**Complete genome sequence of *Vibrio alginolyticus* ATCC
33787^T isolated from seawater with three native
megaplasms**

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Abstract

Vibrio alginolyticus, an opportunistic pathogen, is commonly associated with vibriosis in fish and shellfish and can also cause superficial and ear infections in humans. *V. alginolyticus* ATCC 33787^T was originally isolated from seawater and has been used as one of the type strains for exploring the virulence factors of marine bacteria and for developing vaccine against vibriosis. Here we sequenced and assembled the whole genome of this strain, and identified three megaplastids and three Type VI secretion systems, thus providing useful information for the study of virulence factors and for the development of vaccine for *Vibrio*.

Keywords

Vibrio alginolyticus, genome sequence, plasmid, T6SS

1. Introduction

Vibrio alginolyticus is Gram-negative, halophilic and motile, and strains from this species have been isolated from diverse environments including seawater, aquaculture farms, and hospital patients (Gomez-Leon et al., 2005; Hlady and Klontz, 1996; Lajnef et al., 2012; Schmidt et al., 1979). *V. alginolyticus* is an opportunistic pathogen, causing typical signs of vibriosis which resulted in economic losses due to high mortality in aquaculture systems (Gomez-Leon et al., 2005; Lee et al., 1996). It also affects humans who have been in direct contact with the infected fish or shellfish (Caccamese and Rastegar, 1999), causing severe gastroenteritis and extra-intestinal diseases (Hlady and Klontz, 1996; Newton et al., 2012). Increased incidence of infection and the wide spectrum of antibiotic resistance (e.g. ciprofloxacin, streptomycin, thiostrepton, tetracycline, ampicillin, and erythromycin) have made *V. alginolyticus* a threat to public health (Lajnef et al., 2012; Ye et al., 2008)

There are major differences both between and within different bacterial species in their ability to cause infection. A major driving force in the emergence and evolution of pathogenic isolates with antibiotic resistance is acquisition of virulence factors and resistance genes through horizontal gene transfer (Hastings et al., 2004). Identification of horizontally transferred genes among *V. alginolyticus* depends on the availability of whole genome sequences from different strains. Up to now, sixteen strains of *V. alginolyticus* have draft genome sequences, and one strain (*V. alginolyticus* ATCC 17749^T) has complete genome sequence. *V. alginolyticus* ATCC 17749^T contains two chromosomes, but no plasmid was identified (Liu et al., 2015). Strain *V. alginolyticus* ATCC 33787^T was originally isolated from the seawater of Oahu, Hawaii (Baumann et al., 1971), and recent study suggested that outer

membrane protein from *V. alginolyticus* ATCC 33787^T can be potentially used as shared antigen among *Vibrios*, suggesting its importance in vaccine development for *Vibrio* (Lun et al., 2014). In this study, we present the complete genome of *V. alginolyticus* ATCC 33787^T, to provide useful information for the study of virulence factors and for the development of animal vaccine for *Vibrio*.

2. Data description

General features of strain *V. alginolyticus* ATCC 33787^T were summarized in **Table 1**. *V. alginolyticus* ATCC 33787^T was grown overnight in 2216E medium (BD Difco) at 30°C. The bacterial cells were pelleted by centrifugation and the supernatants discarded. The genomic DNA was purified using Agencourt AMPure XP Kit (Beckman Coulter) and a 10-kb SMRTbell template library was constructed from 5 µg purified genomic DNA at Shanghai OE biotech Corporation (Shanghai, China). The library was sequenced on two SMRT cells on Pacific Biosciences (PacBio) RSII sequencing platform with P4-C2 chemistry for a total of 118,023 reads. A 300-bp paired end library was prepared using Illumina TruSeq DNA prepare kit and sequenced on Illumina Hiseq2500 platform for 1,509 megabases. The 196,712 subreads were *de novo* assembled using HGAP algorithm version 3 into 10 contigs with average genome coverage of 102x. Refine the assembly using Illumina short reads resulted into a genome of 5,750,107bp with a G+C content of 45.5%. Gaps between contigs were filled through sequencing of PCR products by primer walking. Protein-coding sequences were predicted by Glimmer v.3.0 (Delcher et al., 2007) and Genome annotation was completed using the NCBI PGAAP Pipeline. tRNA and rRNA genes were identified by tRNAscan-SE v.1.3 (Lowe and Eddy, 1997) and RNAmmer v.1.2 (Lagesen et al., 2007), respectively. The

Type VI secretion system (T6SS) gene clusters were predicted by T346Hunter (Martinez-Garcia et al., 2015).

Table 1

General features of *V. alginolyticus* ATCC 33787^T and MIGS mandatory information.

Items	Description
<i>General features</i>	
Gram stain	Negative
Cell shape	curved rod
Pigmentation	Non-pigmented
Temperature	4–30 °C
Motility	Motile by means of flagella
<i>MIGS data</i>	
Investigation_type	Bacteria_archaea
Project_name	Genome sequence of <i>Vibrio alginolyticus</i> ATCC 33787 ^T
Lat_lon	20.3 N, 157.3 W
Depth	NA
Geo_loc_name	USA: Oahu
Collection_date	before 1971-04-01
Env_biome	ENVO:02,000,049
Env_feature	ENVO:00,002,150
Env_material	ENVO:00,002,006
Env_package	missing
Num_replicons	5
Extrachrom_elements	3
Estimated_size	5,724,939
Ref_biomaterial	PMID: 4935323
Source_mat_id	ATCC 33787 ^T
biotic_relationship	free living
Trophic_level	chemoorganotroph
Rel_to_oxygen	facultatively anaerobic
Isol_growth_condt	PMID: 4935323
Seq_meth	Illumina Hiseq 2500, PacBio RSII sequencing
Annot_source	GenBank
Finishing_strategy	Complete; 102 x coverage, 5 contigs
<i>Genome assembly data</i>	
Assembly method	HGAP
Assembly name	HGAP algorithm verion 3
Genome coverage	102 x
Sequencing Technology	Illumina; PacBio

The genome of *V. alginolyticus* ATCC 33787^T consists of two circular chromosomes,

named as ChI (3,362,673 bp, 44.7% G+C), ChII (1,851,538 bp, 44.7% G+C), and three circular mega plasmids, named as pMBL128 (128,112 bp, 44.3% G+C), pMBL287 (286,750 bp, 41.4% G+C) and pMBL96 (95,866 bp, 46.0% G+C), respectively (**Table 2** and **Fig. 1**). This genome encodes 125 tRNAs and 34 rRNA genes. Bioinformatics analysis revealed the multiple potential virulence factors on the chromosome and plasmids, such as microbial collagenase, leukocidin, hemolysin, RTX toxin-related calcium-binding protein, and zonular occludens toxin (Zot).

Table 2

Genome features of *V. alginolyticus* ATCC 33787^T.

Features	ChI	ChII	Plasmid pMBL128	Plasmid pMBL287	Plasmid pMBL96
Length (bp)	3,362,673	1,851,538	128,112	286,750	95,866
G+C content (%)	44.6	44.7	44.3	41.3	46.0
Protein coding genes	2943	1583	137	266	98
rRNA genes	31	3	0	0	0
tRNA genes	112	13	0	0	0
Gene	3121	1629	139	281	100
Pseudogene	34	30	2	15	2
GenBank accession no.	CP013484	CP013485	CP013486	CP013487	CP013488

Fig. 1. Circular representation of the *V. alginolyticus* ATCC 33787^T genome. The tracks from the outside to the center: CDSs on forward strand, CDSs on reverse strand, rRNA, tRNA, G+C content and GC skew.

Type VI secretion system (T6SS) is a highly conserved bacterial secretion system to transport proteins across the bacterial envelope, and it is precisely regulated in Gram-negative pathogens. There are two T6SS gene clusters in many previously sequenced *V. alginolyticus* strains in the smaller chromosome ChII (Sheng et al., 2012). *V. alginolyticus* ATCC 33787^T also contains two T6SS gene clusters in chromosome ChII (AT730_17070-AT730_17225, and AT730_19080-AT730_19235). Additionally, a third T6SS gene cluster, which is unique in *V. alginolyticus*, was located in a genomic island of the larger chromosome ChI (AT730_04360-AT730_04470). Plasmid pMBL287 was a putative mobilizable plasmid with genes encoding relaxase NikB protein, and it also contains a CRISPR/Cas system and a

zonular occludens toxin (Zot) gene. Plasmid pMBL96 was a putative conjugative plasmid with genes encoding conjugal transfer proteins. Further functional studies are needed to explore the roles of these unique genomic features in the pathogenicity of the *V. alginolyticus* as well as to develop effective vaccine against *V. alginolyticus* related infections.

Nucleotide sequence accession numbers

The complete genome sequence of *V. alginolyticus* ATCC 33787^T has been deposited in GenBank under the accession numbers CP013484 (ChI), CP013485 (ChII), CP013486 (plasmid pMBL128), CP013487 (plasmid pMBL287) and CP013488 (plasmid pMBL96).

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