



Analysis of whole genome sequencing and virulence factors of *Vibrio vulnificus* 1908-10 isolated from sea water at Gadeok island coast

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Abstract

Vibrio vulnificus is an aquatic bacterium causing septicemia and wound infection in humans. To understand this pathogen at the genomic level, it was performed whole genome sequencing of a cefoxitin-resistant strain, *V. vulnificus* 1908-10 possessing virulence-related genes (*vvhA*, *viuB*, and *vcgC*) isolated from Gadeok island coastal seawater in South Korea. The genome of *V. vulnificus* 1908-10 consisted of two circular contigs and no plasmid. The total genome size was estimated to be 5,018,425 bp with a guanine-cytosine (GC) content of 46.9%. We found 119 tRNA and 34 rRNA genes respectively in the genome, along with 4,352 predicted protein sequences. Virulence factor (VF) analysis further revealed that *V. vulnificus* 1908-10 possess various virulence genes in classes of adherence, antiphagocytosis, chemotaxis and motility, iron uptake, quorum sensing, secretion system, and toxin. In the comparison of the presence/absence of virulence genes, *V. vulnificus* 1908-10 had *fur*, *hlyU*, *luxS*, *ompU*, *pilA*, *pilF*, *rtxA*, *rtxC*, and *vvhA*. Of the 30 *V. vulnificus* comparative strains, 80% of the C-genotype strains have all of these genes, whereas 40% of the E-genotype strains have all of them. In particular, *pilA* were identified in 80% of the C-type strains and 40% of the E-type strains, showing more difference than other genes. Therefore, *V. vulnificus* 1908-10 had similar VF characteristics to those of type C strains. Multifunctional-autoprocessing repeats-in-toxin (MARTX) toxin of *V. vulnificus* 1908-10 contained 8 A-type repeats (GXXGXXXXXG), 25 B.1-type repeats (TXVGXGXX), 18 B2-type repeats (GGXGDXXX), and 7 C-type repeats (GGXGDXXX). The National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) showed that the RtxA protein of *V. vulnificus* 1908-10 had the effector domain in the order of cross-linking domain (ACD)-C58_PaToxP-like domain- α / β hydrolase-C58_PaToxP-like domain.

Keywords: *Vibrio vulnificus*, whole genome sequencing, virulence factor, multifunctional-autoprocessing repeats-in-toxin (MARTX) toxin

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Introduction

Vibrio vulnificus is an aquatic bacterium infected with ingestion of raw or undercooked seafood or with exposure of wounds to seawater causing gastroenteritis, wound infection, and sepsis. In the case of primary sepsis, the fatality rate reaches more than 50% (Hlady & Klontz, 1996; Oliver, 2005; Shapiro et al., 1998). In South Korea, where seafood intake is high, *V. vulnificus* has been steadily detected nationwide, and 37 to 88 cases of *Vibrio* sepsis have been reported every year from 2000 to 2020, and the mortality rate is 45.7% from 2011 to 2020 (Cho & Park, 2019; KDCA, 2021; Kim et al., 1987; 1990; Park et al., 2019). *V. vulnificus* has several virulence factors (VF) such as capsular polysaccharide (CPS), iron uptake, hemolysin (*vvhA*), protease (*vvpE*), repeats-in-toxin (RTX) toxin, lipopolysaccharide, pili, and flagella, and various factors regulating them have been reported for the pathogenicity of *V. vulnificus*, and studies such as identification of the toxic activation mechanisms have been conducted, too (Jones & Oliver, 2009; Lee et al., 2019).

Along with the development of next-generation sequencing technology, genome analysis of many pathogenic bacteria important in public health has been performed, and in the case of *V. vulnificus*, starting with the first genome report in 2003, whole genome analysis has been conducted to identify genomic characteristics and various attempts are being made such as comparison between clinical and environmental genotypes (Chen et al., 2003; Morrison et al., 2012; Pan et al., 2017; Pang et al., 2020; Roig et al., 2018). However, compared to other pathogenic bacteria such as *Salmonella enterica* and *Staphylococcus aureus*, the genome analysis of *V. vulnificus* is insignificant, and even compared to the same genus *Vibrio*, it is less than that of *Vibrio parahaemolyticus* and *Vibrio cholerae* (NCBI, 2023).

The eastern coast of Gadeok island is provided good conditions for the habitat of *V. vulnificus* due to the inflow of the Nakdong river. Additionally, the final treated water from three sewage treatment plants is flowing in this area, and it is possible to influx pollutants from the land. Our previous studies identified the detection tendency of *V. vulnificus* in the seawater of the eastern coast of Gadeok island, the genetic characteristics related to pathogenicity, and the antibiotic resistance characteristics of the isolates (Oh et al., 2020, 2021). In this study, whole genome sequencing analysis was performed on the pathogenic *V. vulnificus* isolated from the coast of Gadeok island, and the characteristics of VFs were looked for.

Materials and Methods

Bacterial strain and DNA extraction

V. vulnificus 1908-10 used for whole genome sequencing analysis was isolated in August 2019 from the seawater of the eastern coast of Gadeok island in a previous study (Oh et al., 2020, 2021). This strain possessed virulence-related genes (*vvhA*, *viuB*, and *vcgC*), β -hemolysis activity, and cefoxitin resistance (minimal inhibitory concentration 32 $\mu\text{g}/\text{mL}$). The strain was cultured at 35°C with Luria-Berani broth (NEOGEN, Lansing, MI, USA) supplemented with 1% NaCl for 12 h, and the genomic DNA was extracted using Genomic DNA extraction kit (Bioneer, Daejeon, Korea) according to the manufacturer's procedure.

Whole genome sequencing and annotation

Two different genomic DNA libraries were constructed according to the manufacturer's instructions for the Illumina and the PacBio platform. Sequencing was performed using PacBio Sequel I System (Pacific Biosciences, Menlo Park, CA, USA) and Illumina HiSeqX ten sequencer (Illumina, San Diego, CA, USA). CANU v1.7 (Koren et al., 2017) and Pilon v1.21 (Walker et al., 2014) were used for *de novo* assembly. The completeness of the genomic data was assessed by BUSCO v5.1.3 (Manni et al., 2021). The genome sequences of *V. vulnificus* 1980-10 were deposited in the National Center for Biotechnology Information (NCBI) GenBank server under the accession numbers CP118438 and CP118439 for chromosome I and II. Gene annotation was conducted using prokka v1.13 (Seemann, 2014), eggNOG 4.5 (Huerta-Cepas et al., 2016) and PATRIC v3.6.12 (Wattam et al., 2017). The functional classification for coding sequences was performed through Position-Specific Iterative Basic Local Alignment Search Tool (PSI-BLAST; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) based on the Clusters of Orthologous Genes (COGs) database (2014 update version; <http://www.ncbi.nlm.nih.gov/COG/>) and was visualized with a circular map using CIRCOS v0.69-63 (<http://circos.ca>).

Comparative genome analysis

The identity of *V. vulnificus* 1908-10 was confirmed by comparative phylogenetic analysis using MEGA11 v.11.08 (Tamura et al., 2021) against 16S rRNA sequences of the genus *Vibrio*. Average nucleotide identity (ANI) matrix was constructed via EDGAR 3.0 (Dieckmann et al., 2021). The NCBI dataset was used for comparative genomic analysis of *V. vulnificus* in this study (Table 1).

Table 1. Attributes of *Vibrio vulnificus* used for comparative genomic analysis in this study

Strain name	Genotype	Isolation source	Country	Collected year	Size (Mb)	Accession No.
<i>V. vulnificus</i>						
07-2444	C	Blood	USA	2007	5.23	GCA_009764115.1
1676-80	C	Water	USA	Unknown	5.04	GCA_012275045.1
2497-87	E	Blood	USA	Unknown	5.03	GCA_014211935.1
93U204	C	<i>Oreochromis</i>	Taiwan	2004	5.13	GCA_000746665.1
ATCC 27562	E	Human blood	USA	1979	5.01	GCA_002224265.1
CECT4606	E	Healthy eel	Spain	1990	5.19	GCA_002891755.1
CECT4999	E	Diseased eel	Spain	1990	5.16	GCA_002215135.1
CECT7030	E	Eel	Denmark	2004	5.11	GCA_002903505.1
CECT898	E	Eel	Japan	1979	5.20	GCA_002903765.1
CG100	C	Oyster	Taiwan	1993	5.21	GCA_002903465.1
CladeA-yb158	C	Tilapia	Israel	2005	5.29	GCA_001013325.1
CMCP6	C	Human	South Korea	2003	5.13	GCA_004355205.1
Env1	E	Oyster	USA	Unknown	4.95	GCA_003047125.1
FDAARGOS_119	E	Human	USA	Unknown	4.98	GCA_001558515.2
FDAARGOS_663	E	Human	USA	Unknown	4.97	GCA_008693685.1
FORC_017	C	Human blood	South Korea	2014	5.23	GCA_001675245.1
FORC_036	C	<i>Macra veneriformis</i>	South Korea	Unknown	6.07	GCA_002117205.1
FORC_037	C	<i>Mya arenaria oonogai Makiyama</i>	South Korea	Unknown	5.12	GCA_002204915.1
FORC_053	E	<i>Macra veneriformis</i>	South Korea	2013	6.02	GCA_003522555.1
FORC_054	E	<i>Konosirus punctatus</i>	South Korea	2014	5.12	GCA_002863725.1
FORC_077	C	Human	South Korea	2017	5.02	GCA_004319645.1
JY1305	E	Oyster	USA	Unknown	4.95	GCA_000269725.1
JY1701	E	Oyster	USA	Unknown	4.94	GCA_000269765.1
LSU1015	C	Human	USA	Unknown	5.59	GCA_002906245.1
MO6-24/O	C	Human	South Korea	Unknown	5.01	GCA_000186585.1
Vv180806	C	Human blood	China	2018	5.36	GCA_014107515.1
VV2014DJH	E	Human blood	China	2014	5.07	GCA_002850455.1
VVyb1(BT3)	E	Tilapia	Israel	2004	5.75	GCA_000342305.2
YJ016	C	Human blood	Taiwan	< 2003	5.26	GCA_000009745.1
<i>Vibrio parahaemolyticus</i> RIMD 2210633		Human	Japan	1996	5.17	GCA_000196095.1
<i>V. parahaemolyticus</i> DLM1799		Seawater	China	2019	5.11	GCA_023205915.1
<i>Vibrio cholerae</i> RFB16		Fresh water	USA	2017	4.14	GCA_008369605.1
<i>V. cholerae</i> 1154-74		Diarrhea	India	1974	3.93	GCA_000969235.1
<i>Clostridium perfringens</i> ATCC13124		Type strain	Unknown	Unknown	3.26	GCA_000013285.1

Identification of virulence factors and multifunctional-autoprocessing repeats-in-toxin (MARTX) toxin

The VFs of the *V. vulnificus* 1908-10 genome were analyzed using the virulence factor database (VFDB) 2019 (Liu et al., 2019) and their locations were edited on a visualized circular map using Adobe Photoshop 2023. Virulence gene presence/absence comparison was performed by obtaining comparison

strains information from the NCBI dataset and reannotating using prokka. We used PROSITE (Sigrist et al., 2012) to find multifunctional-autoprocessing repeats-in-toxin (MARTX) toxin repeat regions and used MEGA 11 to identify the number and location of repeat sequences described by Roig et al. (2011). NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to perform and compare searches for the effector domains.

Antimicrobial resistance genes

The search for antibiotic resistance genes in *V. vulnificus* 1908-10 was performed using ResFinder (Bortolaia et al., 2020) and Comprehensive Antibiotic Resistance Database (CARD) (Alcock et al., 2020).

Results and Discussion

Genome properties and annotation

As a result of whole genome analysis, *V. vulnificus* 1908-10 was composed of two circular contigs, and no plasmid was identified. The total length of the genome was 5,018,425 bp, and its GC content was 46.9%. Contigs 1 and 2 were 3,273,700 bp and 1,744,725 bp long, respectively, with 46.7% and 47.3% corresponding guanine-cytosine (GC) contents. Total coding sequences (CDS) were 4,352, with 2,890 in contig 1 and 1,462 in contig 2. 119 tRNAs and 34 rRNAs were identified and were mainly distributed in contig 1 (Table 2).

As a result of analysis based on the COGs, the genome of *V. vulnificus* 1908-10 had the most genes related to metabolism at 31.6%, genes related to cell processing and signals at 29.3% and genes related to information storage and process were 16.2%. Mobilome-related genes such as prophages and transposons were 1.8% (Fig. 1). In detail, genes involved in signal transduction mechanisms were the most common at 8.3%, followed by genes involved in transcription at 7.1%, genes involved in amino acid

Table 2. Summary of whole-genome sequencing for *Vibrio vulnificus* 1908-10

Property	<i>V. vulnificus</i> 1908-10		
Methods reads			
PacBio Sequel I System			
Total filtered subreads	169,959		
N50	12,142		
Illumina HiSeqX ten			
Total filtered reads	8,211,314		
Q30 (%)	98.16		
Results of assembly	Contig 1	Contig 2	Total
Contigs	1	1	2
Total contig bases	3,273,700	1,744,725	5,018,425
N50	3,273,700		
GC (%)	46.7	47.3	46.9
Depth	326.8	272.0	307.8
Genome annotation			
CDS	2,890	1,462	4,352
tRNA	106	13	119
rRNA	31	4	34

GC, guanine-cytosine; CDS, coding sequence.

transport and metabolism at 6.8%. Genes involved in cell wall/membrane/envelope biogenesis were 5.7%. The COGs of *V. vulnificus* Vv180806 and *V. vulnificus* VV2014DJH have been reported and generally show similar trends. However, in the

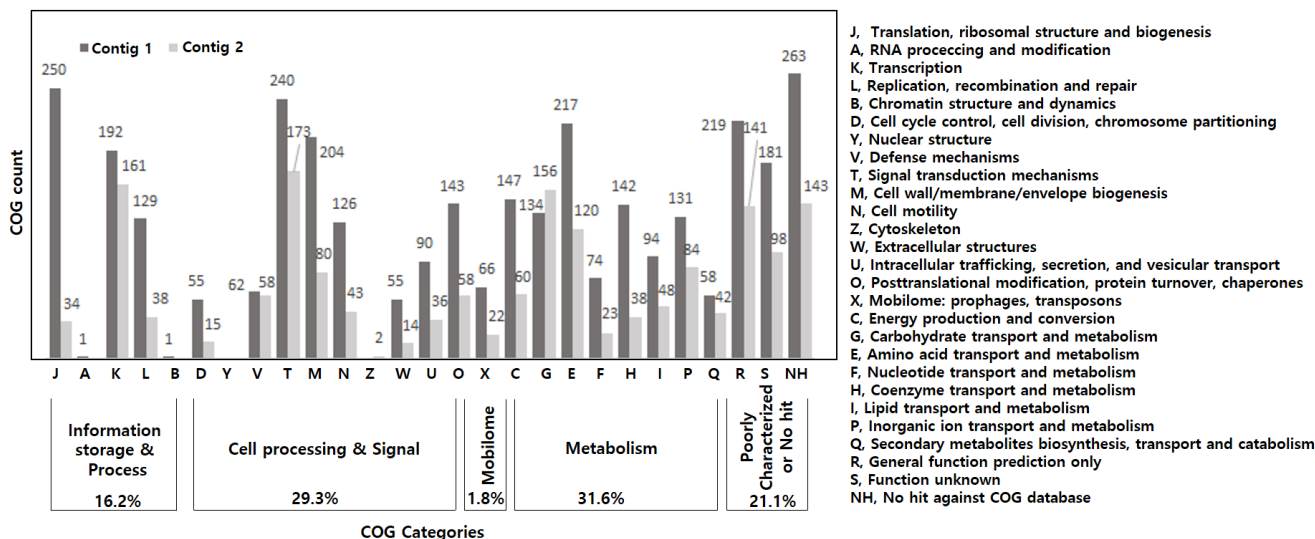


Fig. 1. Functional classification of the protein coding sequence in *Vibrio vulnificus* 1908-10 based on COG categories. COG, clusters of orthologous gene.

cytoskeleton category, *V. vulnificus* Vv180806 and *V. vulnificus* VV2014DJH had no corresponding genes, while two genes were identified in *V. vulnificus* 1908-10 showing differences (Pan et al., 2017; Pang et al., 2020).

Phylogenetic tree and average nucleotide identity (ANI)

In a phylogenetic analysis comparing 16s rRNA using MEGA 11 software, this strain was identified as *V. vulnificus* distinct from other *Vibrio* spp. and outgroup (Fig. 2A). When comparing the similarity of genomes using EDGAR based on the NCBI dataset, *V. vulnificus* 1908-10 was the most similar to *V. vulnificus* FORC_077, with about 99.41% similarity, followed by *V. vulnificus* CMCP6 and *V. vulnificus* FORC_053 was 99.02%. *V. vulnificus* FORC_017 followed with 98.92%. Strain FORC_077, CMCP6, and FORC_017 were of type C, often found in clinically isolated strains when classified based on genotype. *V. vulnificus* FORC_053 was of type E. Twelve (80%) of the top 15 isolates with an ANI greater than 98% were type C, so *V. vulnificus* 1908-10 was similar to type C overall (Fig. 2B). 95% ANI corresponded to the recommended cut-off point for species delineation (Goris et al., 2007).

Identification of virulence factors

As a result of analyzing through VFDB, *V. vulnificus* 1908-10 had genes of VFs such as mannose-sensitive hemagglutinin type IV pilus, CPS, flagella, metalloproteinase, vibriobactin related to iron absorption, heme receptor, and periplasmic binding ATP binding cassette (ABC) protein transport system. It possessed the *luxS* gene of autoinducer-2 in the quorum sensing class, and among the secretion systems, the EPS type II secretion system was identified. Toxin factors like *vvhA*, *tlh*, and RTX gene clusters (*rtxABCD*) were identified (Table 3). Genes related to attachment, motility, and secretion systems were located in contig 1 of the genome, while genes for metalloproteinases, iron uptake vibriobactin, transport systems, and toxins such as hemolysin and RTX were identified in contig 2. CPS genes were identified in both contig 1 and contig 2 (Fig. 3). CPS has been reported to be biochemically and genetically diverse among strains (Pettis & Mukerji, 2020), and *V. vulnificus* 1908-10 was found to have *cpsABCDFHII*, *hp1*, *wbfU*, *wbfV/wcvB*, *wbfY*, *wza*, *wzb*, and *wzc* (Table 3).

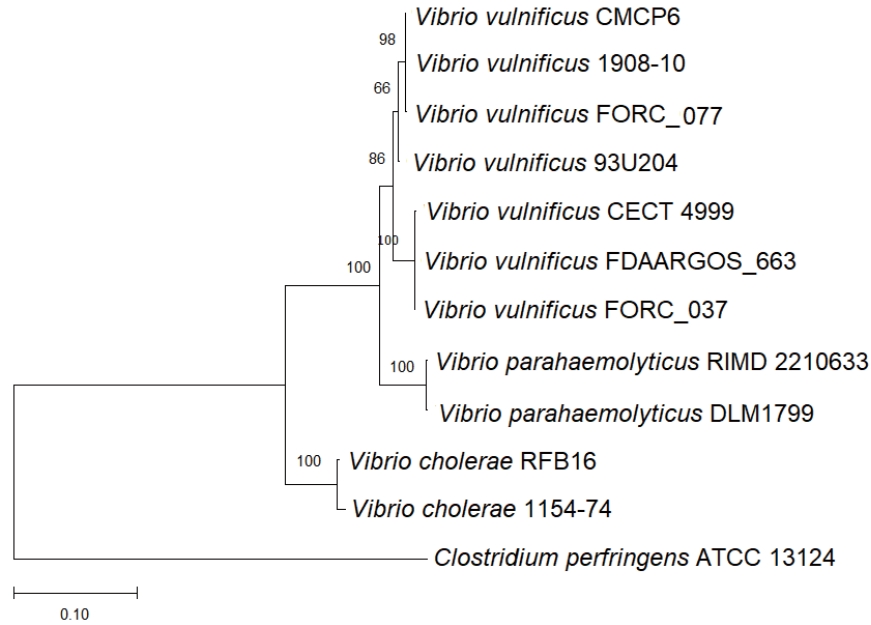
Compared to the 29 *V. vulnificus* strains in the NCBI dataset, *V. vulnificus* 1908-10 had *fur*, *hlyU*, *luxS*, *ompU*, *pilA*, *pilF*, *rtxA*, *rtxC*, and *vvhA* genes. All 30 strains including 1908-10, had *hlyU*, *ompU*, *pilF*, and *vvhA* genes in common. On the other hand, *fur* was identified in 96.7%, *rtxA* in 90%, *luxS* in 83.3%, *rtxC* in 76.7%

and *pilA* in 60% of the strains. Particularly, *pilA*, which is part of the type IV pili operon, was found in 80% of type C strains but in 40% of type E strains, showing differences between genotypes (Table 3 and Fig. 4). *hlyU* regulates the expression of *rtxA1* at the transcriptional level, affecting its cytotoxicity and virulence (Liu et al., 2007). *ompU* is a factor involved in bacterial adhesion to host cells (Goo et al., 2006). *pilF* is a protein gene required for the assembly of type IV pili, whose functions include surface motility of the strain, colony, and biofilm formation, and host cell adhesion (Alm & Mattick, 1997; Hobbs & Mattick, 1993). *vvhA* is a hemolysin/cytolysin gene of *V. vulnificus*. *Fur* regulates the production of hemolysin, *rtxA* encodes the RTX toxin and *rtxC* encodes the toxin activator (Lee et al., 2013; Lin et al., 1999). *luxS* is an autoinducer-2 synthase gene in the quorum sensing system and has been reported to affect the transcription of *vvhA* and *vvpE* (Kim et al., 2003). Overall, *V. vulnificus* 1908-10 had more similar virulence characteristics to type C strains, as 80% of type C strains had all nine genes compared, while 40% of type E strains did.

Multifunctional-autoprocessing repeats-in-toxin (MARTX) toxin

MARTX toxins are large single polypeptide toxins produced by various gram-negative bacteria. They deliver numerous effector proteins from the bacteria to the host cell to alter the target cell physiology. In contrast to the conservation of the MARTX toxin domain structure among most of the *V. cholerae* isolates, the *V. vulnificus* MARTX toxins are strikingly diverse (Kim, 2018; Satchell, 2015). Ten different effector domains have been recognized among the various MARTX toxins of *Vibrio* spp., although any one toxin carries only two to five effectors (Satchell, 2015). MARTX toxin of *V. vulnificus* 1908-10 contained 8 A-type repeats (GXXGXXXXXG), 25 B.1-type repeats (TXVXGXGX), 18 B2-type repeats (GGXGXDXXX), and 7 C-type repeats (GGXGXDXXX). NCBI BLAST showed that the RtxA protein of *V. vulnificus* 1908-10 had the effector domain in the order of actin cross-linking domain (ACD)-C58_PaToxP-like domain- α / β hydrolase-C58_PaToxP-like domain (Fig. 5), with the same domain sequence as *V. vulnificus* FORC_017, *V. vulnificus* FORC_053, and *V. vulnificus* CECT4999 (NCBI, 2023). Their amino acid sequence of the effector domain was 100% identical to *V. vulnificus* FORC_017, but there were differences in some amino acids when compared to *V. vulnificus* FORC_053 and *V. vulnificus* CECT4999. ACD disrupts the cell cytoskeleton and inhibits the engulfing activity of phagocytic immune cells of the

A



B

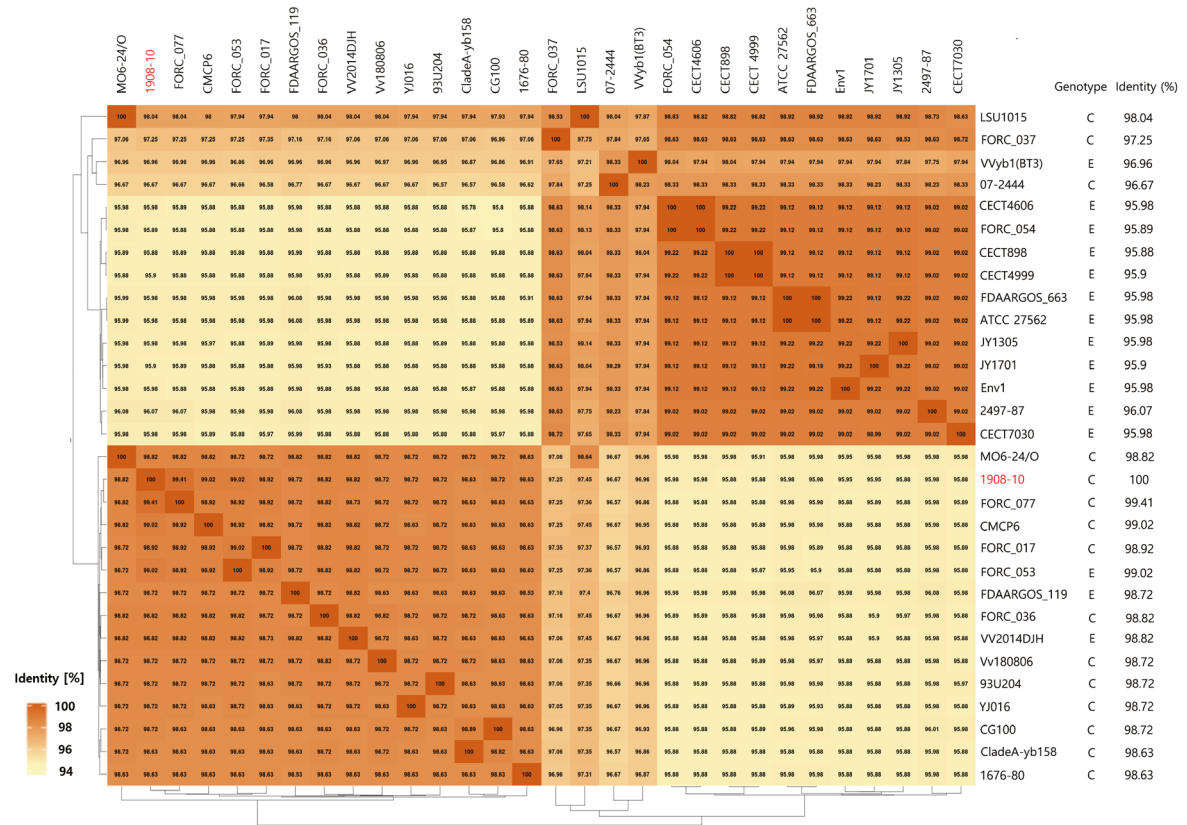


Fig. 2. Phylogenetic tree based on 16S rRNA sequence (A) and median ANI matrix tree (B) of *Vibrio vulnificus* 1908-10. ANI, average nucleotide identity.

Table 3. Virulence factors of *Vibrio vulnificus* 1908-10 identified using the virulence factor database

Virulence class	Virulence factors and genes	
	Presence	Absence
Adherence	MSHA type IV pilus - <i>mshA, mshB, mshC, mshD, mshE, mshF, mshG, mshH, mshI, mshJ, mshK, mshL, mshM, mshN</i> Type IV pilus - <i>pilA, pilB, pilC, pilD</i>	Accessory colonization factor - <i>acfA, acfB, acfC, acfD</i> Toxin-coregulated pilus (typeIVB pilus) - <i>tcpD, tcpE, tcpF, tcpH, tcpI, tcpJ, tcpN/toxT, tcpP, tcpQ, tcpR, tcpS, tcpT</i>
	Antiphagocytosis	Capsular polysaccharide - <i>cpsA, cpsB, cpsC, cpsD, cpsF, cpsH, cpsI, cpsJ, hp1, wbfU, wbfV/wcvB, wbfY, wza, wzb, wzc</i>
Chemotaxis and motility	Flagella - <i>cheA, cheB, cheR, cheW, cheY, cheZ, flm, flaA, flaB, flaC, flaD, flaE, flaG, flal, flgA, flgB, flgC, flgD, flgE, flgF, flgG, flgH, flgI, flgJ, flgK, flgL, flgM, flgN, flhA, flhB, flhF, flhG, flhI, flhJ, flhK, flhL, flhM, flhN, flhO, flhP, flhQ, flhR, flhS, flrA, flrB, flrC, motA, motB, motX, motY</i>	Flagella - <i>flaC</i>
	Enzyme	Metalloproteinase - <i>hap/vvp</i>
Iron uptake	Heme receptors - <i>hutA, hutR</i> Periplasmic binding protein dependent ABC transport systems - <i>vctC, vctD, vctG, vctP</i> Vibriobactin - <i>vibA, vibB, vibC, vibD, vibE, vibF, vibH, viuA, viuB</i>	Enterobactin receptors - <i>irgA, vctA</i> Heme receptors - <i>hasR</i> Periplasmic binding protein dependent ABC transport systems - <i>viuC, viuD, viug, viup</i>
	Quorum sensing	Autoinducer-2 - <i>luxS</i>
Secretion system	EPS type II secretion system - <i>epsC, epsE, epsF, epsG, epsH, epsI, epsJ, epsK, epsL, epsM, epsN, gspD</i>	VAS effector proteins - <i>hcp-1, hcp-2, vgrG-1, vgrG-2, vgrG-3</i> VAS type VI secretion system - <i>vasA, vasB, vasC, vasD, vasE, vasF, vasG, vasH, vasI, vasJ, vasK</i>
	Toxin	Hemolysin/cytolysin - <i>vhA</i> RTX toxin - <i>trxA, rtxB, rtxC, rtxD</i> Thermolabile hemolysin - <i>tlh</i>

MSHA, mannose-sensitive hemagglutinin; ABC, ATP binding cassette; EPS, extracellular protein secretion; VAS, virulence associated secretion; RTX, repeats-in-toxin.

host. α/β hydrolases reduce the intracellular phosphatidylinositol 3-phosphate levels and blocked autophagic/endosomal pathways. The C58_PaToxP-like domain, also known as makes caterpillars floppy-like domain (MCF), is associated with apoptotic cell death (Kim, 2018).

V. vulnificus FORC_077, which had a high ANI value for its genome, had the effector domain sequence of membrane localization domain (MLD)- α/β hydrolase-C58_PaToxP-like domain. *V. vulnificus* CMCP6 and YJ016 had domains of MLD- α/β hydrolase-C58_PaToxP-like domain-toxin_MLD (toxin effector region membrane localization domain)-RtxA-like domain (C2-

2 like domain of various multidomain toxins) (NCBI, 2023). Biochemical mechanisms and direct target molecules for specific effector domains of MARTX toxins have been characterized, but some still require further study (Kim, 2018). Meanwhile, the diversity of effector domains in the MARTX toxin was independent of genotype (data not shown).

Antimicrobial resistance genes

Regarding antibiotic resistance in *V. vulnificus* 1908-10, no acquired resistance genes were identified in the analysis using ResFinder. On the other hand, CARD analysis identified the

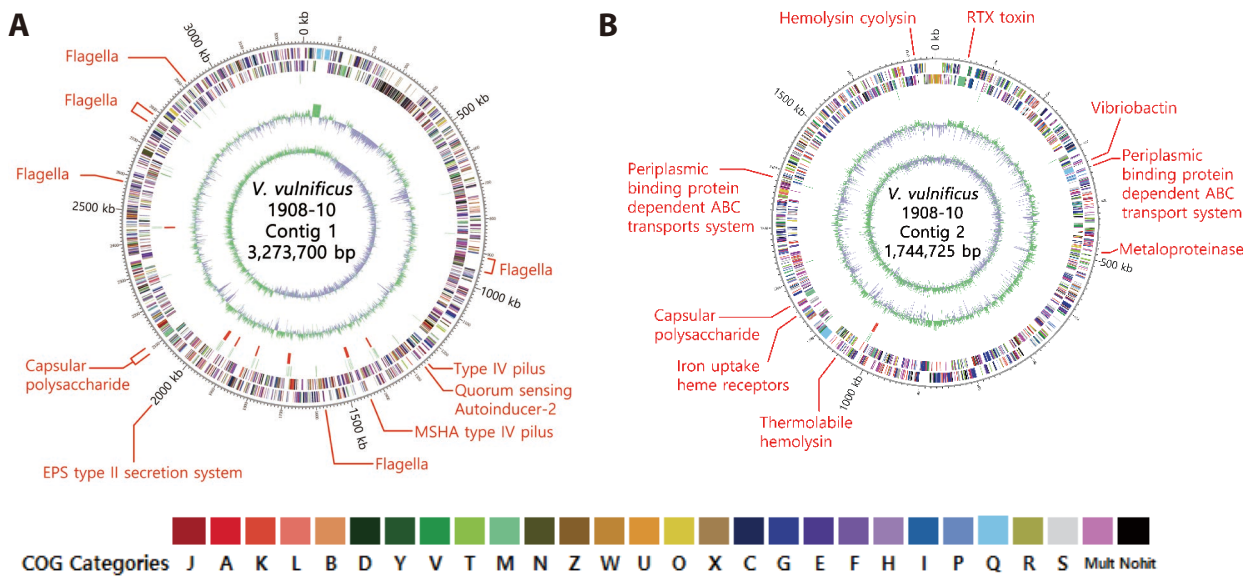


Fig. 3. Circular genome maps of *Vibrio vulnificus* 1908-10 based on COGs and VFDB analysis. A: Contig 1. B: Contig 2. Marked characteristics are shown from outside to the center; CDS on forward strand, CDS on the reverse strand, tRNA (green), rRNA (red), GC content and GC skew. All annotated ORFs were colored differently according to the COG assignments. COG, clusters of orthologous gene; VFDB, virulence factor database; CDS, coding sequence; GC, guanine-cytosine.

Strain name	Genome Size (Mb)	vcg	fur	hlyU	luxS	ompU	pilA	pilF	rtxA	rtxC	w/hA
1908-10	5.02	C									
07-2444	5.23	C									
1676-80	5.04	C									
2497-87	5.03	E									
93U204	5.13	C									
ATCC27562	5.01	E									
CECT4606	5.19	E									
CECT4999	5.16	E									
CECT7030	5.11	E									
CECT898	5.20	E									
CG100	5.21	C									
CladeA-yb158	5.29	C									
CMCP6	5.13	C									
Env1	4.95	E									
FDAARGOS_119	4.98	E									
FDAARGOS_663	4.97	E									
FORC_017	5.23	C									
FORC_036	6.07	C									
FORC_037	5.12	C									
FORC_053	6.02	E									
FORC_054	5.12	E									
FORC_077	5.02	C									
JY1305	4.95	E									
JY1701	4.94	E									
LSU1015	5.59	C									
MO6-24/O	5.01	C									
Vv180806	5.36	C									
VV2014DJH	5.07	E									
Vyb(BT3)	5.75	E									
YJ016	5.26	C									

Fig. 4. Comparison of virulence genes of *Vibrio vulnificus* 1908-10 and other *V. vulnificus* strains.

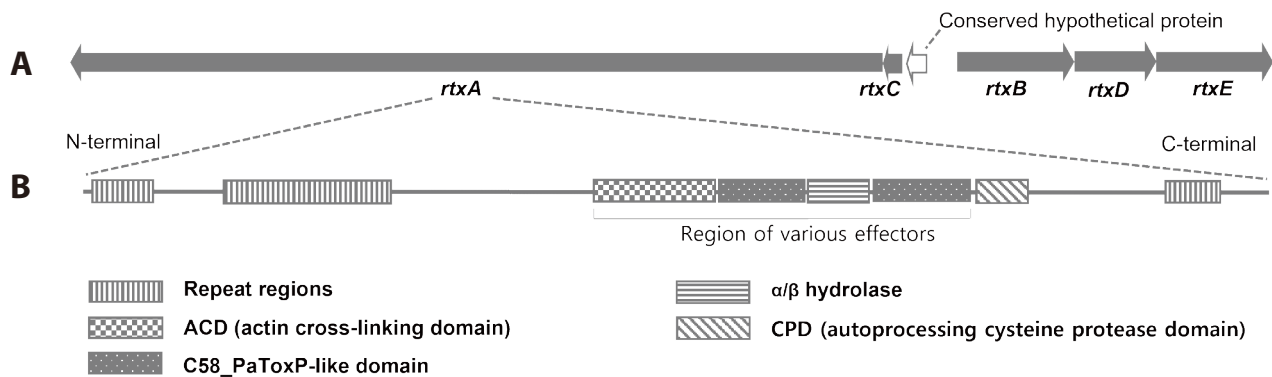


Fig. 5. *rtx* gene cluster (A) and domain organization of the MARTX toxin (B) of *Vibrio vulnificus* 1908-10. MARTX, multifunctional-autoprocessing repeats-in-toxin.

antibiotic resistance genes *crp*, *adeF*, *varG*, *parE*, and *ftsI* in this strain. Of these, *ftsI* was detected from a protein variant model associated with antibiotic target alteration in cephalosporins, but whether it was a factor of cefoxitin resistance in this strain requires further study (data not shown).

Genome analysis of *V. vulnificus* 1908-10 isolated from the eastern coast of Gadeok-do revealed that this strain was similar to C-genotype *V. vulnificus* in terms of ANI and VFs. MARTX toxin sequence was identified also. *V. vulnificus* is continuously threatening food hygiene and public health. Among strains that genomes have been reported in South Korea, there has been no report of isolates from riverine seawater. The genomic information of this strain can be used as basic data for the genome of the strain according to the isolation environment as well as understanding the genomic characteristics and virulence of *V. vulnificus*.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Availability of data and materials

Upon reasonable request, the datasets of this study can be avail-

able from the corresponding author.

Ethics approval and consent to participate

This study conformed to the guidance of animal ethical treatment for the care and use of experimental animals.

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