



Complete genome sequence of pathogenic *Vibrio alginolyticus* EBVib0155 isolated from seawater, South Korea

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해수에서 분리된 병원성 *Vibrio alginolyticus* EBVib0155의 전장 유전체 서열 분석

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Vibrios are a group of Gram-negative bacteria known for causing food poisoning outbreaks globally. This study unveils the complete genome sequence of *Vibrio alginolyticus* strain EBVib0155, isolated from seawater in the southeast sea of South Korea. The complete genome of *Vibrio alginolyticus* strain EBVib0155 comprises two chromosomal DNA. The first chromosome spans 3,324,308 bp, while the second chromosome spans 1,889,638 bp, with 45.4% and 45.3% GC content, respectively. Gene prediction analysis revealed 2,985 CDSs, 118 tRNAs and 34 rRNAs in the first chromosome, along with 1,690 CDSs, 14 tRNAs and 3 rRNAs in the second chromosome.

Keywords: *Vibrio alginolyticus*, complete genome, food poisoning

Vibrios are a group of Gram-negative bacteria commonly associated with food poisoning, typically found in marine, estuarine, and coastal environments (Oliver *et al.*, 2013; Letchumanan *et al.*, 2014). Strains of *Vibrio* spp. are often linked to waterborne gastroenteritis, primarily associated with the consumption of raw or undercooked seafood (McLaughlin *et al.*, 2005). These bacteria have a significant history of public health concern, contributing to major food poisoning

outbreaks on a global scale (Hasan *et al.*, 2015; Onohuean *et al.*, 2022), notably affecting shellfish harvesting environments or shellfish such as oysters and clams (CDC, 1999). *Vibrio* species within the Vibrionaceae family (Letchumanan *et al.*, 2014) is frequently present in shellfish and marine environment. *Vibrio parahaemolyticus* is known to possess two well-known hemolytic virulence factors, thermostable direct hemolysin (tdh) and TDH-related hemolysin (trh). These pore-forming proteins contribute to the bacterium's invasiveness in humans (Raghunath, 2015). In this study, we investigated the isolation of a unique *Vibrio alginolyticus* strain possessing virulence factors commonly associated with pathogenic *Vibrio*, particularly those found in *Vibrio parahaemolyticus*.

The *Vibrio alginolyticus* strain EBVib0155 was isolated from seawater adjacent to Mireuk-do in southeast sea (Gyeongsangnam-do province) of South Korea. Genomic DNA extraction for EBVib0155 was conducted using a Bacterial Genomic DNA Extraction Kit Ver. 3.0 (Takara, MiniBEST), and genome sequencing was performed using the PacBio Sequel sequencing platform. The high-quality genomic DNA was sheared using a G-tube device (Covaris), and the sheared DNA was used to prepare a PacBio library. The library was then sequenced using a single-molecule real-time (SMRT)

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Table 1. Genome features of *Vibrio alginolyticus* EBVib0155

Feature	Chromosome 1 (EBVib0155C1)	Chromosome 2 (EBVib0155C2)
Size (bp)	3,324,308	1,889,638
G + C contents (%)	45.4%	45.3%
Total number of CDSs	2,985	1,690
rRNA genes (5S, 16S, 23S)	34 (12, 11, 11)	3 (1, 1, 1)
tRNA genes	118	14
Pseudo genes	570	332
GenBank Accession No.	CP128370	CP128371

sequencing system, resulting in a total of 583,475 sequence reads with an N50 value of 10,129, and a mean length of 8,599 that were assembled into 2 contigs. The raw sequence reads were *de novo* assembled using HGAP, resulting in a final assembly with a coverage (depth) of 962X. Additionally, the resulting asymmetric structure was validated using Circlator (version 1.5.5) to ascertain its circular or linear form. Genome annotation was performed using the NCBI prokaryotic genome annotation pipeline (Tatusova *et al.*, 2016).

The genome of *Vibrio alginolyticus* strain EBVib0155 comprises two chromosomal DNA. The first chromosome spans 3,324,308 bp, while the second chromosome spans 1,889,638 bp, with 45.4% and 45.3% GC content, respectively. Gene prediction analysis revealed 2,985 CDSs, 118 tRNAs and 34 rRNAs in the first chromosome, along with 1,690 CDSs, 14 tRNAs and 3 rRNAs in the second chromosome (Table 1).

The study of bacteria with multiple virulence factors is important. As in the case of the food poisoning bacteria that emerged in Germany in 2011 and subsequently spread throughout Europe and North America (Karch *et al.*, 2012), it was identified as a hybrid strain with one or more pathogenic factors. The *Vibrio alginolyticus* strain EBVib0155 isolated in this study, the *in silico* Virulence Factor Database (Liu *et al.*, 2019) identified virulence and related genes (Fig. 1), including thermostable direct hemolysin (tdh, locus tag: QSJ11_19040) and TDH-related hemolysin (trh, locus tag: QSJ11_20010). This finding is unusual, as in the case of *Vibrio alginolyticus*, it is rare for it to possess virulence factors such as tdh and trh. Furthermore, these virulence factors are primarily associated

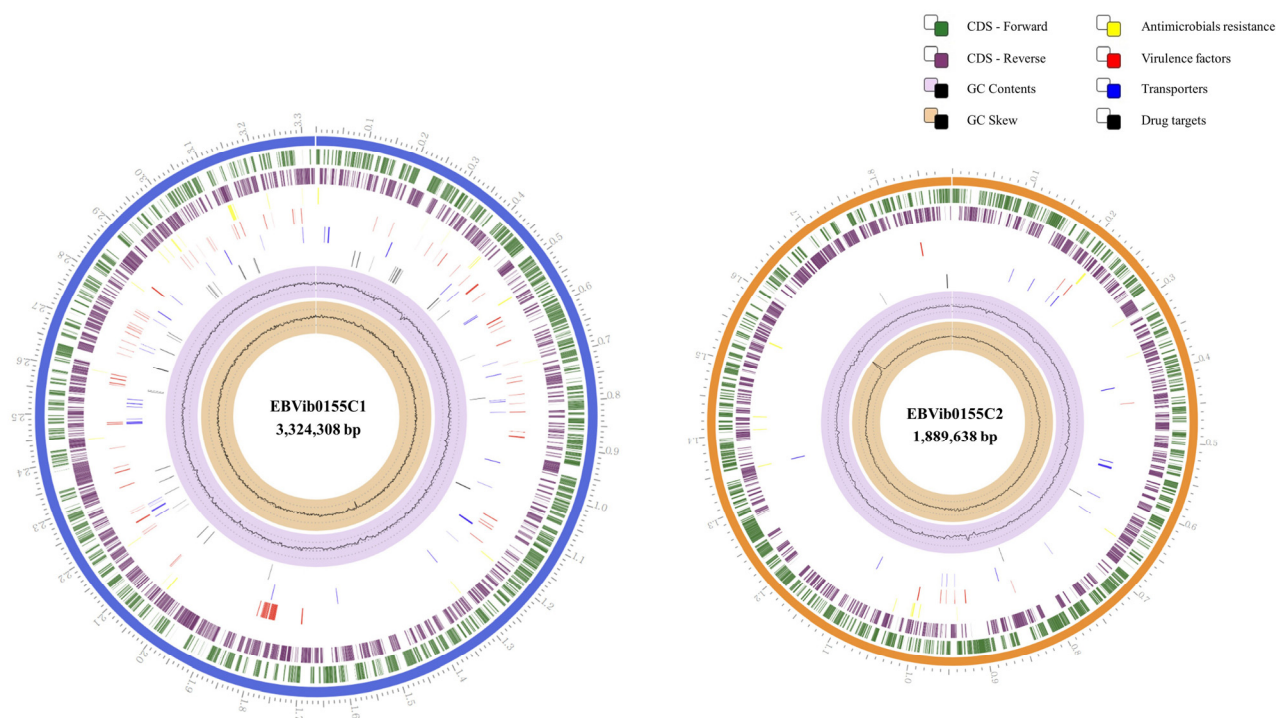


Fig. 1. Genome map of pathogenic *Vibrio alginolyticus* EBVib0155. The graphical circular maps are shown of the alignment of genes and other genomic data. The tracks on the viewer are displayed as concentric rings, from outermost to innermost: Forward CDS, Reverse CDS, GC Skew, GC contents, AMR genes, VF genes, transporters and drug target. Genes with specialized functions labeled with different colors as virulence-related genes in red, and other functional genes such as antimicrobials resistance, transporters, drug target as yellow, blue, and black.

with genes found in *Vibrio parahaemolyticus*. Understanding the genetic makeup and virulence factors of food poisoning bacteria is crucial for public health and food safety. This information can contribute to the development of strategies aimed at mitigating the risks associated with its consumption and preventing outbreaks of foodborne illnesses.

Nucleotide sequence accession number(s)

Nucleotide sequence accession numbers. The complete genome sequence of *Vibrio alginolyticus* EBVib0155 has been deposited at the NCBI GenBank database under the accession numbers CP128370 (chromosome 1, EBVib0155C1), CP128371 (chromosome 2, EBVib0155C2) and the strain has been deposited in the Korean Culture Collection of Aquatic Microorganisms (KoCAM) under the strain number EBVib0155.

적 요

비브리오는 전 세계적으로 식중독 발병을 일으킬 수 있는 그람 음성 세균이다. 본 연구에서 분리된 균주는 대한민국 남동해 해역 해수에서 분리된 *Vibrio alginolyticus* EBVib0155 균주로 전장 유전체 서열 분석을 실시하였다. *Vibrio alginolyticus* EBVib0155의 전장 유전체 분석결과 두 개의 염색체가 확인되었으며, 첫 번째 염색체는 3,324,308 bp, 두 번째 염색체는 1,889,638 bp로 각 45.4% 및 45.3%의 GC 함유량이 확인되었다. 또한, 유전자 예측 분석에 따르면 첫 번째 염색체에는 2,985개의 CDSs, 118개의 tRNA 및 34개의 rRNA, 두 번째 염색체에는 1,690개의 CDSs, 14개의 tRNA 및 3개의 rRNA로 확인되었다.

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Conflict of Interest

The authors have no conflict of interest to report.

References

- CDC, Centers for Disease Control and Prevention.** 1999. Outbreak of *Vibrio parahaemolyticus* infection associated with eating raw oysters and clams harvested from Long Island Sound-Connecticut, New Jersey, and New York, 1998. *MMWR Morb. Mortal. Wkly. Rep.* **48**, 48–51.
- Hasan NA, Grim CJ, Lipp EK, Rivera IN, Chun J, Haley BJ, Taviani E, Choi SY, Hoq M, Munk AC, et al.** 2015. Deep-sea hydrothermal vent bacteria related to human pathogenic *Vibrio* species. *Proc. Natl. Acad. Sci. USA* **112**, E2813–E2819.
- Karch H, Denamur E, Dobrindt U, Finlay BB, Hengge R, Johannes L, Ron EZ, Tønjum T, Sansonetti PJ, and Vicente M.** 2012. The enemy within us: lessons from the 2011 European *Escherichia coli* O104:H4 outbreak. *EMBO Mol. Med.* **4**, 841–848.
- Letchumanan V, Chan KG, and Lee LH.** 2014. *Vibrio parahaemolyticus*: a review on the pathogenesis, prevalence, and advance molecular identification techniques. *Front. Microbiol.* **5**, 705.
- Liu B, Zheng DD, Jin Q, Chen LH and Yang J.** 2019. VFDB 2019: a comparative pathogenomic platform with an interactive web interface. *Nucleic Acids Res.* **47**, D687–D692.
- McLaughlin JB, DePaola A, Bopp CA, Martinek KA, Napolilli NP, Allison CG, Murray SL, Thompson EC, Bird MM, and Middaugh JP.** 2005. Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. *N. Engl. J. Med.* **353**, 1463–1470.
- Oliver JD, Pruzzo C, Vezzulli L, and Kaper JB.** 2013. *Vibrio* species, pp. 401–440. In Doyle MP and Buchanan RL (eds.), *Food Microbiology: Fundamentals and Frontiers*. ASM Press, Washington DC, USA.
- Onohuean H, Agwu E, and Nwodo UU.** 2022. A global perspective of *Vibrio* species and associated diseases: three-decade meta-synthesis of research advancement. *Environ. Health Insights* **16**. <https://doi.org/10.1177/11786302221099406>.
- Raghunath P.** 2015. Roles of thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) in *Vibrio parahaemolyticus*. *Front. Microbiol.* **5**, 805.
- Tatusova T, DiCuccio M, Badretdin A, Chetverin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, and Ostell J.** 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res.* **44**, 6614–6624.