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Complete genome sequence of the biotype 1 *Vibrio vulnificus* isolated from diseased Pacific shortfin eel (*Anguilla bicolor pacifica*) cultured in Korea§

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국내 양식 동남아산 뱀장어 (*Anguilla bicolor pacifica*) 병어에서 분리된 biotype I *Vibrio vulnificus*의 전장 유전체 분석§

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Vibrio vulnificus is an important waterborne zoonotic pathogen that causes several life-threatening infections in humans and other aquatic animals. Here, we report the complete genome of indole-positive biotype 1 *V. vulnificus* strain GCU-01, isolated from a diseased Pacific shortfin eel (*Anguilla bicolor pacifica*) cultured in Korea. The genome consists of two circular chromosomes and three plasmids. Multilocus sequence typing analysis revealed the sequence type of strain GCU-01 was assigned to 502, and genes coding virulence, antimicrobial resistance, and prophages were also analyzed. *Tet(A)* encoding tetracycline efflux pump was detected in pVv_GCU-01-1, which was determined as a conjugative IncA/C plasmid widespread in *Enterobacteriaceae*. This indicates a possible association with increased antimicrobial resistance in aquaculture and public health. This work enhances our understanding of eel-pathogenic BT1 *V. vulnificus* and its management in the Korean eel culture and seafood industries.

Keywords: aquaculture, conjugative plasmid, multilocus sequence typing, ST 502, *tet(A)*

Vibrio vulnificus is a halophilic, Gram-negative bacteria that is common in estuarine and coastal waters worldwide (Oliver, 2006). Additionally, it is a zoonotic pathogen that infects both humans and aquatic animals, usually via the consumption of contaminated seafood or direct exposure to seawater or seafood products (Jones and Oliver, 2009). Infection in aquatic animals is usually termed “warm water vibriosis” and freshwater eels (mainly anguillid eel) are considered the most susceptible animals to this vibriosis (Austin and Austin, 2012). The anguillid eel is one of the most important fish species in global aquaculture, and a total of four species (*Anguilla japonica*, *A. rostrata*, *A. bicolor pacifica*, and *A. marmorata*) are currently farmed in

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Korea (Lee *et al*., 2015). Among those, the Pacific shortfin eel (*A. bicolor pacifica*) is the second preferred choice to *A. japonica* and is now considered an economically important species in terms of global market demand (Marini *et al*., 2021). Although the pathogenic and genomic characteristics of *V. vulnificus* infecting *A. japonica* are relatively well investigated (Amaro *et al*., 2015), those infecting *A. bicolor pacifica* have not been reported until yet.

Based on the biochemical characteristics, *V. vulnificus* is currently divided into three biotypes (BT1, BT2, and BT3), and the BT2 isolates are mainly considered the causative agent of eel vibriosis (Amaro *et al*., 2015). Although the indole-positive BT1 *V. vulnificus* is mainly associated with clinical infections in humans, it is responsible for several fatal infections in cultured eels (Amaro *et al*., 1995; Coleman *et al*., 1996; Haenen *et al*., 2014). Moreover, several studies have reported the occurrence of antimicrobial-resistant *V. vulnificus* strains isolated from Korean aquatic environments and clinical specimens (Yoon *et al*., 2020; Park *et al*., 2021) threatens public health and can lead to tremendous economic losses in the aquaculture industry. Therefore, the clinical importance of *V. vulnificus* has prompted the recent analyses of several genomes from Korean isolates (Chung *et al*., 2016; Oh *et al*., 2023). This study describes the first complete genome of the BT1 *V. vulnificus* isolated from a cultured *A. bicolor pacifica* in Korea.

Moribund juvenile Pacific shortfin eels (*A. bicolor pacifica*) with hemorrhagic dermal ulcerations of the skin and mouth were collected from a private eel culture farm (Gyeonggi-do, Republic of Korea) that experienced cumulative mortality in 2023. During the necropsy, congestion of the liver and enlarged kidneys with petechial hemorrhages were observed. The indole-positive representative strain GCU-01 was isolated from a liver abscess in a fish by overnight culture on tryptic soy agar (Difco) at 25°C. Based on the *gyrB* gene sequence analysis, the isolate exhibited 97.9% nucleotide similarity against *V. vlunificus* ATCC 27562^{T} (CP012881.1), a human-isolated BT1 type strain (Li *et al*., 2012). Therefore, the isolate was finally classified as BT1 *V. vulnificus* (Supplementary data Fig. S1).

The total genomic DNA of *V. vulnificus* strain GCU-01 was extracted using the DNeasy Blood & Tissue Kit (Qiagen) and sequenced using a hybrid approach, as previously described (Kwon *et al*., 2023). The PacBio RSII System (Pacific Biosciences) was used to construct a 20-kb SMRTbell^{TM} template library. The HiSeq X-10 platform (Illumina) was used to prepare a DNA library using the TruSeq Nano DNA Library Prep Kit (Illumina). The Microbial Genome Assembly application (http://www.pacb.com/products-and-services/pacbio-systems/sequel/sequel-software/) was used to assemble the PacBio long reads (413,033,630 bp; 50,013 reads) and Illumina paired-end reads (2,659,030,080 bp; 17,617,624 reads) for error correction using Pilon v1.21 (Walker *et al*., 2014). Genome annotation was performed using the National Center for Biotechnology Information Prokaryotic Genome Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/).

The sequenced GCU-01 genome comprised 5,239,895 bp (46.8% G + C content, 78× coverage), consisting of two circular chromosomes and three plasmids encoding 4,591 coding DNA sequences (CDSs), 111 tRNAs, 28 rRNAs, four noncoding RNAs, and 75 pseudogenes. Chromosome I is 3,216,867 bp in length (72× coverage) with a G + C content of 46.5%. Chromosome II is 1,761,186 bp in length (69× coverage) with a G + C content of 47.0%. Plasmid pVv_GCU-01-1 is 126,955 bp in length (131× coverage) with 50.5% G + C content. Plasmid pVv_GCU-01-2 is 77,318 bp in length (382× coverage) with 43.5% G + C content. Finally, the plasmid pVv_GCU-01-3 was 57,569

Table 1. General genomic features of *Vibrio vulnificus* strain GCU-01

Features	Value
Genome size (bp)	5,239,895
Chromosome I	3,216,867
Chromosome II	1,761,186
Plasmid (pVv_GCU-01-1)	126,955
Plasmid (pVv_GCU-01-2)	77,318
Plasmid (pVv_GCU-01-3)	57,569
G + C content (%)	46.8
Coverage (total)	78 ×
No. of contigs	5
Chromosomes	2
Plasmids	3
Total genes	4,734
Protein coding genes	4,516
tRNAs	111
rRNAs	28
ncRNAs	4
Pseudogenes	75

bp in length (170× coverage) with 45.5% G + C content (Table 1).

The circular chromosomes I and II of the strain GCU-01 were compared with the six representative *V. vulnificus* strains of the three biotypes using BLAST Ring Image Generator (BRIG) software (Alikhan *et al*., 2011) (Fig. 1A). The genomic similarities between GCU-01 and those strains were further assessed using the Orthologous ANI tool software (Lee *et al*., 2016) (Fig. 1B). Comparative genome analysis revealed that the strain GCU-01 exhibited high genetic relatedness to several clinical BT1 *V. vulnificus* strains. The OrthoANI analyses also confirmed that chromosome I of GCU-01 showed the highest ANI value (98.4%) with strain CMCP6 (AE016795.3) and chromosome II displayed the highest ANI value (98.2%) with strain FORC_017 (CP012739.1) (Oh *et al*., 2023). These findings strongly suggest that the newly-isolated strain GCU-01 belongs to biotype 1, which is typically associated with human infections.

Multilocus sequence typing (MLST) was performed to determine the sequence type (ST) of *V. vulnificus* strain GCU-01. A total of 10 house-keeping genes (*dtds*, *glp*, *gyrB*, *lysA*, *mdh*, *metG*, *pntA*, *purM*, *pyrC*, and *tnaA*) of the sequenced genome were compared *in silico* with those available in the PubMLST database (https://pubmlst.org/organisms/vibrio-spp) and their respective allele numbers and ST were generated. The allele profile of strain GCU-01 was 149-22-3-148-97-1-37-20-3-109 and its ST was assigned to 502.

Potential virulence- and antimicrobial resistance-associated genes in GCU-01 were screened as described previously (Kwon *et al*., 2023). Among the major virulence factors of the species, *V. vulnificus hemolysin* (*vvhA*) and metalloproteinase (*vvp*) were detected on chromosome II, and clusters of RTX toxins (*rtxA, rtxB,* and *rtxD*) were detected in the plasmid pVv_GCU-01-2 (Supplementary data Table S1). Moreover, *vep07*, a conserved sequence associated with eel serum resistance in eel-pathogenic BT2 *V. vulnificus* (Amaro *et al*., 2015), was observed in the plasmid pVv_GCU-01-2. In addition, *tet(A)* (tetracycline efflux pump; locus_tag: MUN71_RS19745) was detected in the plasmid

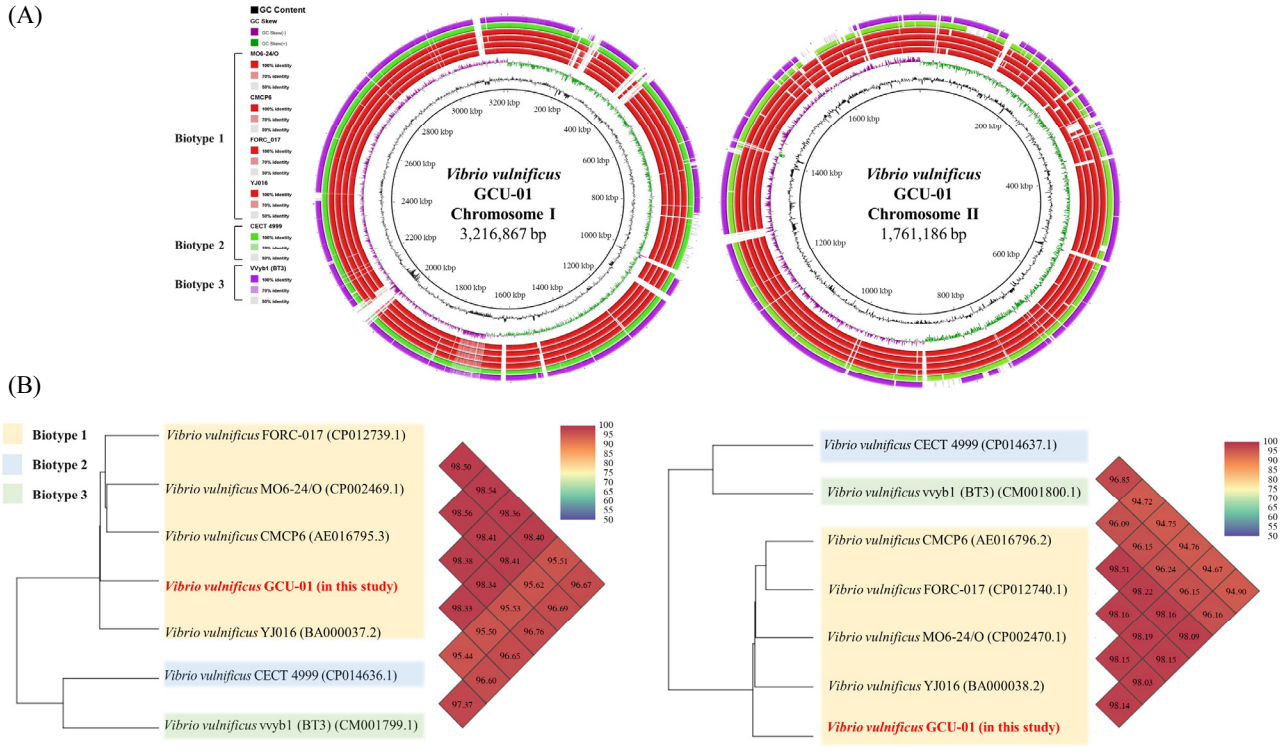

Fig. 1. Overall genome relatedness (A) and the Orthologous ANI value-based heatmaps (B) determined using the chromosomes I (left) and II (right) of strain GCU-01 against the representative six *Vibrio vulnificus* strains of the three biotypes. Each chromosome was aligned and visualized based on BLAST identity using BLAST Ring Image Generator (BRIG) software (Alikhan *et al*., 2011). The ANI values were calculated and heatmaps were visualized using OrthoANI tool software (Lee *et al*., 2016), respectively.

pVv_GCU-01-1 with >99.8% amino acid sequence identity (Supplementary data Table S2). Interestingly, the plasmid pVv_GCU-01-1 showed high genetic similarity (> 99.9%) to conjugative plasmids of *Enterobacteriaceae* (*Escherichia*, *Klebsiella*, and *Proteus*) which were reported to be transferrable between different genera and can encode multiple antimicrobial resistance genes (Zhang *et al*., 2020). Plasmid MLST (https://pubmlst.org/organisms/plasmid-mlst) using genes *repA, parA, parB*, and *A053* has classified the plasmid pVv_GCU-01-1 into the A/C incompatibility group sequence type 3, which was previously reported in aquatic bacteria including *Aeromonas*, *Vibrio*, and *Photobacterium* spp. (Kim *et al*., 2008; Folster *et al*., 2014; Vincent *et al*., 2014). These findings strongly suggest that the *tet(A)* gene in *V. vulnificus* strain GCU-01 might be transferred via a conjugative plasmid to other-related aquatic bacterial pathogen and could be associated with tetracycline-related antimicrobial resistance in the aquaculture industry and public health. Moreover, a total of four prophage regions were identified in the GCU-01 chromosome using the PHASTER (Phage Search Tool Enhanced Release) server (Arndt *et al*., 2016), one of which was intact (score >90) and three incomplete (score <70) (Supplementary data Table S3). The intact prophage region (Region 1; 5.3 kb) observed on chromosome II was similar to *Vibrio* phage Vf12 (NC_005949).

Although BT2 of *V. vulnificus* is mainly considered the causative agent of eel vibriosis, we first confirmed that BT1, which is generally associated with human clinical infections, could pose lethal infections in Korean eel culture farms, causing cumulative mortality. These results provide important insights into the development of control strategies against *V. vulnificus,* which causes serious economic losses in the Korean aquaculture industry and public health. This is the first report of the BT1 genome of *V. vulnificus* isolated from a Korean eel culture farm. Genome-based investigations of recent Korean *V. vulnificus* isolates are urgently required to ensure the safety of fishery products.

Nucleotide sequence accession number

Vibrio vulnificus strain GCU-01 was deposited in the Korean Collection for Type Cultures (KCTC) under the deposition number KCTC 8599. The complete genome sequences of the strain GCU-01 were deposited in the GenBank database under accession numbers NZ_CP134783.1 (chromosome I), NZ_CP134784.1 (chromosome II), NZ_CP134785.1 (pVv_GCU-01-1), NZ_CP134786.1 (pVv_GCU-01-2), and NZ_CP134787.1 (pVv_GCU-01-3).

적 요

*Vibrio vulnificus*는 해양 환경에 상재하며 사람 및 수생동물에서 치명적인 감염을 일으키는 인수공통 병원성 세균으로, 보고된 세 종류의 biotype 중 BT1은 인간 패혈증을 BT2는 주로 장어에서 비브리오증(vibriosis)을 유발한다. 본 연구에서는 국내 양식 동남아산 뱀장어(*Anguilla bicolor pacifica*) 병어에서 분리한 BT1 *V. vulnificus* GCU-01 균주의 전장 유전체를 분석하였다. GCU-01 균주는 두 개의 염색체와 세 개의 플라스미드(pVv_GCU-01-1, pVv_GCU-01-2, pVv_GCU-01-3)으로 구성되었다. 다좌위 서열 형별 분석(MLST) 결과 GCU-01 균주는 502 타입으로 분류되었으며, 유전체 내에서 다양한 병원성 인자, 항생제 내성 유전자 및 prophage region의 존재가 확인되었다. 흥미롭게도, 접합성 Inc A/C형 플라스미드로 확인된 pVv_GCU-01-1에서 tetracycline efflux pump를 암호화하는 *tet(A)* 유전자가 검출되었다. 본 연구 결과는 국내 양식 산업 및 식품 안전 분야에서 인수공통 병원성 BT1 *V. vulnificus*를 이해하고 관리 방안을 개발하는 데 기여할 것이다.

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Conflict of Interest

All authors disclose no conflicts of interest relevant to this research.

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