Quorum sensing and genomic studies of a marine bacterium *Vibrio variabilis* strain T01

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Abstract

Vibrio variabilis strain T01 was isolated from the coastal waters in Hulu Selangor, Malaysia and its genome sequenced. This curved gram-negative bacterium shows cell-to-cell communication properties. The characteristics of the sequenced genome and its annotation processes are described here. The finished assembled whole genome of T01^T exhibits genome size of 4,529,728 bp in 83 contigs with 46.22% G+C content, 4053 protein coding genes and 94 RNA genes. The whole genome analysis revealed the presence of quorum sensing signalling molecule synthase gene (*luxM*) which is crucial to understand the quorum sensing dependent phenotypes in this isolate.

Keywords: *Vibrio variabilis*, Quorum sensing, *Vibrio*, Whole genome sequencing, *N*-acylhomoserine lactone, marine

Introduction

Vibrio spp. are gram-negative bacteria, highly motile and can be found in almost all aquatic environment including marine, estuarine and coastal swamps [1]. The varieties of the *Vibrio* spp. are dependent on the environmental parameters such as the salinity, temperature, geographical locations, nutrient contents, pH level and the amount of aquatic macrophytes such as zooplanktons, mollusks, fish and crustacean [2, 3, 4]. The advanced development in classification and taxonomical technology such as DNA-DNA hybridization (DDH), multilocus sequence analysis (MLSA) and fluorescent amplified fragment length polymorphism (FAFLP) have been the key tools in the emergence of many new novel *Vibrio* species [5, 6].

Example of famous headline by the Vibrio species is the major outbreaks caused by *Vibrio cholerae* on 1992 in Southern Bangladesh which caused 1473 deaths [7]. Not only affecting humans, *Vibrio harveyi* [8] and *Vibrio coralliilyticus* [9] are one of the many pathogenic bacteria that affect the aquatic life. Mentioned above are only a few of the vast species of Vibrios discovered so far and quite a number of them are actually non-pathogenic. The genetic make-up of a particular species of *Vibrio* plays a pivotal role to the morphology and their cell properties. The occurrence of events such as insertion, deletion, mutation and addition in the DNA sequences leads to the discovery of new species and previously not reported phenotypes in a species [10]. *Vibrio variabilis* is a non-pathogenic bacterium recently discovered species in 2011

by Chimetto and his colleagues [11]. *V. variabilis* strain T01 is isolated in the coastal waters of Morib beach, Malaysia, and its genome properties is described here.

Classification and features

Discovered in the coastal marine waters of Morib beach, Hulu Selangor (Malaysia), *Vibrio variabilis* strain T01 is a gram-negative, non-sporulating, motile bacterium which thrives on growing media containing 1–3% (w/v) NaCl concentration. It is a member of the *Vibrionaceae* family in the *Gammaproteobacteria* class. Synonym with its species name *variabilis*; the colony changes its colour from cream coloured beige into blackish when cultured in limited light conditions [11]. The optimum temperature for growth was observed at 28°C with smooth rounded colonies (1mm) forming after 24 hours of incubation on Luria-Bertani Agar (LBA) with 3% w/v NaCl. The cells are shaped like curved-rods with 0.9 μm width and 1.35 μm long when viewed under the Scanning Electron Microscope (SEM) (data not shown). Carbon utilization, nitrogen utilization, fatty acid composition and polar lipid analysis were previously described here [11]. Table 1 below shows the Minimum Information about the Genome Sequences (MIGS).

Table 1. Classification and general features of *Vibrio caribbeanicus* T01 according to the MIGS recommendations [12]

MIGS ID	Property	Term	Evidence code
		Domain *Bacteria*	TAS [13]
		Phylum *Proteobacteria*	TAS [14]
		Class *Gammaproteobacteria*	TAS [15, 16, 17]
		Order *Vibrionales*	TAS [18]
	Current classification	Family *Vibrionaceae*	TAS [19, 20]
		Genus *Vibrio*	TAS [19, 21–23]
		Species *Vibrio variabilis*	TAS [11]
		Type strain T01	
	Gram stain	Negative	IDA
	Cell shape	Curved rods (vibroids)	IDA
	Motility	Motile via single polar flagellum	TAS [11]
	Sporulation	Non-sporulating	IDA
	Temperature range	4–37°C	IDA
	Optimum temperature	28°C	TAS [11]
	Salinity	Considerably hydrophilic; 1–3% w/v NaCl (optimum)	IDA
MIGS-22	Oxygen requirement	Aerobic	IDA
	Carbon source	Highly diverse	IDA
	Energy metabolism	Highly diverse	IDA
MIGS-6	Habitat	Marine environment	TAS [11], IDA
MIGS-15	Biotic relationship	Free-living	NAS
MIGS-14	Pathogenicity	Non-pathogenic	NAS

	Biosafety level	1	NAS
MIGS-23.1	Isolation	Coastal marine waters	IDA
MIGS-4	Geographic location	Morib Beach, Hulu Selangor, Malaysia	IDA
MIGS-5	Sample collection time	10 a.m	IDA
MIGS-4.1	Latitude	2° 45' 2.7" N	
MIGS-4.2	Longitude	101° 26' 34.7" E	
MIGS-4.3	Depth	5 cm from water surface	IDA

Evidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [24]. If the evidence is IDA, the property was directly observed by one of the authors.

By amplifying the 16S rRNA of *V. variabilis* strain T01 shares 95–98% sequence similarities to the *Vibrio* clade. The highest matching sequence of T01 was with *Vibrio variabilis* R–40492^T (98.77%) isolated from the mucus of zoanthids (soft coral) in São Paulo, Brazil [11]. Ez-Taxon database was used to compare the 16S rRNA sequences with highest matching percentage [25]. The phylogenetic tree (Figure 1) was built using the MEGA 6.0 software [26] by using the Maximum Likelihood method based on the Tamura-Nei model [27].

Cell-to-cell communication abilities

Preliminary test to determine the cell-to-cell communication or quorum sensing (QS) properties was done by using biosensor; *Chromobacterium violaceum* CVO26 respond to exogenous QS signal (autoinducer) by producing purple pigment. CVO26 does not produce its own autoinducer signals because its autoinducer synthase gene has been mutated rendering it malfunction [28]. In this case, the presence of autoinducer signals produced from *V. variabilis* strain T01 is confirmed by the purple pigmentation of the biosensor (CVO26) (Figure 2). For QS screening, *Erwinia caratovora* (GS101) and *Erwinia caratovora* (PNP22) served as positive and negative controls, respectively.

Genome sequencing and annotation

Genome project history

Strain T01 of was chosen for sequencing based on its phylogeny similarities based on its 16S rRNA to *V. variabilis* which is a recently discovered species in 2011 [11] as well as its QS activity. The whole sequence of T01 has been deposited into GenBank under the accession number JRWM00000000. The version described in this paper is the first version JRWM00000000. The whole genome was also deposited in the Genome On-Line Database (GOLD) and has obtained its respective GOLD ID [29]. Strain T01 genome sequences consist of 83 contigs and annotated using an online server namely Rapid Annotation using Subsystem Technology (RAST) [30].

Growth condition and DNA isolation

Strain T01 was isolated using serial dilution method and conventional streaking was done to obtain pure colony. The growth medium used was Luria-Bertani agar (LBA) with 3% (w.v) NaCl concentration and incubated at 28°C for 24 hours. The genomic DNA was extracted using the QIAamp DNA minikit (Qiagen, Germany) following the manufacturer's instruction.

Genome sequencing and assembly

The purified genomic DNA of strain T01 was used for sequencing performed using Illumina MiSeq (Illumina Inc., CA) located in Microbiome Laboratory 1, High Impact Research, University of Malaya. Approximately 1,549,093 reads were obtained with 61.96× coverage. Assembly of T01 genome was done using CLC Genomics Workbench 5.1 (CLC Bio, Denmark) and a total of 83 contigs were generated.

Genome annotation

By using an online server database namely Rapid Annotation using Subsystem Technology (RAST) [30], annotation of the assembled sequences of strain T01 was carried out to determine the open reading frames (ORFs) based on the SEED database [31] available on the RAST server. To identify the non-coding sequences and the miscellaneous genes, RNAmmer [32] and tRNA scan-SE [33] online servers were used, respectively. Additional annotations of both functional and predicted genes were performed using the Integrated Microbial Genomes (IMG-ER) platform [34].

Genome properties

The genome of *V. variabilis* strain T01 consists of 4,529,728 bp with 46.22% G+C content. The assembled genomes produced 83 contigs with 97.73% (4,053) belongs to the protein coding genes category. Functional protein coding genes was estimated at 87.29% (3,620) while the remaining percentages (10.44%) are hypothetical protein coding genes. There are 94 RNA genes predicted from the total amount of protein coding genes with rRNA, tRNA and other RNA genes included. The unanimous rRNA genes are the 5S rRNA (3 genes; 0.07%), 16S rRNA (2 genes; 0.05%) and 23S rRNA (4 genes; 0.10%) while a total of 83 genes (2%) are included in the tRNA category. Strain T01 possessed QS abilities based on the preliminary test done aforementioned on top. Hence the gene responsible for the QS traits was selected from the annotated gene pool and it lies in contig 16 (1,203 bp). This gene (*luxM*) of *V. variabilis* strain T01 encodes LuxM protein which is a homologue to the QS synthase LuxI found in its closely related species *viz. Vibrio fischeri* [35]. The LuxM synthase gene directs the production of the signal molecule termed the autoinducer (*N*-(3-hydroxybutanol)-L-homoserine lactone) [36]. Once the signal reached a threshold level, it will bind to its respective cognate receptor; transcriptional activator which stimulates a series of cascade reaction that is responsible for many distinct physiological traits such as pathogenicity, bioluminescence, swarming and many more [36].

Conclusion

This report summarizes the genome and genes properties in *V. variabilis* strain T01. The relevance of this project is to discover the QS gene and its whole genome analysis. With the genome sequences available, this will path the way to understanding further on the functionality and the biological processes that is QS-dependent as QS modulates diverse phenotypes in proteobacteria including strain T01. To our best knowledge, this is the first report on the QS gene found in the *V. variabilis* strain T01.

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Figure 1: Phylogenetic tree highlighting the position of *Vibrio variabilis* T01 relative to the type strains of other species within the genus *Vibrio*. The strains and their corresponding GenBank accession numbers of 16S rRNA genes are indicated in parentheses. The sequences were aligned using MEGA 6.06 and the phylogenetic inferences were obtained using Neighbour-

joining method with MEGA version 6 [22]. The numbers at nodes are the percentage of bootstrap values obtained by 500 replicates and there were a total of 1,269 positions in the final dataset. *Catenococcus thiocycli* (HE582778) was used as outgroup

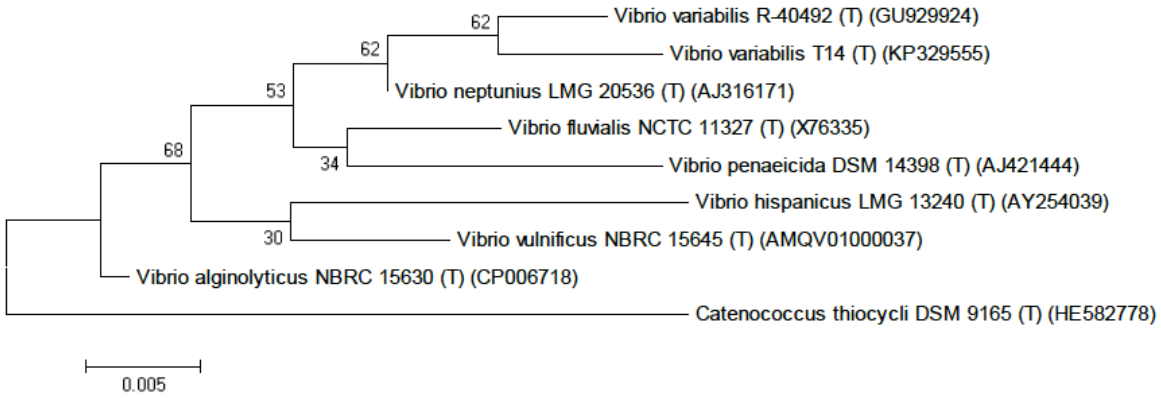

Figure 2: Preliminary QS test of T01. CVO26 served as the biosensor; GS101 as the positive control and PNP22 as the negative control