Genome Report

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Genome sequence of *Vibrio* sp. OULL4 isolated from shellfish

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Abstract: The members of Vibrionaceae family are Gram-negative bacterium are ubiquitous in marine and estuarine environments. This diverse group of bacteria include many pathogenic strains that potentially cause infection to human and aquaculture animals. *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* are among the few recognized as a major, worldwide cause gastroenteritis, particularly in countries where seafood consumption is high. The control of these vibrios has been a hurdle due to the rising numbers of antibiotic resistant strains in the environments. We report the genome sequence of *Vibrio* sp. OULL4 isolated from shellfish. The availability of this genome sequence will facilitate the study of its antimicrobial traits, as well as add our knowledge of *Vibrio* sp. diversity and evolution.

Keywords: Vibrionaceae; infection; gastroenteritis; antibiotic; genome

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Introduction

Seafood production has double over the years to meet the rising consumer demand for seafood. This involuntary action has exposed aquatic animals to bacterial infections[1,2]. This situation gets complicated and worsen by the emergence of resistant *Vibrio* sp. strains, which hampers medical care. *Vibrio* sp. is a Gram-negative halophilic bacteria that belongs to the Vibrionaceae family[3–8]. They naturally inhabit the aquatic surroundings and associated with aquatic animals for example crustaceans, molluscs and fish[9–13].

The World Health Organization (WHO) has acknowledged antibiotic resistance as a public health hazard that affects millions of people worldwide[14]. Due to excessive use of antibiotics in the aquaculture sector, the incidence of resistance accelerated, mostly among foodborne pathogens such as *Vibrio* sp.[15–23], *Listeria* sp.[24–26], and *Salmonella* sp.[27–32]. The resistant foodborne pathogens poses a threat and challenge to drug discovery programmes worldwide[33,34]. Therefore, it is important to continuously monitor and manage the resistant *Vibrio* sp. in seafood and environments.

Vibrio sp. OULL4 strain was isolated from shellfish originated from a supermarket in Selangor, Malaysia. The strain presented a large yellow colony on selective media—thiosulphate citrate bile salt sucrose (TCBS) agar. The antibiotic susceptibility test was performed to determine to resistance phenotype of *Vibrio* sp. OULL4 strain. The strain was resistant to 11/14 antibiotics tested, namely the ampicillin, ampicillin/sulbactam, 3rd generation cephalosporin (cefotaxime, ceftazidime), aminoglycoside (amikacin, gentamicin, kanamycin), suphamethox/trimethoprim, oxytetracycline, tetracycline, and chloramphenicol. This is a worrying scenario as the antibiotic resistant profile exhibited by the strain is among the recommended antibiotics agents used in treatment if *Vibrio* sp. infection[35–37]. The *Vibrio* sp. OULL4 strain was selected for genome sequencing to further explore and understand the antibiotic resistant traits.

Data description

The genomic DNA of *Vibrio* sp. OULL4 was extracted using Masterpure™ DNA purification kit (Epicentre, Il lumina Inc., Madison, WI, USA) prior to RNase (Qiagen,

USA) treatment[38,39]. The DNA quality was quantified using NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and a Qubit version 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). Illumina sequencing library of genomic DNA was prepared using Nextera™ DNA Sample Preparation kit (Illumina, San Diego, CA, USA) and library quality was validated by a Bioanalyzer 2100 high sensitivity DNA kit (Agilent Technologies, Palo Alto, CA) prior to sequencing. The genome of OULL4 strain was sequenced on MiSeq platform with MiSeq Reagent Kit 2 (2 x 250bp; Illumina Inc, San Diego, CA, USA)[40]. The trimmed sequences were *de novo* assembled with CLC Genomic Workbench version 5.1 (CLC Bio, Denmark). Contigs with at least 200bp and 30-fold coverage were selected for gene prediction and annotation. The bacteria identity was also checked by local BLAST against NCBI prokaryotic 16S rRNA database. Prodigal (version 2.6.1) was utilized to predict the bacteria gene coding sequence (CDS) from the draft genome[41]. Gene annotation was performed by local BLAST of translated predicted CDS against NCBI-nr database and on Rapid Annotation using Subsystem Technology (RAST) server[42]. Presence of rRNA and tRNA genes were detected using RNAmmer and tRNAscan SE version 1.21[43,44]A total of 59 contigs were generated with N50 size of 201,133bp. The assembled genome size of *Vibrio* sp. OULL4 contains 4,146,642 bp, with an average genome coverage of 54-fold with a G + C content of 45.4% (Table 1). The whole genome project was deposited at DDBJ/EMBL/GenBank under accession MQVK00000000. The version described in this paper is the first version MQVJ00000000. It is composed of 59 contigs and there were 3,743 protein coding genes (out of a total of 3,898 predicted gene) (Table 1).

Table 1. General features of *Vibrio* sp. OULL4 draft genome.

Attribute	Value
Genome size (bp)	4,146,642
G + C content %	45.4
DNA scaffold	59
Total genes	3,898
Protein coding genes	3,743
RNA genes (5S, 16S, 24S)	5, 3, 1
Pseudo genes	55

The analysis obtained from RAST server revealed 493 subsystems (Figure 1). The annotated genome has 63 genes responsible for resistance to antibiotic and toxic compounds including 25 genes for multidrug resistance efflux pumps, one gene for beta-lactamase, and two genes for tetracycline resistance. The presences of these genes in the genome is closely related to the phenotypic resistance exhibited by the strain toward ampicillin, cefotaxime, oxytetracycline, and tetracycline.

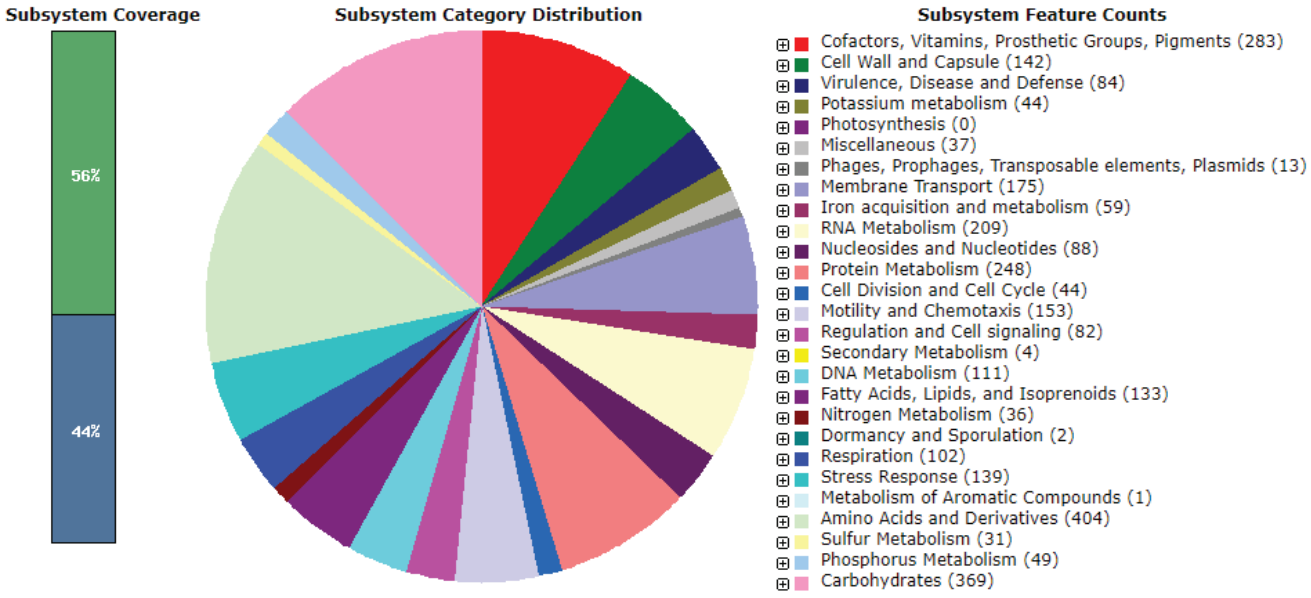

Figure 1. Subsystem category distribution of *Vibrio* sp. OULL4 (based on RAST annotation server).

Vibrio sp. OULL4 is a multidrug resistant strain—resistant to 11/14 antibiotics tested. The resistant phenotype and genes of genome illustrates how extensive antibiotics have been used in aquaculture sector. Some of the resistance phenotype seen in this strain possibly due to the misuse of permitted antibiotics in Asian aquaculture industry namely tetracycline, quinolone, oxytetracycline, sulphonamide, and trimethoprim[45]. Soon, our dependency to antibiotics will eventually deprive the efficacy of clinical antibiotics. We will need to resort to non-antibiotic approach such as bacteriophage application or natural plant antimicrobials to manage *Vibrio* infections in the aquaculture[46–50]. We also could adapt quorum sensing method to understand the various signalling molecules of *Vibrio* sp. These information are useful in the management of virulence traits[51]. In summary, the application of antibiotics in aquaculture should be reviewed and monitored in order to ensure the efficacy of these antibiotics for treatment.

Conflict of Interest

The authors declare that the research was conducted in

the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors Contributions

The research and manuscript writing were performed by VL and W-ST. W-FY and K-GC provided vital guidance and support for the success of the project. The project was founded by VL and K-GC.

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