environment result in the co-selection of MDR and MHMR strains such as PH1009. Similar resistance profiles were also observed in *V. parahaemolyticus* strains from cultured crustaceans and shellfish [23,27] and enteric bacteria such as *Escherichia coli* [43] exposed to various concentrations of heavy metals such as cadmium, copper, and nickel.

The presence of genes encoding mannose-sensitive hemagglutinin (MSHA) and flagella in the PH1009 (Table 2) suggests the potential adaptive mechanisms of the PH1009 strain. Flagella were known to be necessary for chemotaxis, adhesion, and host cell invasion [18], while studies of MSHA in *V. cholerae* determined its role in biofilm formation, colonization, hemolymph adhesion, and as phage receptor [29,38,58,60]. The virulence factor Ati2 was also detected in PH1009. Ati2 encodes for an effector protein that destabilizes the host cell membrane and disrupts phagocytosis indicating its importance in host cell invasion and lysis [15]. The presence of genes encoding these resistance determinants and virulence factors in PH1009 shows the likelihood that PH1009 was a virulent strain.

3.3. Prophages were identified in the genome of PH1009

PHASTER [5] identified one 5.8 kb questionable (score = 70–90) and one 20.7 kb incomplete (score< 70) prophage in the genome of PH1009 (Fig. 3). The first phage identified has a GC percentage of 41.82% and was composed of 8 CDS, with 4 of these annotated as hypothetical proteins. It was mostly identical to Vibrio phage VCY phi (66.66%) in the PHASTER database. Zona occludens toxin (Zot)

Table 1
Tetranuclemplemented in Jspecies webserver. The genome of PH1009 is highly related to the genome of *Vibrio harveyi* strains (=>0.99) in the Jspecies database.

Position	Species	Z-Score
1	*Vibrio harveyi* VH2	0.99977
2	*Vibrio harveyi* HENC-01	0.99966
3	*Vibrio harveyi* NBRC 15634 = ATCC 14126	0.99964
4	*Vibrio harveyi* NBRC 15634 = ATCC 14126 NBRC 15634	0.99963
5	*Vibrio harveyi* NCTC12970	0.99954
6	*Vibrio harveyi* FDAARGOS_109	0.99953
7	*Vibrio harveyi* VH5	0.99949
8	*Vibrio harveyi* CAIM 1792	0.99947
9	*Vibrio harveyi* Hep-2a-10	0.99938
10	*Vibrio harveyi* HENC-02	0.99792
11	*Vibrio owensii* CAIM 1854 = LMG 25443 DY05	0.98166
12	*Vibrio* sp. HENC-03	0.98
13	*Vibrio harveyi* 1DA3	0.9797
14	*Vibrio owensii* 47,666-1	0.97947
15	*Vibrio* sp. JCM 19052	0.97821
16	*Vibrio campbellii* UMTGB204	0.97764
17	*Vibrio campbellii* HY01	0.97735
18	*Vibrio harveyi* BSW5	0.97677
19	*Vibrio campbellii* CAIM 519 = NBRC 15631 CAIM 519	0.97581
20	*Vibrio harveyi* BSW7	0.97452

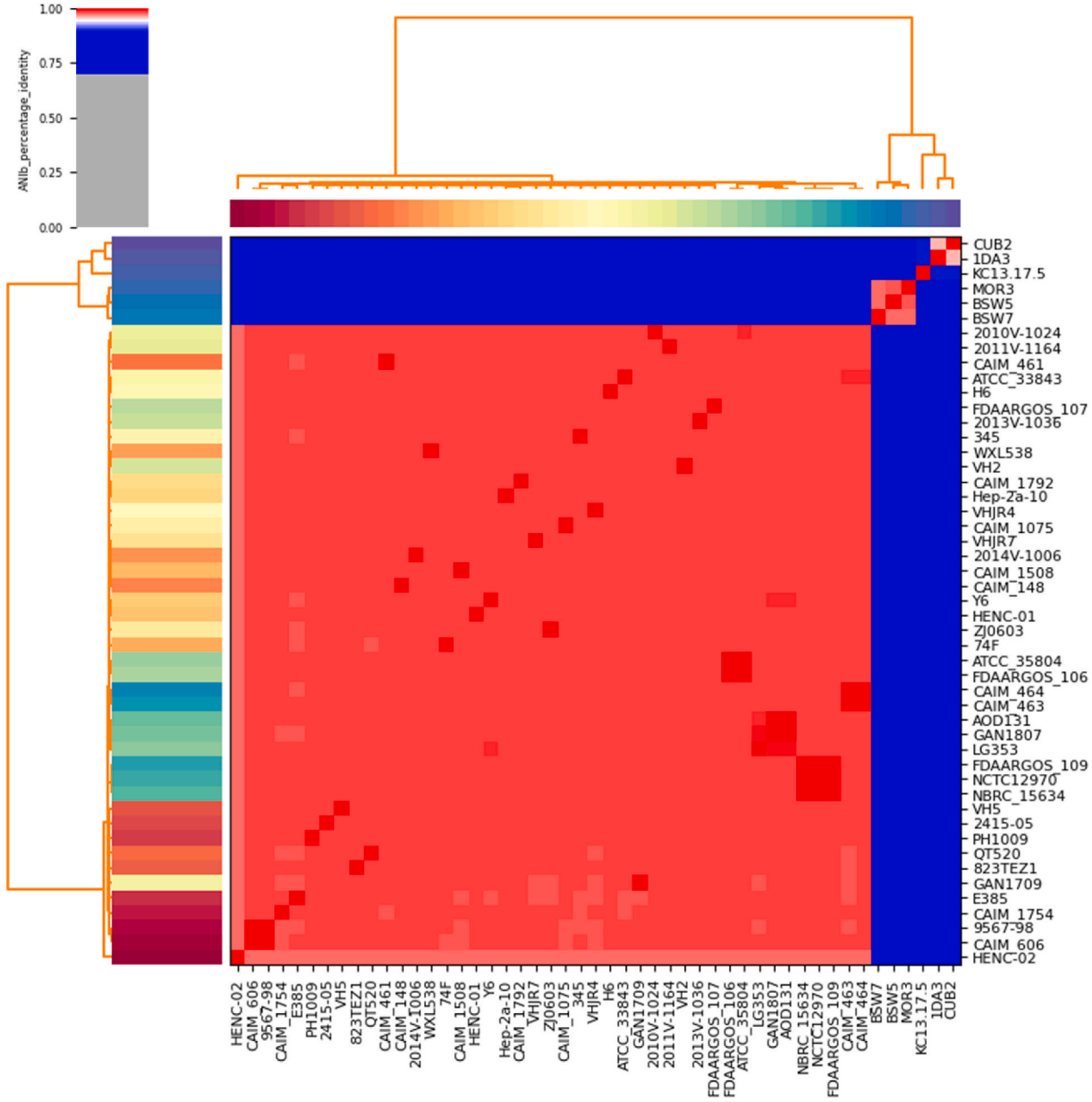

Fig. 1. Average Nucleotide Identity (ANI) similarities of *Vibrio harveyi* strains from Refseq including PH1009. PH1009 have more than 95% genome similarity with the reference genome of *Vibrio harveyi* ATCC 33843. Strains CUB2, 1DA3, KC13.17.5, MOR3, BSW5 and BSW7 have less than 95% similarities against other *V. harveyi* genomes indicating they are of different species.

and Accessory cholera enterotoxin (Ace), RstA phage replication protein, and RstB phage integrase were detected. Zot is an enterotoxin targeting the tight junction of intestinal epithelia, leading to increased gut permeability, and Ace causes increased fluid secretion in the gut. The *zot* and *ace* genes comprise two of the three crucial genes for virulent strains of *V. cholerae*, missing only the *ctx* gene. RstA and RstB, both crucial proteins for the replication and integration of phages in the bacterial chromosome, represent part of the critical genes in the phage life cycle.

The PH1009 Zot was highly related to the Zot found in *Vibrio* Phage VCY phi and *Vibrio cholerae* phage KSF phi (Fig. 4). *Vibrio* Phage VCY phi and *Vibrio cholerae* phage KSF phi were both described in environmental *V. cholerae* strains. Both lack the ctxA and ctxB toxin of toxigenic *V. cholerae* strains [19,59]. Zot from these phages also lacks the "FCIGRL" amino acid residue, the biologically active domain of Zot from the CTX phage. However, the biological effects of Zot on the epithelial barrier were hypothesized to be due to its toxin structure rather than its amino acid sequence [47]. For instance, Zot from cytotoxic *V. parahaemolyticus* also lacks the "FCIGRL" domain, but its activity against Caco-2 cells suggested it was responsible for the loss of integrity of the actin skeleton in Caco-2 cells

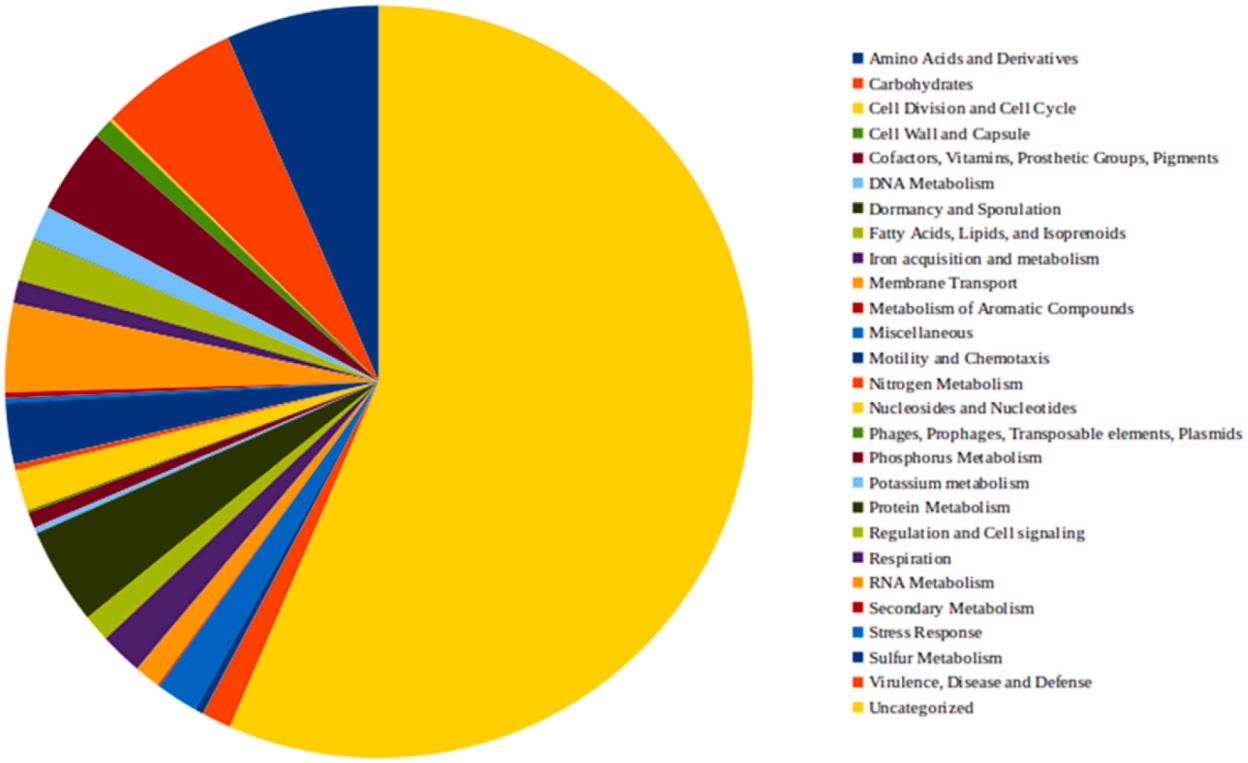

Fig. 2. Feature counts of the annotated genome of *Vibrio harveyi* PH1009. More than half (56%) of the total features of the annotated genome are uncategorized. Genes for adhesion, resistance against antibiotics and toxic compounds, and toxins are present in the genome of *V. harveyi* PH1009 under the Virulence, Disease and Defense category.

[48]. The differences in the Zot amino acid sequences from species such as the PH1009 suggest that they could have different toxic effects and merits further investigations on their activity and mechanisms.

The second prophage region was closest to Vibrio phage PV94 (33.33%) with a GC percentage of 40.2%. It has 22 CDS, 15 of which were hypothetical proteins. Monothiol glutaredoxin, phage protein, phage integrase, phage replication protein, two transcriptional regulators, and a putative site-specific DNA methylase were annotated in this prophage region. Since it is mainly composed of hypothetical proteins, its relevance to PH1009 pathogenicity cannot be determined using the methods used in this study.

3.3.1. The pan-genome of V. harveyi

The total number of genes of all the *Vibrio harveyi* strains (n = 43) used in this study was 17,344 genes, while 1778 genes comprised the core genome (99–100% of strains). The total number of genes in the pan-genome increased and the number of core genes decreased upon the introduction of a new genome, implying the *Vibrio harveyi* pan-genome as "open." This result agrees with the data obtained by Kayansamruaj and co-authors [31] using a different pan-genome pipeline and 33 *V. harveyi* genomes. Clusters of Orthologous Group (COG) category of the core genes were assigned using eggNOG mapper [24]. The *V. harveyi* core genome was mainly comprised of genes necessary for primary cellular function and growth (Fig. 5). Several genes in the core genome had unknown functions or had no homologs in the database, thus, they cannot be categorized. This problem was not confined to the core genome of *V. harveyi* and was observed even in the smallest bacterial genome [16]. Currently, only less than 1% of the protein sequences in Uniprot [8] have been experimentally validated because functional annotation of gene products cannot keep up with the increasing number of available genomes [3]. Identification of the function of these "hypothetical proteins" can elucidate the importance of these proteins on the survival of *V. harveyi* in different environments.

Phylogenetic analysis using the core genome of *V. harveyi* strains is shown in Fig. 6. The PH1009 strain was closest to the *V. harveyi* strains, QT520, CAIM 1754, and 823tez1. Although the location of the isolates may have a role in the result of the analysis, some virulence factors such as AcfA, IlpA, MAM7, OmpU, Type IV pili, Flagellin, Hemolysin, and RTX of strain QT520 [56] were also present in the annotated genome of PH1009 (Supplementary), indicating a probable similarity in pathogenicity. Interestingly, PH1009 Zot was not found in the QT520, CAIM 1754, and 823tez1 genomes but was present in strains CAIM 148 and HENC-01, emphasizing the role of horizontal gene transfer in acquiring new virulence factors. Despite being highly related, QT520, CAIM 1754, 823tez1, and PH1009 may have different acquired virulence factors because they had different hosts. *V. harveyi* CAIM 148, like PH1009, was isolated from a penaeid shrimp, suggesting a similar infection and pathogenicity mechanism since expression of some virulence factors tend to be host-linked [44].

Table 2
Virulence and Resistance Determinants of *Vibrio harveyi* PH1009 predicted by RAST Annotation (Virulence, Defense and Disease category) and ARIBA (VFDB and CARD). Toxins such as the Zona occludens toxin and Accessory cholera enterotoxin are found in the genome of PH1009. Resistance antibiotics and toxic compounds such as acridine are also present. Resistance against aminoglycoside, elfamycin and oxazolidinone antibiotic classes are predicted using ARIBA (CARD) while virulence factors such as motility and chemotaxis, effector proteins, type IV pilus were predicted by ARIBA using VFDB.

Role	Feature	Method
Accessory colonization factor AcfA	fig|6666666.713212.peg.1241	RAST Annotation of the assembled genome
Accessory cholera enterotoxin	fig|6666666.713212.peg.3065	RAST Annotation of the assembled genome
Zona occludens toxin	fig|6666666.713212.peg.3064	RAST Annotation of the assembled genome
Transcriptional activator ToxR	fig|6666666.713212.peg.712, fig|6666666.713212.peg.3259	RAST Annotation of the assembled genome
Transmembrane regulatory protein ToxS	fig|6666666.713212.peg.713	RAST Annotation of the assembled genome
Conserved uncharacterized protein CreA	fig|6666666.713212.peg.1070	RAST Annotation of the assembled genome
Inner membrane protein CreD	fig|6666666.713212.peg.2818	RAST Annotation of the assembled genome
Multicopper oxidase	fig|6666666.713212.peg.5195	RAST Annotation of the assembled genome
Cytochrome *c* heme lyase subunit CcmF	fig|6666666.713212.peg.3992	RAST Annotation of the assembled genome
Cytochrome *c* heme lyase subunit CcmH	fig|6666666.713212.peg.3989	RAST Annotation of the assembled genome
Copper-translocating P-type ATPase (EC 3.6.3.4)	fig|6666666.713212.peg.560, fig|6666666.713212.peg.567, fig|6666666.713212.peg.3754, fig|6666666.713212.peg.4300, fig|6666666.713212.peg.4909	RAST Annotation of the assembled genome
Multidrug resistance transporter, Bcr/CflA family	fig|6666666.713212.peg.921, fig|6666666.713212.peg.1773, fig|6666666.713212.peg.3436, fig|6666666.713212.peg.4146, fig|6666666.713212.peg.4963, fig|6666666.713212.peg.5184	RAST Annotation of the assembled genome
CopG protein	fig|6666666.713212.peg.4301	RAST Annotation of the assembled genome
Choloylglycine hydrolase (EC 3.5.1.24)	fig|6666666.713212.peg.3128	RAST Annotation of the assembled genome
DNA-binding heavy metal response regulator	fig|6666666.713212.peg.2121	RAST Annotation of the assembled genome
Cobalt-zinc-cadmium resistance protein	fig|6666666.713212.peg.1294	RAST Annotation of the assembled genome
Heavy metal sensor histidine kinase	fig|6666666.713212.peg.2122	RAST Annotation of the assembled genome
Transcriptional regulator, MerR family	fig|6666666.713212.peg.396, fig|6666666.713212.peg.3520, fig|6666666.713212.peg.4050, fig|6666666.713212.peg.5057	RAST Annotation of the assembled genome
DNA gyrase subunit B (EC 5.99.1.3)	fig|6666666.713212.peg.4934	RAST Annotation of the assembled genome
DNA gyrase subunit A (EC 5.99.1.3)	fig|6666666.713212.peg.4566	RAST Annotation of the assembled genome
Secreted protein, suppressor for copper-sensitivity ScsC	fig|6666666.713212.peg.2752	RAST Annotation of the assembled genome
Suppression of copper sensitivity: putative copper-binding protein ScsA	fig|6666666.713212.peg.2753, fig|6666666.713212.peg.3740	RAST Annotation of the assembled genome
Magnesium and cobalt efflux protein CorC	fig|6666666.713212.peg.4327	RAST Annotation of the assembled genome
Membrane protein, suppressor for copper-sensitivity ScsB	fig|6666666.713212.peg.3739	RAST Annotation of the assembled genome
Copper homeostasis protein CutE	fig|6666666.713212.peg.4328	RAST Annotation of the assembled genome
Cytoplasmic copper homeostasis protein CutC	fig|6666666.713212.peg.4480	RAST Annotation of the assembled genome
Membrane protein, suppressor for copper-sensitivity ScsD	fig|6666666.713212.peg.3738	RAST Annotation of the assembled genome
Periplasmic divalent cation tolerance protein CutA	fig|6666666.713212.peg.1035	RAST Annotation of the assembled genome
Chromate transport protein ChrA	fig|6666666.713212.peg.2441	RAST Annotation of the assembled genome
RND efflux system, membrane fusion protein CmeA	fig|6666666.713212.peg.1489, fig|6666666.713212.peg.3261	RAST Annotation of the assembled genome
Multi antimicrobial extrusion protein (Na(+)/drug antiporter), MATE family of MDR efflux pumps	fig|6666666.713212.peg.2483, fig|6666666.713212.peg.3614, fig|6666666.713212.peg.4707	RAST Annotation of the assembled genome

(*continued on next page*)

Table 2 (*continued*)

Role	Feature	Method
Acriflavin resistance protein	fig|6666666.713212.peg.1093, fig|6666666.713212.peg.1559, fig|6666666.713212.peg.2506	RAST Annotation of the assembled genome
Multidrug efflux pump component MtrF	fig|6666666.713212.peg.2361	RAST Annotation of the assembled genome
SSU ribosomal protein S7p (S5e)	fig|6666666.713212.peg.4631	RAST Annotation of the assembled genome
Translation elongation factor G	fig|6666666.713212.peg.4630	RAST Annotation of the assembled genome
Translation elongation factor Tu	fig|6666666.713212.peg.4629, fig|6666666.713212.peg.5162, fig|6666666.713212.peg.5196, fig|6666666.713212.peg.5246, fig|6666666.713212.peg.5285	RAST Annotation of the assembled genome
SSU ribosomal protein S12p (S23e)	fig|6666666.713212.peg.4632	RAST Annotation of the assembled genome
DNA-directed RNA polymerase beta' subunit (EC 2.7.7.6)	fig|6666666.713212.peg.5204	RAST Annotation of the assembled genome
DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	fig|6666666.713212.peg.5203	RAST Annotation of the assembled genome
Quinolinate synthetase (EC 2.5.1.72)	fig|6666666.713212.peg.448	RAST Annotation of the assembled genome
Quinolinate phosphoribosyltransferase [decarboxylating] (EC 2.4.2.19)	fig|6666666.713212.peg.2553	RAST Annotation of the assembled genome
L-aspartate oxidase (EC 1.4.3.16)	fig|6666666.713212.peg.2610	RAST Annotation of the assembled genome
LSU ribosomal protein L35p	fig|6666666.713212.peg.3339	RAST Annotation of the assembled genome
Translation initiation factor 3	fig|6666666.713212.peg.3340	RAST Annotation of the assembled genome
LSU ribosomal protein L20p	fig|6666666.713212.peg.3338	RAST Annotation of the assembled genome
Role	Feature	Method
Escherichia coli 23S rRNA with mutation conferring resistance to oxazolidinone antibiotics	Escherichia_coli_23S	ARIBA (filtered reads) against CARD database
Escherichia coli EF-Tu mutants conferring resistance to Enacyloxin IIa	Escherichia_coli_EF_Tu	ARIBA (filtered reads) against CARD database
Escherichia coli 16S rRNA (rrsH) mutation conferring resistance to spectinomycin	*rrsH*	ARIBA (filtered reads) against CARD database
Aeromonas salmonicida Type III secretion system effector, Inositol polyphosphate 5-phosphatase	*ati2*	ARIBA (filtered reads) against VFDB database
Vibrio parahaemolyticus RIMD 2210633 chemotaxis methyltransferase	*cheR*	ARIBA (filtered reads) against VFDB database
Vibrio parahaemolyticus RIMD 2210633 chemotaxis	*cheV*	ARIBA (filtered reads) against VFDB database
Vibrio parahaemolyticus RIMD 2210633 chemotaxis protein	*cheY*	ARIBA (filtered reads) against VFDB database
Vibrio vulnificus YJ016 chemotaxis protein CheY	*cheY*	ARIBA (filtered reads) against VFDB database
Vibrio parahaemolyticus RIMD 2210633 flagellin flaB	*flaB*	ARIBA (filtered reads) against VFDB database
Vibrio parahaemolyticus RIMD 2210633 flagellar basal body rod protein	*flgC*	ARIBA (filtered reads) against VFDB database
Vibrio parahaemolyticus RIMD 2210633 flagellar basal body rod modification protein	*flgD*	ARIBA (filtered reads) against VFDB database
Vibrio parahaemolyticus RIMD 2210633 flagellar hook protein	*flgE*	ARIBA (filtered reads) against VFDB database
Vibrio parahaemolyticus RIMD 2210633 flagellar biosynthesis sigma factor FliA	*fliA*	ARIBA (filtered reads) against VFDB database
Vibrio parahaemolyticus RIMD 2210633 Mannose-sensitive hemagglutinin (MSHA type IV pilus)	*mshA*	ARIBA (filtered reads) against VFDB database
Vibrio parahaemolyticus RIMD 2210633 type III secretion system chaperone	*vcrH*	ARIBA (filtered reads) against VFDB database

3.3.2. The genome of PH1009 encodes several unique genes

The PH1009 has 141 unique genes compared to reference *V. harveyi* strains based on the Roary analysis. Sixty-two (62) of these genes had no COG assignments, while six (6) had unknown functions. Genes for polysaccharide production (*cps2J*), detoxification (*gloA, gloB*), fatty acid synthesis (*accB*), and tellurite resistance (*terB*) were among the unique genes in PH1009. Several hypothetical proteins had functions related to phages such as transposases, integrases, and transcription regulators, implying that the phages could have highly contributed to the distinct features of the PH1009 genome and its potential virulence. Phages are known to carry genes beneficial to their host. In some species, susceptibility to phage infection was linked to acquired resistance against antibiotics and

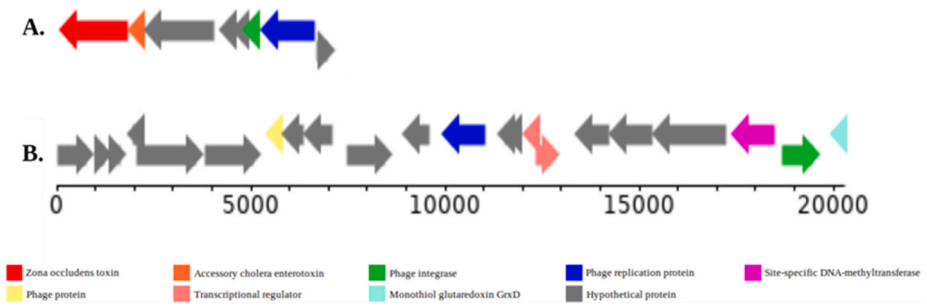

Fig. 3. Graphical Representation of bacteriophages in the genome of PH1009. The first phage (A.) (5.8 kb) detected contains virulence factors, Zona occludens toxin and Accesory cholera enterotoxin found in CTX phage of pathogenic *V. cholerae* strains. The second phage (B.) (20.7 kb) within the genome consists of 22 CDS, 15 of which are hypothetical proteins.

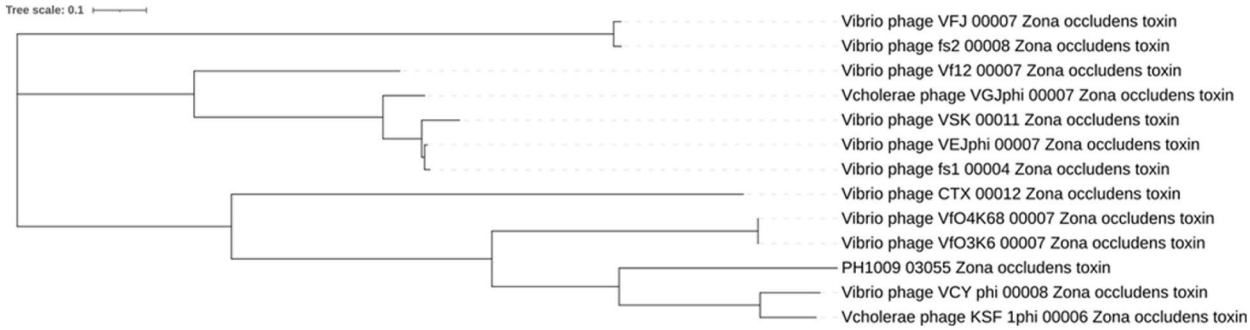

Fig. 4. Phylogenetic tree topology of Zona occludens toxin (Zot) from bacteriophages in the genome of Different *Vibrio* species inferred using maximum likelihood model and Whelan And Goldman model. Zot from *V. harveyi* PH1009 was more related to Zot found in *Vibrio* phage VCY phi and *V. cholerae* phage KSF phi which were described in *V. cholerae* environmental strains.

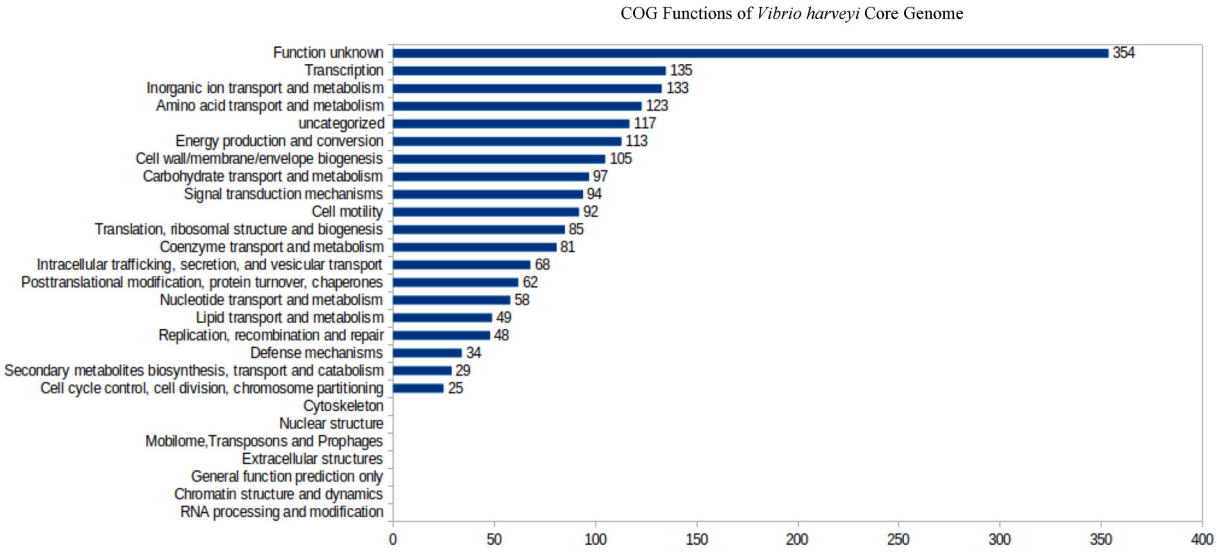

Fig. 5. Clusters of Orthologous Groups (COG)Functional Annotation of the core genome of *Vibrio harveyi*. Most genes in the core genome have unknown function and mainly comprised of genes necessary for primary cellular function and growth.

heavy metals [37]. Some phages may also supplement their host during nutrient-limited environmental conditions by carrying genes for antioxidation, carbon metabolism, cell protection, cycling of nutrients (such as nitrogen, phosphorus, and sulfur), fatty acid metabolism, iron-sulfur clusters, photosynthesis, purine and pyrimidine metabolism, and protein synthesis [9]. Bacteriophages carrying potential toxins have been identified from *Vibrio* isolates. An example of this is *V. harveyi* myovirus like (VHML) phage which

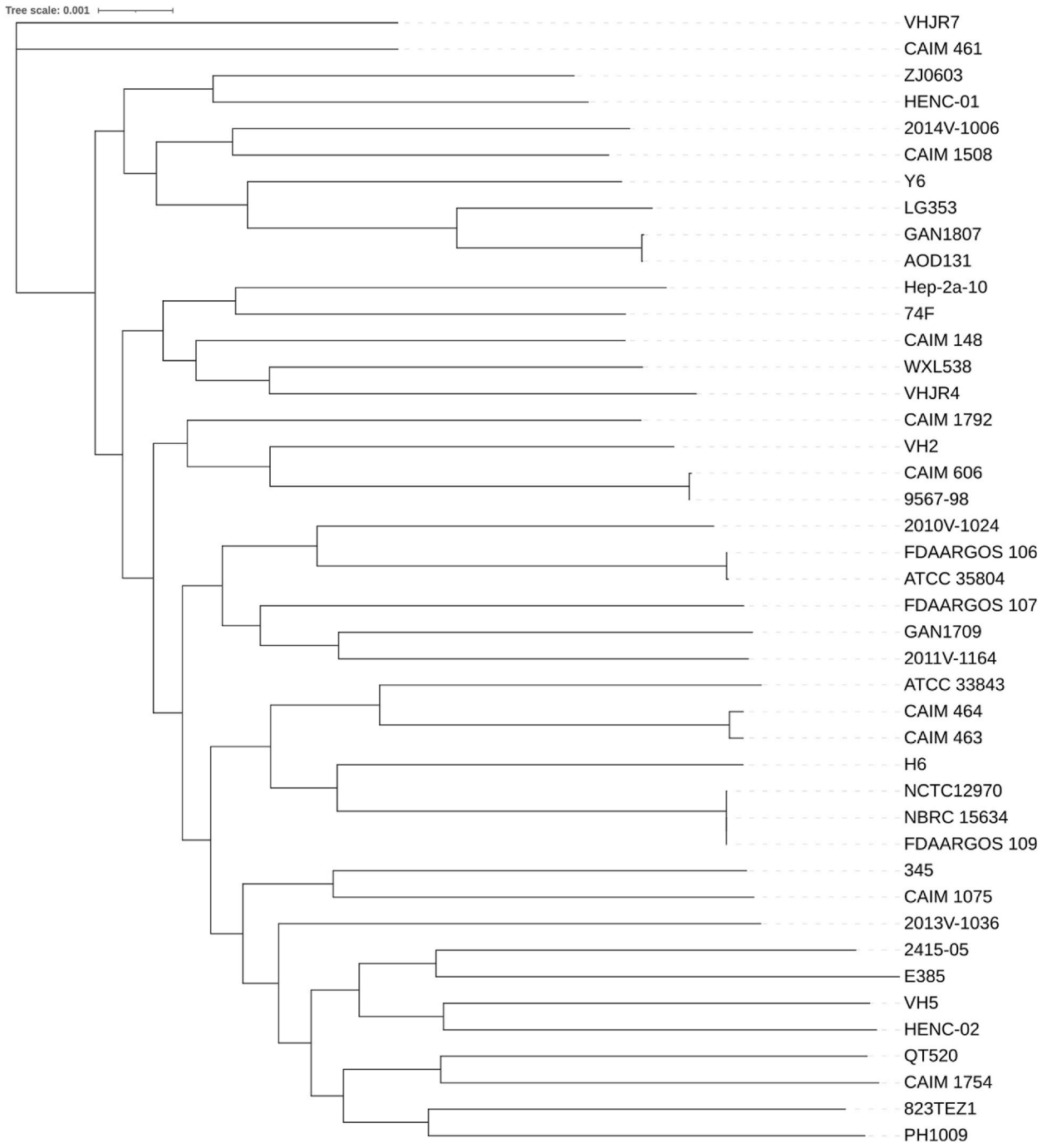

Fig. 6. Phylogenetic tree topology of *V. harveyi* strains based on MAFFT core genome alignment implemented in FastTree. The V. harveyi strain PH1009 from Masbate Island, Philippines was most related to *V. harveyi* strains 823TEZ1, CAIM 1754 and QT520.

infected isolates and were found to have increased hemolytic activity and can even turn avirulent strains to virulent ones in Atlantic salmon. Some isolates virulent to Tiger prawn were also found to harbor a novel siphovirus-like phage 1 (VHS1) which was suggested to mediate toxicity by providing toxin genes or genes controlling toxin production [61].

4. Conclusions

The PH1009 strain isolated from *P. monodon* infected with WSSV from Masbate, Philippines, was identified as *Vibrio harveyi* by Average Nucleotide Identity. The identified virulence and resistance determinants in the PH1009 genome suggest it was a potential

virulent MDR and MHMR strain. A prophage region containing genes coding for Zona occludens toxin (Zot) and Accessory cholera enterotoxin (Ace) were also identified, suggesting that phage infection may have contributed to the potential virulence of PH1009. It is likely that the presence of Zot and Ace predisposed the host to WSSV via the weakening of epithelial barriers in the hepatopancreas.

Author contribution statement

Czarina Anne De Mesa; Remilyn M Mendoza; Sarah Mae U Penir: Analyzed and interpreted the data; Wrote the paper. Leobert D Dela Pena; Edgar C Amar: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper. Cynthia Palmes Saloma, PhD: Conceived and designed the experiments; Wrote the paper.

Data availability statement

Data associated with this study has been deposited at "DDBJ/ENA/GenBank & SRA (NCBI)" under the accession number "JAENDI000000000 & SRR13267183" respectively.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

Supplementary content related to this article has been published online at [URL].

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Czarina Anne De Mesa reports a relationship with Republic of the Philippines Department of Science and Technology that includes: funding grants. Remilyn M. Mendoza reports a relationship with Republic of the Philippines Department of Science and Technology that includes: funding grants.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e14926.

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