Genome Report

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Genome sequence of *Vibrio* sp. SALL 6 isolated from shellfish

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Abstract : Members of the *Vibrionaceae* family are well known as foodborne pathogen that cause hazard to human in many forms of clinical infection and also affecting aquaculture via infection to livestock. This pathogen has caused seafood associated gastroenteritis cases in many countries including United States, Asian, and South East Asian countries. Antibiotics are usually used as prophylactic and therapeutic to manage the rising *Vibrio* infections, however, this in turn led to emergence of antibiotic resistant strains in the environments. *Vibrio* sp. SALL 6 isolated from shellfish was selected for genome sequencing to further explore its antimicrobial traits. Here, a high-quality genome sequence of *Vibrio* sp. SALL 6 is reported, while its genome reveals a potential for future antibiotic resistance managements.

Keywords: *Vibrionaceae*; foodborne; gastroenteritis; antibiotics; genome

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Introduction

Vibrio species are natural inhabitant of aquatic environments and are the main cause of seafood-borne gastroenteritis[1,2]. This Gram-negative halophilic bacteria belong to the *Vibrionaceae* family[3–5] and many of them are linked with aquatic animals such as crustaceans, molluscs and fish[6]. Of the 12 identified pathogenic *Vibrio* sp., the three commonly reported are *Vibrio cholerae* and *Vibrio parahaemolyticus* — associated with seafood contamination, and *Vibrio vulnificus* — related via wound infections[7,8]. The increase in seafood consumption worldwide lead to the global rise of seafood production from aquaculture. This causes the marine animals to be prone to bacterial infections[9]. The occurrence of *Vibrio* sp. in our environments does raise a public concern on food safety due to the rising number of reported foodborne cases worldwide[10]. This situation has worsen by the emergence of antibiotic resistant bacteria which cause a delay in treatment, prolong hospitalization and even mortality. Antibiotics are used in the aquaculture sector to treat bacterial infection, however, the misuse of them has resulted in the rising number of resistant foodborne pathogens such as *Vibrio* sp.[11–18], *Listeria* sp.[19,20], and *Salmonella* sp.[21–24]. Antibiotic resistance among foodborne pathogens is a major health issue and a great challenge to worldwide drug discovery programmes[25]. The clinical and environmental Vibrio sp. strains are reported to exhibit antibiotic resistance traits[26]. Hence, it is vital to continuously monitor and manage the occurrence of *Vibrio* in seafood and environments.

Vibrio sp. SALL 6 strain was isolated from shellfish originated from a wetmarket in Selangor, Malaysia. It formed green colony on thiosulphate citrate bile salt sucrose (TCBS) agar. The strain exhibited multidrug resistance profile towards 3/14 antibiotics tested. Based on the antibiotic susceptibility phenotype, *Vibrio* sp. SALL

6 strain was resistant to ampicillin, 3rd generation cephalosporin (cefotaxime) and aminoglycoside (amikacin). This is a distressing condition as the antibiotic resistant profile shown by this strain is among the recommended antimicrobials agents used in treatment of *Vibrio* sp. infections[27,28]. As an attempt to further explore its antimicrobial resistance traits, this strain was selected for genome sequencing.

Data description

Genomic DNA of *Vibrio* sp. SALL 6 was extracted using Masterpure™ DNA purification kit (Epicentre, Il lumina Inc., Madison, WI, USA) before performing RNase (Qiagen, USA) treatment[29,30]. 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 SALL 6 strain was sequenced on MiSeq platform with MiSeq Reagent Kit 2 (2 x 250bp; Illumina Inc, San Diego, CA, USA)[31]. The trimmed sequences were *de novo* assembled with CLC Genomic Workbench version 5.1 (CLC Bio, Denmark). Contigs with at least 200 bp 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. Bacteria gene coding sequence (CDS) was predicted from the draft genome using Prodigal (version 2.6.1)[32]. Gene annotation was performed by local BLAST of translated predicted CDS against NCBI-nr database and also on Rapid Annotation using Subsystem Technology (RAST) server[33]. Presence of rRNA and tRNA genes were detected using RNAmmer and tRNAscan SE version 1.21[34,35] A total of 81 contigs were generated with N50 size of 193,737 bp.

The assembled genome size of *Vibrio* sp. SALL 6 contains 4,989,632 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 MQVK01000000. It is composed of 81 contigs and there were 4,500 protein coding genes (out of a total of 4,681 predicted gene) (Table 1).

Table 1. General features of *Vibrio* sp. SALL 6 draft genome

Attribute	Value
Genome size (bp)	4,989,632
G + C content %	45.4
DNA scaffold	81
Total genes	4,681
Protein coding genes	4,500
RNA genes (5S, 16S, 24S)	2, 6, 1
Pseudo genes	66

The analysis obtained from RAST server revealed 544 subsystems (Figure 1). The annotated genome has 74 genes responsible for resistance to antibiotic and toxic compounds including 28 genes for multidrug resistance efflux pumps, one gene for beta-lactamase, four genes for resistance to fluoroquinolones, and two genes for tetracycline resistance. The phenotypic resistance shown by *Vibrio* sp. SALL 6 toward ampicillin and cefotaxime is closely related to the gene coding beta-lactamase in the genome.

Multidrug resistance profile seen in the phenotype and genes of *Vibrio* sp. SALL 6 genome illustrates how extensive antibiotics have been utilized in agriculture and aquaculture settings. The efficacy of clinical antibiotics are declining, thus there is a need for non-antibiotic method such as bacteriophage application or natural plant antimicrobials to manage *Vibrio* infections in the aquaculture[36,37].

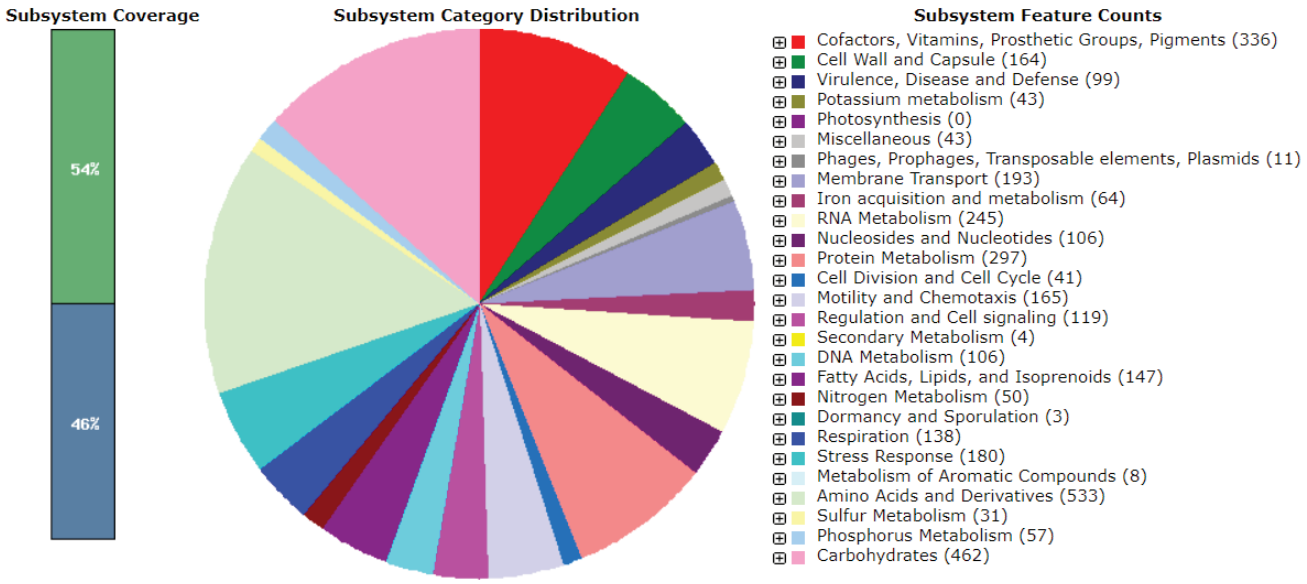

Figure 1. Subsystem category distribution of Vibrio sp. SALL 6 (based on RAST annotation server

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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