Journal of Global Antimicrobial Resistance 26 (2021) 323–325

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Draft genome of a multidrug and multi-heavy metal resistant *Vibrio parahaemolyticus* ST165 strain of *Penaeus vannamei* from seawater farms in Zhejiang, China ☆

Jiehong Fang[a], Xue Jiao[a], Hui Cheng[b], Biao Tang[c], Wen Wang[c], Ting Yu[a], Han Jiang[a,*]

[a] *Key Laboratory of Marine Food Quality and Hazard Controlling Technology of Zhejiang Province, College of Life Sciences, China Jiliang University, Hangzhou, Zhejiang 310018, China*

[b] *Research and Develop Department, Hangzhou Wahaha Group Co. Ltd., Hangzhou, Zhejiang 310018, People's Republic of China*

[c] *State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products & Institute of Agro-product Safety and Nutrition, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China*

ARTICLE INFO

Article history:
Received 4 May 2021
Accepted 14 July 2021
Available online 5 August 2021

Editor: Mr Marc Stegger

Keywords:
Vibrio parahaemolyticus
Multidrug and multi-heavy metal resistance
Genomics

ABSTRACT

Objectives: *Vibrio parahaemolyticus* ST165 strain (FB-11) was first isolated in 2019 in China from *Penaeus vannamei* from seawater farms. The strain includes multidrug resistant (MDR) and multi-heavy metal resistant (MHMR) phenotypes. In this study, we aimed to determine the draft genome sequence of strain FB-11 and analyze the genetic features with a special focus on MDR and MHMR genes.

Methods: The genomic DNA was sequenced using Nanopore PromethION and the Illumina Novaseq6000 platform, and the reads were de novo assembled into contigs using Unicycler. Genome function elements were predicted, the coding sequences were annotated, and whole-genome sequencing (WGS) analysis was performed.

Results: WGS analysis revealed that the genome comprised two chromosomes of 3,328,286 bp (GC content, 45.37%) and 1,805,825 bp (GC content, 45.36%). It harbored 26 important drug resistant genes and six important heavy metal resistant genes; all of these were located on the chromosomes. Multilocus sequence type of the strain was ST165.

Conclusions: This is the first report of the complete genome sequence of a *V. parahaemolyticus* ST165 strain isolated from *P. vannamei* in China. This genome sequence provides useful information on the genomic features associated with antimicrobial and heavy metal resistance in *V. parahaemolyticus* ST165.

Vibrio parahaemolyticus is a widespread bacterium in the marine environment and one of the leading causes of seafood-borne bacterial poisoning worldwide [1]. Along with rapid industrialization and urbanization, the inappropriate use of antibiotics in aquaculture has contributed to the development of drug resistant bacteria including *V. parahaemolyticus* [2]. Additionally, heavy metals pollutants have frequently been detected in marine animals and related environments in recent years. Heavy metals have been suggested to enhance selection for antibiotic resistance in the environment and vice versa through co- or cross-resistance pathways [3]. Here, we report the first complete genome sequence of a multidrug resistant (MDR) and multi-heavy metal resistant (MHMR) *V. parahaemolyticus* ST165 strain in China, designated FB-11, in an effort to understand its genomic features, particularly the presence of MDR and MHMR genes.

The *V. parahaemolyticus* strain FB-11 was isolated in 2019 of *P. vannamei* from seawater farms in Zhejiang province using chromogenic *Vibrio* agar plates (CHROMagar Microbiology, Paris, France). Polymerase chain reaction (PCR) was used to detect the *V. parahaemolyticus* highly conserved species-specific gene *toxR* and the virulence genes *tdh* and *trh*. The antimicrobial resistance profile was assessed using the disk diffusion method on Mueller–Hinton (MH) agar (Oxoid Ltd., Basingstoke, United Kingdom) in accordance with the guidelines of the Clinical and Laboratory Standards In-

☆ Key Laboratory of Marine Food Quality and Hazard Controlling Technology of Zhejiang Province, College of Life Sciences, China Jiliang University, Hangzhou, Zhejiang 310018, China

* Corresponding authors: Han Jiang, Key Laboratory of Marine Food Quality and Hazard Controlling Technology of Zhejiang Province, College of Life Sciences, China Jiliang University, Hangzhou, Zhejiang 310018, China
E-mail address: jianghan825@126.com (H. Jiang).

https://doi.org/10.1016/j.jgar.2021.07.015

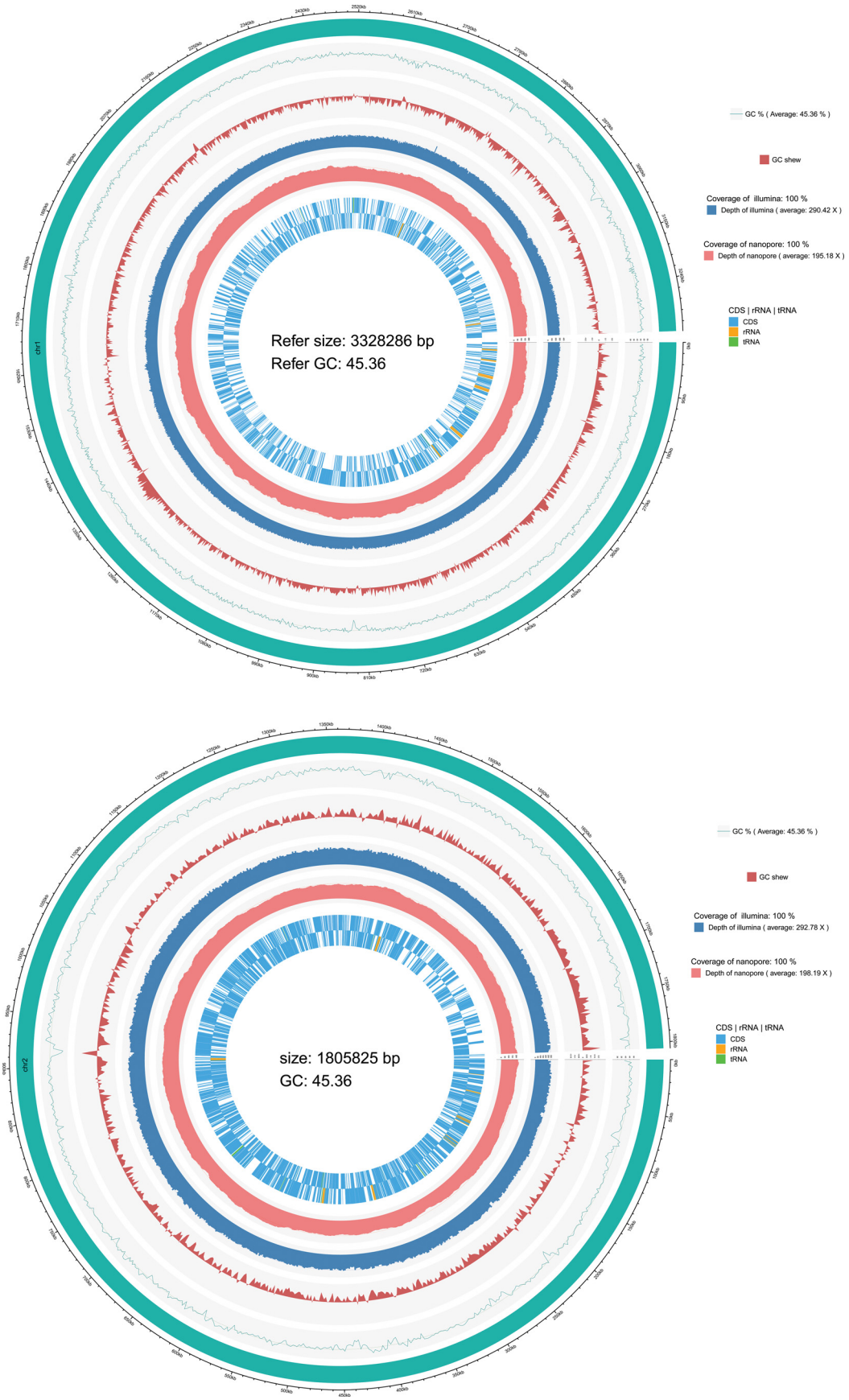

Fig. 1. Genome map of the two chromosomes of *V. parahaemolyticus* FB-11. A: Chr1 (3,328,286 bp, 45.37% GC content); B: Chr2 (1,805,825 bp, 45.36% GC content).

stitute (CLSI, 2017). According to the method described by Malik and Aleem, the minimum inhibitory concentration (MIC) values for heavy metals were determined using MH agar containing Zn^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Pb^{2+}, or Cr^{3+} in varying concentrations (100–3200 μg/mL) [4].

Bacterial genomic DNA was extracted using an Agencourt AMPure XP Kit (Beckman Coulter, Brea, CA, USA) and was quantified with a NanoDrop One spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). Genomic DNA libraries were prepared using the SQK-LSK109 ligation kit (ONT, Oxford, United Kingdom) and sequenced with a Nanopore PromethION (ONT) and Illumina Novaseq6000 platform (Illumina Inc., San Diego, CA, USA) using the standard protocol by Benagen (Wuhan, China). The filtered reads were assembled by Unicycler version 3 and bwa version 0.7.17. Genome function element prediction including the prediction of coding sequences (CDSs), clustered regularly interspaced short palindromic repeats (CRISPRs), genomics islands (GIs), and prophages was determined by Prokka (http://www.vicbioinformatics.com/software.prokka.shtml), CRISPRfinder (https://crispr.i2bc.paris-saclay.fr/), IslandViewer 4 (http://www.pathogenomics.sfu.ca/islandviewer/), and PHASTER (http://phaster.ca/). CDSs were annotated using BLAST against the COG, KEGG, Swiss-Prot, and RefSeq databases. The Comprehensive Antibiotic Resistance Database (CARD) and Virulence Factor Database (VFDB) were used to retrieve MDR and virulence genes.

The assembled genome contained two circular chromosomes, designated Chr1 (3,328,286 bp, 45.37% GC content) and Chr2 (1,805,825 bp, 45.36% GC content) (Fig. 1). FB-11 is an multilocus sequence type (MLST) sequence type 165 strain according to the 7 loci scheme and is the first ST165 strain recorded in China [5]. The genome contained 4,544 predicted CDSs, 127 tRNA genes, 37 rRNA genes, and 1 tmRNA gene. Consistent with the results of the antimicrobial susceptibility testing, strain FB-11 encodes a chromosomally encoded β-lactamase gene, *CARB β-lactamase*, and the tetracycline resistance genes *tetR* and *tet35*. In addition, strain FB-11 encodes extensive drug resistance determinants, such as resistance to chloramphenicol (*catB*), macrolide (*macA* and *macB*), phenazine (*ehpR*), and quaternary ammonium compound (*sugE*). The strain also harbored multidrug efflux pump genes (*acrA, acrB, acrD, acrE, acrF, mdtA, mdtL, mdtE, mdtG, mdtN, mexA, mexB, emrD*, and *emrK*, and multidrug efflux pump related regulator genes (*marA, marR, robA*, and *norM*).

The MIC values for Cu^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} were 3200, 800, 1600, 1600, and 3200 μg/mL, respectively. Thus, strain FB-11 was found to be resistant to these heavy metals. This resistance pattern was consistence with its genetic background, wherein sequence analysis revealed that strain FB-11 harbored *copA* and *cusS* genes, which confer resistance to Cu^{2+}, and the *zntA* gene, which encodes a zinc/cadmium/lead-transporting P-type ATPase that confers resistance to Zn^{2+}, Cd^{2+}, and Pb^{2+}. In addition, the stain harbored *czcA* and *czcB* genes encoding cobalt-zinc-cadmium resistance proteins CzcA and CzcB, and harbored the *czcD* gene encoding cadmium-cobalt-zinc/H(+)-K(+) antiporter CzcD, which both confer resistance to Cd^{2+}, Co^{2+}, and Zn^{2+}. There was no plasmid detected in the whole genome, and both MDR and MHMR genes were located on the chromosomes. Additionally, the mobile genetic element genes of IS*6110*, Tn*3*, Tn*10*, and Tn*552* had been annotated in the genome sequence, which were related to the dissemination of MDR and MHMR genes. Furthermore, the virulence genes *tdh* and *trh* were not detected by PCR, and the genome contained secretion system types II (T2SS), III (T3SS), and VI (T6SS), and 12 GIs. No CRISPRs were predicted.

In conclusion, this is the first report of the complete genome sequence of *V. parahaemolyticus* ST165 strain isolated from *P. vannamei* in China. All the MDR and MHMR genes were located on the chromosomes and the virulence genes *tdh* and *trh* were absent. Our findings provide the foundation for future investigations of the MDR and MHMR mechanisms of *V. parahaemolyticus* ST165.

Funding

This study was supported by the National Natural Science Foundation of China (31901792 and 31801655) and the Fundamental Research Funds for the Provincial Universities of Zhejiang (2020YW34) and the Key Research and Development Program of Zhejiang Province (2020C02031).

Competing interests

None declared.

Ethical approval

Not required.

GenBank accession number

The complete genome sequence has been deposited in GenBank with accession no. CP073068 (chromosome 1), CP073069 (chromosome 2).

References

[1] He Y, Wang S, Zhang J, Zhang X, Sun F, He B, et al. Integrative and conjugative elements-positive *Vibrio parahaemolyticus* isolated from aquaculture shrimp in Jiangsu. China. Front Microbiol 2019;10:1574.
[2] Lopatek M, Wieczorek K, Osek J. Antimicrobial resistance, virulence factors, and genetic profiles of *Vibrio parahaemolyticus* from seafood. Appl Environ Microbiol 2018;84:e00537 -18.
[3] Nguyen CC, Hugie CN, Kile ML, Navab-Daneshmand T. Association between heavy metals and antibiotic-resistant human pathogens in environmental reservoirs: a review. Front Environ Sci Eng 2019;13:46.
[4] Malik A, Aleem A. Incidence of metal and antibiotic resistance in *Pseudomonas* spp. from the river water, agricultural soil irrigated with wastewater and groundwater. Environ Monit Assess 2011;178:293–308.
[5] Jiang H, Yu T, Yang Y, Yu S, Wu J, Lin R, et al. Co-occurrence of antibiotic and heavy metal resistance and sequence type diversity of *Vibrio parahaemolyticus* isolated from *Penaeus vannamei* at freshwater farms, seawater farms, and markets in Zhejiang Province. China. Front Microbiol 2020;11:1294.