Whole-genome Sequencing of *Vibrio sinaloensis* T47, a Tropical Marine Isolate with Quorum Sensing Properties

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Published: 2017.03.09

Abstract

A large number of *Vibrio* sp. thrive in the marine environment and they are notable to cause food borne infection associated with undercooked seafood. In this study, we report the whole genome sequence of *Vibrio sinaloensis* T47 which was isolated from coastal marine water in Morib Beach, Hulu Selangor. The genome is made up of approximately 4.59 Mbp with 80 contigs and 46% G+C content. From the annotated genome, genes associated with quorum sensing (QS) were identified. This research provides a genetic basis for better understanding of QS pathway which contributes to the physiological traits of strain T47 to thrive in the marine environment.

Key words: *Vibrio sinaloensis*, whole genome sequencing, quorum sensing, autoinducer synthase, virulence factor

Introduction

*Vibrio* sp. is a very common bacterium which can be found in almost all water-borne environments including sea, estuary and fresh water. *Vibrio* sp. first made its debut into the scientific world through the discovery of bioluminescence properties. It was reported that *Vibrio fischeri* forms a symbiotic relationship with its host, the Hawaiian bobtail squid (*Euprymna scolopes*) and this underlying symbiosis is associated with quorum sensing (QS) [1]. QS is well known as a mechanism of virulence and colonization when the population in bacteria surpasses a threshold. This feature seems to be a common trait in the members of the genus *Vibrio*.

Since the development of advance taxonomical tools such as DNA-DNA hybridization, fluorescent amplified fragment length polymorphism and multilocus sequence analysis, the number of novel species from *Vibrio* family being discovered is constantly expanding [2, 3]. Among the vast members of *Vibrio* genus, a number of them were demonstrated to possess QS abilities such as *V. harveyi* [4], *V. cholera* [5], and *V. anguillarum* [6]. There is mounting data suggesting that QS is responsible for many unique traits such as pathogenicity, swarming abilities and biofilm production [7, 8, 9]. In this work, we study on *V. sinaloensis* strain T47 which was isolated from a tropical marine in Morib Beach, Selangor (2° 45' 2.7" N, 101° 26' 34.7" E). A water sample was collected approximately 15 cm from the water surface.

*V. sinaloensis* was first documented by Gomez-Gil and colleagues [10] from the spotted rose snapper (*Lutjanus guttatus*) which causes infection and vibriosis. In fact, this bacterium is a major threat to the aquaculture sector due to its pathogenicity properties.
The colonization of *V. sinaloensis* has been reported in crustaceans, for example, the white-leg shrimp, *Litopenaeus vannamei* [11]. Here, the sequencing strategy and data of the whole genome of strain T47 is presented to provide better understanding of the marine bacterium as well as insights to the physiological behaviors associated to QS activity.

*V. sinaloensis* strain T47 was cultured in aseptic condition on Luria Bertani Agar (LBA) with 3% NaCl concentration (w/v) and incubated at 28°C overnight. The genomic DNA of strain T47 was extracted using QIAamp DNA Minikit (Qiagen, Germany) according to the manufacturer’s instructions. The quality of the extracted DNA was measured using NanoDrop Spectrophotometer (Thermo Scientific) and Qubit 2.0 fluorometer (Life Technologies). Next, Nextera DNA Prep Kit (Illumina Inc., CA) was used to prepare the sequencing library followed by whole genome sequencing using a personal sequencer, Illumina MiSeq (Illumina Inc., CA). The total reads were assembled into 80 contigs with 43.8 × coverage using CLC Genomic Workbench version 5.1 (CLC Bio, Denmark). The draft genome of strain T47 is made up of 4,599,504 bp with G+C content of 46.12%. The genome sequence has been deposited into GenBank under the accession number JXBJ00000000. The 16S rDNA sequence used in identification of strain T47 [12] was also deposited into NCBI under accession number KR058860.

Based on 16S rDNA sequence, strain T47 was found to have more than 99% similarity to several Vibrio sp. such as *V. variabilis*, *V. caribbeanicus*, and *V. sinaloensis*. On the other hand, annotations of both functional and predicted genes were performed using the Integrated Microbial Genomes (IMG-ER) platform with and with GOLD-ID Ga0063884 [13]. As shown in Table 1, the genome was resolved into 4,105 protein coding genes (CDs) and a total of 127 RNA genes which consist of 8 genes responsible for 5S rRNA synthesis, 5 genes for 16S rRNA synthesis, 6 genes for 23S rRNA synthesis and 107 genes for tRNA. From the IMG-ER platform, cluster of orthologous groups (COG) categories showed that a large number of genes are responsible for basic life-sustaining needs of the bacterium. It was found that 310 genes were predicted to contribute to amino acid transport and metabolism, 245 genes are linked to carbohydrate transport and metabolism, 295 involves in signal transduction mechanisms, and 91 genes are related to the virulence and defense regulation (Table 2).

From the annotated genome sequences, a gene associated with QS was found in contig 14. The 1203 bp of luxM homologue is analogous to an N-acyl homoserine lactone (AHL) synthase, AinS, which can be also found in *V. fischeri* [14, 15]. Hence, it is highly postulated that the autoinducer synthase LuxM is responsible for the production of signaling molecules in strain T47. In this study, the availability of the sequence could contribute to a better understanding of QS system and its role in *V. sinaloensis*.

### Table 1. Genome features of *V. sinaloensis* strain T47

| Attributes                                      | Number         | % of Total |
|------------------------------------------------|----------------|------------|
| DNA, total number of bases                     | 4,599,504      | 100.00     |
| DNA coding number of bases                     | 4,053,747      | 88.13      |
| DNA G + C number of bases                      | 2,121,494      | 46.12      |
| DNA scaffolds                                  | 80             | 100.00     |
| Genes total number                             | 4232           | 100.00     |
| Protein coding genes                           | 4105           | 97.00      |
| RNA genes                                      | 127            | 3.00       |
| rRNA genes                                     | 19             | 0.45       |
| 5S rRNA                                        | 8              | 0.19       |
| 16S rRNA                                       | 5              | 0.12       |
| 23S rRNA                                       | 6              | 0.14       |
| tRNA genes                                     | 106            | 2.50       |
| Other RNA genes                                | 2              | 0.05       |
| Protein coding genes with function prediction  | 3428           | 81.00      |
| Pseudo genes                                   | 65             | 1.54       |
| Without function prediction                    | 677            | 16.00      |
| Protein coding genes with enzymes              | 1124           | 26.56      |
| Without enzymes but with candidate KO based enzymes | 5         | 0.12       |
| Protein coding genes connected to Transporter Classification | 614       | 14.51      |
| Protein coding genes connected to KEGG pathways | 1310         | 30.95      |
| Not connected to KEGG pathways                 | 2795           | 66.04      |
| Protein coding genes connected to KEGG Orthology (KO) | 2448   | 57.84      |
| Not connected to KEGG Orthology (KO)           | 1657           | 39.15      |
| Protein coding genes connected to MetaCyc pathways | 954       | 22.54      |
| Not connected to MetaCyc pathways              | 3151           | 74.46      |
| Protein coding genes with COGs                 | 3085           | 72.90      |
| Chromosomal Cassettes                          | 392            | -          |
| Biosynthetic Clusters                          | 8              | -          |
| Genes in Biosynthetic Clusters                 | 125            | 2.95       |
| Fused Protein coding genes                     | 136            | 3.21       |
| Protein coding genes coding signal peptides    | 445            | 10.52      |
| Protein coding genes coding transmembrane proteins | 1034       | 24.43      |
Table 2. Cluster of orthologous groups for strain T47 IMG-ER platform

| Name                                    | Count | Percentage (%) |
|-----------------------------------------|-------|----------------|
| Amino acid transport and metabolism     | 310   | 8.8            |
| Carbohydrate transport and metabolism   | 245   | 6.95           |
| Cell cycle control, cell division,      | 39    | 1.11           |
| chromosome partitioning                 |       |                |
| Cell motility                           | 135   | 3.83           |
| Cell wall/membrane/envelope biogenesis  | 220   | 6.24           |
| Chromatin structure and dynamics        | 1     | 0.03           |
| Coenzyme transport and metabolism       | 173   | 4.91           |
| Defense mechanisms                      | 91    | 2.58           |
| Energy production and conversion        | 198   | 5.58           |
| Extracellular structures                | 49    | 1.39           |
| Function unknown                        | 207   | 5.87           |
| General function prediction only        | 238   | 6.75           |
| Inorganic ion transport and metabolism  | 177   | 5.02           |
| Intracellular trafficking, secretion and vesicular transport | 77 | 2.19 |
| Lipid transport and metabolism          | 122   | 3.48           |
| Mobiome: prophages and transposons      | 12    | 0.34           |
| Nucleotide transport and metabolism     | 93    | 2.64           |
| Posttranslational modification, protein turnover, chaperones | 162 | 4.6 |
| RNA processing and modification         | 1     | 0.03           |
| Replication, recombination and repair   | 120   | 3.41           |
| Secondary metabolites biosynthesis,     | 64    | 1.82           |
| transport and catabolism               |       |                |
| Signal transduction mechanisms          | 295   | 8.37           |
| Transcription                           | 257   | 7.29           |
| Translation, ribosomal structure and biogenesis | 240 | 6.81 |
| Not in COG                              | 1147  | 27.1           |

Nucleotide sequence accession numbers

The draft genome sequence of *V. sinaloensis* strain T47 can be obtained from GenBank under the accession number JXB00000000. This version described in the paper is the first version, JXB000000000. The GenBank accession number for 16S rDNA nucleotide sequence for strain T47 is KR058860. This version described in the paper is the first version, KR058860.

Acknowledgement

This work was supported by the High Impact Research Grants, University of Malaya (UM-MOHE HIR Grant UM.C/625/1/HIR/ MOHE/CHAN/14/1, No. H-50001-A000027; UM-MOHE HIR Grant UM.C/625/1/HIR/ MOHE/CHAN/01, No. A000001-50001) awarded to Kok-Gan Chan which are gratefully acknowledged.

Competing Interests

The authors have declared that no competing interest exists.

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