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# Complete genome sequence of an Acute Hepatopancreatic Necrosis Disease (AHPND)-causing *Vibrio parahaemolyticus* 19-021-D1 isolated from cultured pacific white shrimp (*Penaeus vannamei*) at 2019 in Korea§

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# 국내 양식 흰다리새우(*Penaeus vannamei*)에서 분리된 급성간췌장괴사증후군(AHPND) 유발 *Vibrio parahaemolyticus* 19-021-D1의 전장 유전체 분석§

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*Vibrio parahaemolyticus* is one of the most important seafood-borne pathogens that cause several life-threatening infections or large food poisoning outbreaks. Some strains of species harboring the pVA1-type plasmid containing the two toxin subunit genes (*pirA* and *pirB*) are the major etiological agents of acute hepatopancreatic necrosis disease (AHPND), causing significant economic losses in the global shrimp aquaculture industry. Previously, we reported the first complete genome of the pVA1-type plasmid (pVp_Kor-D1-2) of *V. parahaemolyticus* 19-021-D1, which caused a severe AHPND outbreak in cultured pacific white shrimp (*Penaeus vannamei*) at 2019 in Korea. Here, we present the whole genomic characteristics of the bacterial chromosomes and plasmids of strain 19-021-D1. The genome consisted of two circular chromosomes and two plasmids (including pVp_Kor-D1-1 and pVp_Kor-D1-2). Several genes encoding virulence, antimicrobial resistance, and prophage regions were detected in the 19-021-D1 genome. Multilocus sequence typing analyses identified the sequence type of strain 19-021-D1 as 413 and revealed that the Korean isolate may be associated with those collected from cultured shrimp from different geographical regions. This genomic information provides important insights into control strategies against the prevalent AHPND-causing *V. parahaemolyticus* in Korean aquaculture and seafood industries.

**Keywords:** multilocus sequence typing, pacific white shrimp (*Penaeus vannamei*), pVA1-type plasmid, ST 413

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*Vibrio parahaemolyticus* is a halophilic gram-negative bacterium widely found in aquatic environments, including estuarine, marine, and coastal ecosystems (Ceccarelli *et al*., 2013). This species is a leading causative agent of foodborne human acute gastroenteritis, which can be transmitted by the consumption of uncooked, undercooked, or mishandled marine products (Newton *et al*., 2012). Specific antigen types of virulent *V. parahaemolyticus* cause several life-threatening infections and large food poisoning outbreaks. Moreover, most virulent isolates have acquired resistance to several antimicrobial agents, which could be an important reservoir for various antimicrobial resistance (AMR) genes (Letchumanan *et al*., 2014). Therefore, *V. parahaemolyticus* poses a risk to public health and food safety.

Moreover, *V. parahaemolyticus* is the major etiological agent of acute hepatopancreatic necrosis disease (AHPND or early mortality syndrome), which causes significant economic losses in the global shrimp aquaculture industry. The AHPND-causing *V. parahaemolyticus* ($Vp_{AHPND}$), which produces the PirAB toxin, is characterized by the presence of a large (~70 kb) conjugative plasmid (pVA1) containing two toxin subunit genes (*pirA* and *pirB*) (Lee *et al*., 2015). Since its first outbreak in China in 2009, AHPND has been reported in several countries in the Americas and Asia, including Korea, and has caused serious economic losses up to recently (Han *et al*., 2020). Previously, we reported the first complete genome of the pVA1-type plasmid (pVp_Kor-D1-2) of $Vp_{AHPND}$ 19-021-D1, which caused a severe AHPND outbreak in 2019 among cultured shrimp in Korea (Han *et al*., 2020). In this study, we present the whole genomic characteristics of the bacterial chromosomes and plasmids of $Vp_{AHPND}$ 19-021-D1.

In our previous study, $Vp_{AHPND}$ 19-021-D1 was isolated from the culture pond water of pacific white shrimp (*Penaeus vannamei*) with AHPND (Han *et al*., 2020). The total genomic DNA of $Vp_{AHPND}$ 19-021-D1 was extracted using the DNeasy Blood & Tissue Kit (Qiagen) and sequenced via a hybrid approach: (1) using the PacBio RSII System (Pacific Biosciences) by constructing a 20-kb SMRTbell™ template library and (2) using the HiSeq X-10 platform (Illumina) by preparing a DNA library using the TruSeq Nano DNA Library Prep Kit (Illumina). The PacBio reads (1,387,556,996 bp; 141,754 reads) were *de novo* assembled with the HGAP (v3.0) (Chin *et al*., 2013). Then, the Illumina paired-end reads (898,634,220 bp, 5,951,220 reads) were mapped to the PacBio assembled genome using BWA-MEM v0.7.17 (Li and Durbin, 2009), and the final error correction was conducted with Pilon v1.21 (Walker *et al*., 2014). Genome annotation was performed using the NCBI prokaryotic genome annotation pipeline (http://www.ncbi.nlm.nih.gov/books/NBK174280/).

The sequenced genome of strain 19-021-D1 consisted of two circular chromosomes (encoding 4,923 CDS, 133 tRNAs, 37 rRNAs, and four ncRNAs) and two plasmids (pVp_Kor-D1-1 and pVp_Kor-D1-2) (encoding 200 CDS). Chromosome I was 3,577,848 bp in length (145× coverage) with 45.15% G + C content. Chromosome II was 1,814,246 bp in length (132× coverage) with 45.65% G + C content. The plasmid pVp_Kor-D1-1 was 118,438 bp in length (310× coverage), with 44.29% G + C content. Lastly, the pVA1-type plasmid pVp_Kor-D1-2 was 68,848 bp in length (176× coverage) with 45.81% G + C content (Fig. 1 and Table 1).

Potential virulence-associated genes in the 19-021-D1 strain were compared with those of *V. parahaemolyticus* strains available in the GenBank database and additional virulence- and AMR-related genes (ARGs) were screened as previously described (Lee *et al*., 2018). Among the three major virulence factors in this species, the 19-021-D1 genome possessed only thermolabile hemolysin (TLH) on chromosome II. On the other hand, thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH), which are responsible for human gastroenteritis (Liston, 1990), were not detected (Supplementary data Table S1). Although the $Vp_{AHPND}$ 19-021-D1 showed no significant phenotypical resistance against several antimicrobial agents of the guidelines of the Clinical and Laboratory Standards Institute (Han *et al*., 2020), several ARGs were detected by Resistance Gene Identifier on the criteria of 'perfect and strict' in the Comprehensive Antibiotic Resistance Database (Alcock *et al*., 2020) in the chromosomes of strain 19-021-D1. Overall, the ARGs in strain 19-021-D1 were very similar to those reported in *V. parahaemolyticus* Vp2015094, which was isolated from maricultured shellfish in China (Wang *et al*., 2022); (i) three efflux pump genes, *adeF* (fluoroquinolone and tetracycline resistance), *crp* (macrolide, fluoroquinolone, and penam resistance), and *txR* (tetracycline resistance); and (ii) the antibiotic inactivation gene $bla_{CARB\text{-}18}$ (penam resistance). However, one antibiotic target alteration gene, *vanT* (glyco-

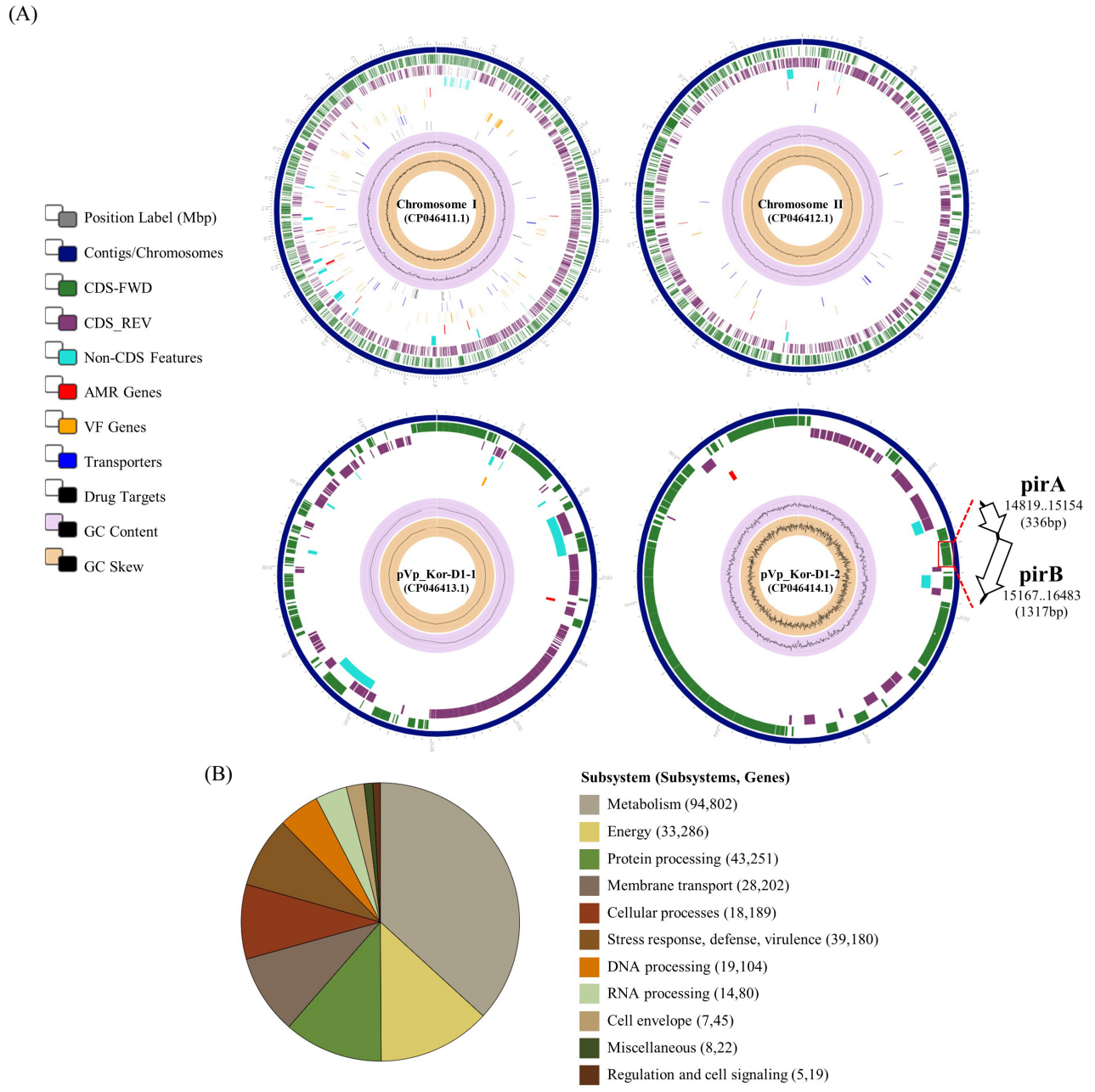


**Fig. 1. The genome map of *Vibrio parahaemolyticus* 19-021-D1.** A circular graphical genome map (A) of strain 19-021-D1 and its subsystems (B) were respectively annotated and generated by using the Pathosystems Resource Integration Center (PATRIC) server v.3.6.12 (Wattam *et al.*, 2017).

peptide antibiotic resistance), was detected only in strain 19-021-D1. Additionally, none of the ARGs was detected in the two plasmids (Supplementary data Table S2).

Eight prophage regions were identified in the 19-021-D1 chromosome (six in chromosome I and two in chromosome II) using the Phage Search Tool Enhanced Release (PHASTER) server (Arndt *et al.*, 2016), one of which was intact (score > 90), and five were incomplete (score < 70). The only intact prophage region of the 19-021-D1 genome was detected in chromosome II consisted of 11.6 kb (Region 8) and showed significant similarity with *Vibrio* phage vf33 (NC_005948) and CTX (NC_015209) (Supplementary data Table S3).

Moreover, we performed multilocus sequence typing (MLST) analyses to determine the sequence type (ST) of $Vp_{AHPND}$

**Table 1. The general genome features of AHPND-causing *V. parahaemolyticus* strain 19-021-D1**

| Features | Value |
|---|---|
| Genome size (bp) | 5,579,380 |
| Chromosome I | 3,577,848 |
| Chromosome II | 1,814,246 |
| Plasmid (pVp_Kor-D1-1) | 118,438 |
| Plasmid (pVp_Kor-D1-2) | 68,848 |
| G + C content (%) | 45.09 |
| No. of contigs | 4 |
| Plasmid | 2 |
| Total genes | 5,297 |
| Protein-coding genes | 5,052 |
| rRNAs | 37 |
| tRNAs | 133 |
| ncRNAs | 4 |
| Pseudogenes | 71 |

19-021-D1. A total of seven housekeeping gene (*dnaE*, *gyrB*, *recA*, *dtdS*, *pntA*, *pyrC*, and *tnaA*) sequences of the sequenced genome were compared *in silico* with those available in the PubMLST database (https://pubmlst.org/organisms/vibrio-spp) and its respective allele numbers and ST were generated. The allele profile of the $Vp_{AHPND}$ 19-021-D1 was 47-8-166-19-28-46-121, and its ST was finally assigned to 413. Interestingly, we found six other strains of ST 413 *V. parahaemolyticus* in the PubMLST database, all of which were isolated from diseased or unknown shrimp products of different geographical origins (including South Korea, China, Thailand, Vietnam, and the USA) (Supplementary data Table S4).

Therefore, we conducted the genome-based comparison of the strain 19-021-D1 against the available genomes of three ST 413 strains and other three $Vp_{AHPND}$ strains using the OAT software (Lee *et al.*, 2016). The results indicated that the chromosomes of strain 19-021-D1 were clustered with all the tested *V. parahaemolyticus* strains, and the four ST 413 strains were nested together within the clade showing high orthologous average nucleotide identity values (≥ 99) (Supplementary data Fig. S1). Although detailed information on diseased shrimp samples is not currently available, it can be speculated that ST 413 *V. parahaemolyticus* is strongly associated with culturing shrimps, and some strains might be involved in the onset of the disease in shrimp. However, further research is required to verify the potential relationship between ST 413 *V. parahaemolyticus* and AHPND. Based on the genetic characterization of $Vp_{AHPND}$ that occurred in Korea in 2019, we confirmed that this shrimp-specific pathogen could pose a threat to public health including food safety due to the presence of TLH. These results provide important insights for the development of strategies to control $Vp_{AHPND}$ which has caused serious economic losses in the Korean shrimp culture industry. This is the first genome report of $Vp_{AHPND}$ in Korea, and further genome-based investigation of recent Korean $Vp_{AHPND}$ isolates is urgently required to ensure the safety of shrimp products.

## Nucleotide sequence accession number

The complete genome sequences of $Vp_{AHPND}$ 19-021-D1 have been deposited in the GenBank database under accession numbers NZ_CP046411.1 (chromosome I), NZ_CP046412.1 (chromosome II), NZ_CP046413.1 (pVp_Kor-D1-1), and NZ_CP046414.1 (pVp_Kor-D1-2).

## 적 요

수산물 유래 식중독 세균인 *Vibrio parahaemolyticus*는 전 세계 공중보건과 식품위생에 심각한 위해를 야기하고 있다. 더불어, *pirA* 및 *pirB* 독성 유전자를 암호화하는 플라스미드(pVA1)를 보유하는 특정 *V. parahaemolyticus* 균주는 양식 새우의 급성간췌장괴사증(AHPND)을 유발하여 전세계 새우 양식산업에 심각한 경제적 손실을 야기하는 주 병원체로 알려져 있다. 이전 연구에서, 2019년도에 최초로 국내 양식 흰다리새우(*Penaeus vannamei*)에서 분리한 AHPND 발병 균주, *V. parahaemolyticus* 19-021-D1의 플라스미드(pVA1) 완전 유전체를 보고한 바 있으며, 본 연구에서는 19-021-D1 균주의 전장유전체 분석을 통해 병원성 및 항생제 내성 관련 유전적 특성을 분석하였다. 분석 결과, 19-021-D1 균주는 두 개의 원형 염색체와 두 개의 플라스미드로 구성되며, 병원성, 항생제 내성, prophage region과 관련이 있을 것으로 추정되는 다양한 유전자가 검출되었다. 다좌위 서열 형별 분석(MLST) 결과 19-021-D1 균주는 413타입으로 분류되었으며, 흥미롭게도, 기존 다른 국가의 양식 새우에서 분리된, 질병과 연관된 것으로 추정되는 균주들과 동일한 타입으로 분류되었다. 이러한 연구 결과는 국내 수산양식 산업 및 식품 공중보건 분야에서 문제를 야기하는 *V. parahaemolyticus*를 이해하고 제어하기 위한 중요한 기반자료로 활용될 것이다.

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## Conflict of Interest

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

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