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ORIGINAL PAPER

Vibrio profundi sp. nov., isolated from a deep-sea seamount

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Abstract A Gram-stain negative, rod-shaped, facultative anaerobic, motile bacterial strain, designated TP187^T, was isolated from a seamount near the Yap Trench in the tropical western Pacific. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain TP187^T is related to members of the genus *Vibrio* and has high 16S rRNA gene sequence similarity with the type strains of *Vibrio chagasii* (97.3%) and *Vibrio gallaecicus* (97.1%). Sequence similarities to all other type strains of current species of the genus *Vibrio* were below 97%. The polar lipids profile was found to contain diphosphatidylglycerol, phosphatidylglycerol, an aminophospholipid, two aminolipids, four phospholipids and eleven unidentified polar lipids. Ubiquinone Q-8 was detected as the predominant quinone. The genomic DNA G + C content of strain TP187^T was determined to be 43.7 mol%. In addition, the maximum values of in silico DNA–DNA hybridization (*is*DDH) and average nucleotide identity (ANI) between strain TP187^T with *V. chagasii* LMG 21353^T were 22.40 and 77.50% respectively. Both values are below the proposed cutoff levels for species delineation, i.e. 70 and 95%, respectively. Combined data from phenotypic,

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phylogenetic, *is*DDH and ANI data demonstrated that the strain TP187^T is representative of a novel species of the genus *Vibrio*, for which we propose the name *Vibrio profundi* sp. nov. (type strain TP187^T = KACC 18555^T = CGMCC 1.15395^T).

Keywords *Vibrio* · Coral · Seamount

Introduction

The genus *Vibrio* belongs to the family *Vibrionaceae* (Baumann and Baumann 1984) and members of the genus *Vibrio* are widespread in aquatic environments (Huq and Colwell 1995, Thompson et al. 2004). *Vibrio cholera* is the type species of the genus *Vibrio* and is a free-living aquatic bacterium that interacts with and infects a variety of organisms. Members of the genus *Vibrio* are typically Gram-stain negative, halophilic, chemoorganotrophic bacteria, which have facultatively fermentative metabolism. They contain ubiquinone-8 as the main respiratory quinone and $C_{16:0}$ as their predominant fatty acid. At the time of writing, more than 120 species are recognised in the genus *Vibrio* (http://www.bacterio.net/vibrio.html).

In this study, we report the characterisation of a novel bacterium of the genus *Vibrio* isolated from a deep-sea seamount, for which we propose the name *Vibrio profundi* sp. nov. (type strain TP187^T = KACC 18555^T = CGMCC 1.15395^T).

Materials and methods

Isolation of the bacterial strain and culture conditions

Strain TP187^T was isolated from a coral, which was collected from a seamount (tentatively named the Yap-3 seamount; 8°51′N, 137°40′E at a depth of 2030 m) near the Yap Trench by the submersible remotely operated vehicle (ROV) Faxian (Discovery) during the seamount cruise of the R/V Kexue (Science) in the tropical western Pacific in 2014 (Zhang et al. 2016, 2017; Liu et al. 2017a, b; Wang et al. 2018). The coral sample was washed immediately with sterile saline solution (0.8%) and appropriate dilutions were then plated on Marine agar 2216 (MA, Difco) at 20 °C. After incubation at 20 °C for 2 weeks on the ship, single colonies were selected and sub-cultured on MA to achieve purity. One of the pure cultures was designated TP187^T. The strain was routinely cultured on MA at 25 °C and stored as a suspension in skim milk (10%, w/v) at –80 °C. *Vibrio gallaecicus* DSM 23502^T and *Vibrio chagasii* LMG 21353^T were obtained from DSMZ and LMG for comparison as reference strains and routinely grown on MA at 25 °C.

Phenotypic determination

Cell morphology was investigated using a transmission electron microscope (JEM-1400, JEOL). Gram-staining was tested by using the bioMérieux Gram stain kit. The nitrate reduction, indole production, citrate, methyl red and Voges–Proskauer tests were done according to Dong and Cai (2001). Degradation of casein, starch, Tween 60, Tween 80, carboxymethyl cellulose, alginic acid, agar and chitin were tested on MA plates supplemented with appropriate substrates as described by Margesin et al. (2003). Hydrolysis of DNA was tested on DNAse agar (Oxoid, CM0321) prepared in sterile seawater. Growth under anaerobic conditions was examined after 7 days of incubation at 25 °C in an anaerobic jar (containing Anaerocult A (Merck) to produce anaerobic conditions) on MA supplemented with 20 mM $NaNO_3$ and 10 mM $NaNO_2$.

Physiological and biochemical characteristics and enzyme activities were determined using API 20 NE, API 20 E and API ZYM kits (bioMérieux) at 25 °C according to the manufacturers' instructions except that the NaCl concentration was adjusted to 2.0% in all tests and cell suspensions for inoculation were prepared in sterile seawater. API ZYM panels were analysed after 4 h at 25 °C and API 20 NE and API 20 E panels after 7 days at 25 °C, except nitrate reduction and indole production, which were analysed after 48 h. Growth at 4, 10, 15, 20, 25, 30, 35, 37 and 40 °C was assessed on MA and in marine broth 2216 (MB; BD). Salt tolerance tests, growth at different temperatures and pH range were performed as described Wang et al. (2018).

Chemotaxonomic characterisation

For fatty acid methyl ester analysis, strain TP187^T and the two reference strains were grown on MA at 25 °C for 3 days. All three strains shared similar growth behaviour and a sufficient amount of cells of comparable physiological age could be harvested from the third streak quadrant of the MA plates after cultivation under the applied conditions. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the Sherlock Microbial Identification System (MIDI, version 6.1) (Sasser 1990), using the data bank TSBA40 for identification. Respiratory quinones were extracted according to Altenburger et al. (1996) and were analysed by HPLC (Stolz et al. 2007). The polar lipid profile was analysed by the TLC methods of Tindall (1990a, b).

Phylogenetic analyses

DNA was extracted and purified as described by Sambrook and Russell (2001). The 16S rRNA gene was amplified, cloned and sequenced according to a previous protocol (Zhang et al. 2006, 2011). Multiple sequence alignments were performed using the clustalw program integrated in the MEGA version 6 (Tamura et al. 2013).

The MLSA was performed using five housekeeping genes, *pyrH* (uridylate kinase),

recA (recombination-repair protein), *rpoD* (polymerase sigma factor), *gapA* (glyceraldehyde-3-phosphate dehydrogenase) and *topA* (topoisomerase I). These housekeeping genes were amplified by PCR and sequenced as described by Sawabe et al. (2007) (Supplementary Table S1). The sequences of these genes were compared with the sequences available from GenBank using the BLASTN program. The phylogenetic trees were reconstructed using the neighbour-joining (NJ; Saitou and Nei 1987) and maximum-likelihood (ML; Felsenstein 1981) methods in MEGA version 6. The topologies of the phylogenetic trees were determined using bootstrap analyses based on 1000 replicates.

Genome sequencing

The draft genomes of strain TP187^T and the two reference strains *V. gallaecicus* DSM 23502^T and *V. chagasii* LMG 21353^T were sequenced using an Illumina NovaSeq PE150 at the Beijing Novogene Bioinformatics Technology Co., Ltd. Reads of each data set were filtered, and high quality paired-end reads were assembled using the SOAP denovo (Li et al. 2008, 2010).

DNA–DNA relatedness

Both the in silico DNA–DNA hybridization (*is*DDH) and the average nucleotide identity (ANI) values were used to determine the similarity of TP187^T with its two closely related type strains of the genus *Vibrio*. isDDH similarity was calculated using the GGDC web server (http://ggdc.dsmz.de/), with 70% similarity as the standard threshold for the bacterial species boundary (Meier-Kolthoff et al. 2013). The ANI values was calculated using the EzBioCloud web (https://www.ezbiocloud.net/tools), with the 95% cut-off value suggested for the bacterial species boundary (Yoon et al. 2017).

Results and discussion

Cells of strain TP187^T were observed to be motile by a polar flagellum and rod-shaped (Supplementary Fig. S1). Cells were observed to be Gram-stain negative, catalase positive and oxidase positive. Strain TP187^T is able to grow on LB medium and requires Na^+ for growth. Strain TP187^T can hydrolyse aesculin, gelatin, DNA, Tween 60, Tween 80, casein, chitin and starch, but not urea, carboxymethyl cellulose and alginic acid. The strain shows distinctive phenotypic features that discriminates it from closely related members of the genus *Vibrio* as shown in Table 1. Strain TP187^T was found to be positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, α-glucosidase, nitrate reduction, indole production, glucose fermentation, assimilation of D-maltose, mannitol, potassium gluconate, trisodium citrate and malic acid; fermentation of glucose, mannitol, melibiose and amygdalin. The strain is negative for cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-fucosidase, α-mannosidase, arginine dihydrolase, lysine dihydrolase, ornithine dihydrolase, H_2S production, urease, tryptophan deaminase, assimilation of D-mannose,

Table 1 Features that distinguish strain TP187^T from the type strains of *Vibrio gallaecicus* and *Vibrio chagasii*

Characteristic	1	2	3
Hydrolysis of chitin	+	−	+
Enzyme activity (API ZYM):			
Esterase (C4)	W	−	+
Lipase (C14)	+	W	−
Trypsin	−	W	+
Naphthol-AS-Bi-phosphohydrolase	+	−	+
N-acetyl-β-glucosaminidase	−	W	−
Assimilation of (API 20 NE)			
Trisodium citrate	+	−	+
D-mannitol	+	−	+
N-acetyl-glucosamine	W	−	+
Potassium gluconate	+	+	−
Fermentation/oxidation of (API 20 E):			
Melibiose	+	−	−

Strains: 1, TP187^T; 2, *Vibrio gallaecicus* DSM 23502^T; 3, *Vibrio chagasii* LMG 21353^T (all data from this study). +, positive; -, negative; W, weak reaction. All strains are positive for catalase and oxidase activity, hydrolysis of aesculin, gelatin, DNA, Tween 60, Tween 80, casein and starch, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, α-glucosidase, nitrate reduction, indole production, glucose fermentation, assimilation of D-maltose and malic acid, fermentation of glucose, mannitol and amygdalin. All strains are negative for hydrolysis of urea, carboxymethyl cellulose and alginic acid, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-fucosidase, α-mannosidase, arginine dihydrolase, lysine dihydrolase, ornithine dihydrolase, H_2S production, assimilation of L-arabinose, capric acid and phenylacetic acid

L-arabinose, capric acid, adipic acid and phenylacetic acid; and fermentation of inositol, sorbitol, rhamnose and sucrose. The polar lipids profile was found to contain diphosphatidylglycerol, phosphatidylglycerol, an aminophospholipid, two aminolipids, four phospholipids and eleven unidentified polar lipids (Supplementary Fig. S2). The quinone system of strain TP187^T was found to consist predominantly of ubiquinone-8 (92.6%) and also traces of ubiquinone-7 (7.4%). The predominant cellular fatty acids were identified as $C_{16:0}$ and summed feature 3 (composed of iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$) (Supplementary Table S2).

The 16S rRNA gene sequence of strain TP187^T was obtained (GenBank/EMBL/DDBJ accession number KT900237). On the basis of pairwise comparisons of 16S rRNA gene sequences using the latest version of EzTaxon-e, strain TP187^T has high 16S rRNA gene sequence similarities with the type strains of *Vibrio chagasii* (97.3%) and *Vibrio gallaecicus* (97.1%). The phylogenetic tree constructed using the NJ algorithm (Saitou and Nei 1987) revealed that strain TP187^T clusters with the members of the genus *Vibrio* and forms a coherent cluster with *V. gallaecicus* CECT 7244^T (Fig. 1). The phylogenetic tree for concatenated sequences of the five housekeeping genes constructed with the maximum-likelihood method confirmed the clustering of strain TP187^T and representatives of the genus *Vibrio* and again forms a coherent cluster with *V. gallaecicus* (Fig. 2). The *pyrH*, *gapA* and *topA* gene sequences of *V. gallaecicus* DSM 23502^T have been deposited as MK840790, MK840792 and MK840795.

Sequence scaffolds of the draft genomes of strains TP187^T, *V. gallaecicus* DSM 23502^T and *V. chagasii* LMG 21353^T have been deposited at DDBJ/ENA/GenBank under the accession numbers RZIS00000000,SZXU00000000 and SZQG00000000, respectively. The DNA G + C content of strain TP187^T determined from the genome sequence is 43.7 mol%, which is within the range observed for other members of the genus *Vibrio*.

The OrthoANI values of TP187^T with the two closely related type strains of the genus *Vibrio* were $\leq$ 77.50% ANI (Table 2). These values are far lower than the 95% ANI cut-off value suggested for the bacterial species boundary (Chun and Rainey 2014). Likewise the isDDH with the two closely related type strains of the genus *Vibrio* were $\leq$ 23.0, again below the standard threshold for the bacterial species boundary (Meier-Kolthoff et al. 2013). These result strongly support the conclusion that TP187^T is a novel species of the genus *Vibrio*.

On the basis of physiological, chemotaxonomic characteristics, phylogenetic analysis, *is*DDH and ANI data, it is proposed that strain TP187^T represents a novel species belonging to the genus *Vibrio*, for which the name *Vibrio profundi* sp. nov. is proposed.

Description of *Vibrio profundi* sp. nov.

Vibrio profundi (pro.fun′di. L. gen. n. *profundi*, of/from the depths of the sea).

Cells are Gram-stain negative, oxidase positive, catalase positive and rod-shaped, 1.3–1.9 μm long and

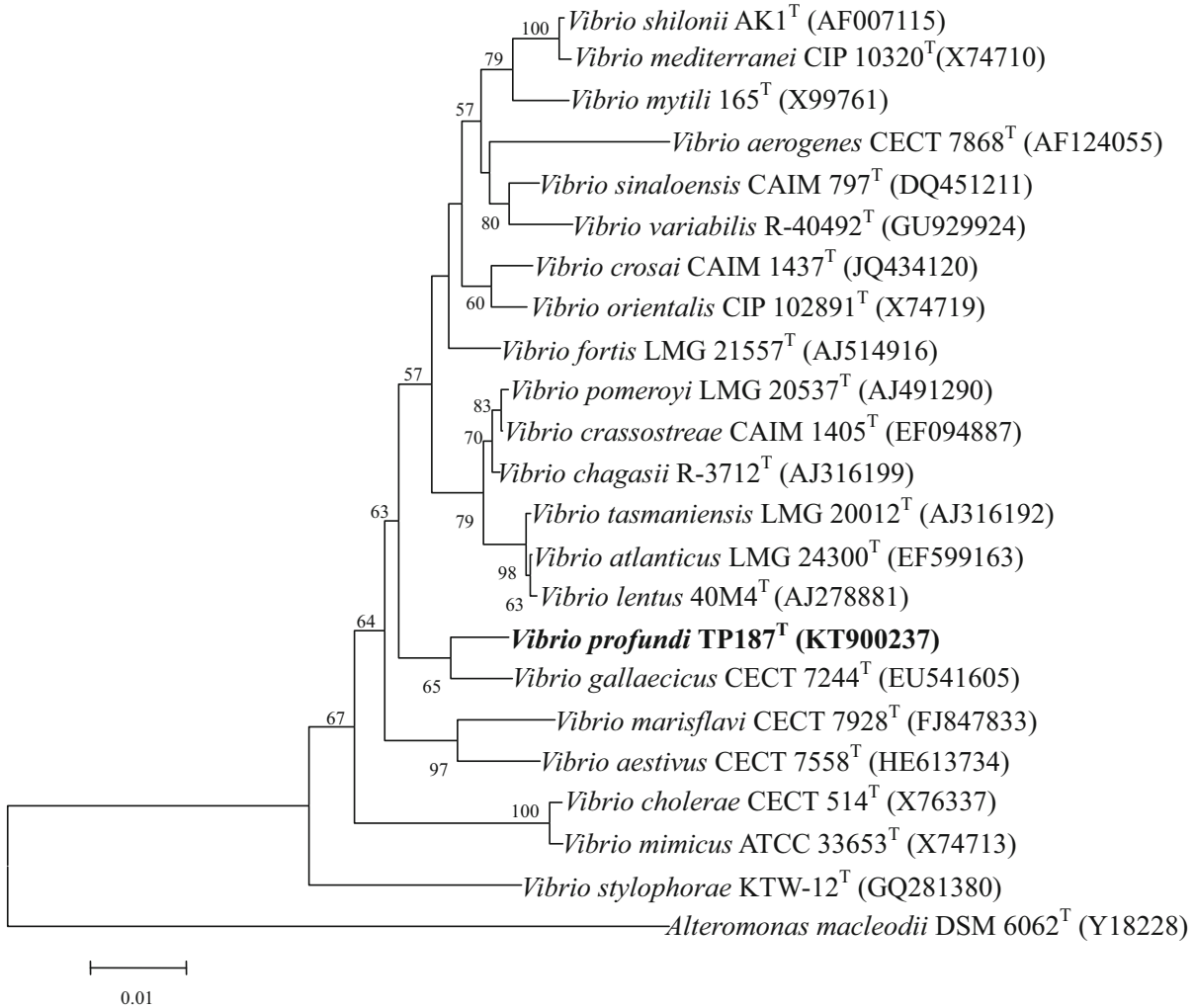

Fig. 1 Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequence data, showing the phylogenetic position of strain TP187^T among other members of the genus *Vibrio*. Bootstrap values (%) are based on 1000 replicates and are shown for branches with more than 50% support. *Alteromonas macleodii* DSM 6062^T was used as an outgroup. GenBank accession numbers of 16S rRNA sequences are given in parentheses. Bar, 0.01 substitutions per nucleotide position

0.8–1.2 μm wide. Motile by a flagellum. Colonies on MA are white, smooth, raised with entire margins and circular. Requires Na^+ for growth. Growth occurs in media with 0.5–6% (w/v) NaCl (optimum 2–3%). Grows at 4–37 °C but not at 40 °C on MA (optimum growth at 22–25 °C). The pH for growth is 6.0–8.5, with the optimum pH 6.5–7.5. The polar lipids profile contains diphosphatidylglycerol, phosphatidylglycerol, an aminophospholipid, two aminolipids, four phospholipids and eleven unidentified polar lipids. The predominant cellular fatty acids are $C_{16:0}$ and summed feature 3 (composed of iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$). Ubiquinone Q-8 is the predominant quinone. The genomic DNA G + C content of the type strain is 43.7 mol%.

The type strain, TP187^T (= KACC 18555^T = CGMCC 1.15395^T), was isolated from a coral, which was collected from a seamount (tentatively named as Yap-3 seamount) (8°51′N, 137°40′E at a depth of 2030 m) near the Yap Trench in the tropical western Pacific. The GenBank/EMBL/DDBJ accession numbers for the draft genome and *recA*, *pyrH*, *gapA*, *rpoD*, *topA* and16S rRNA gene sequences of strain TP187^T are RZIS00000000, MK840788, MK840789, MK840791, MK840793, MK840794 and KT900237.

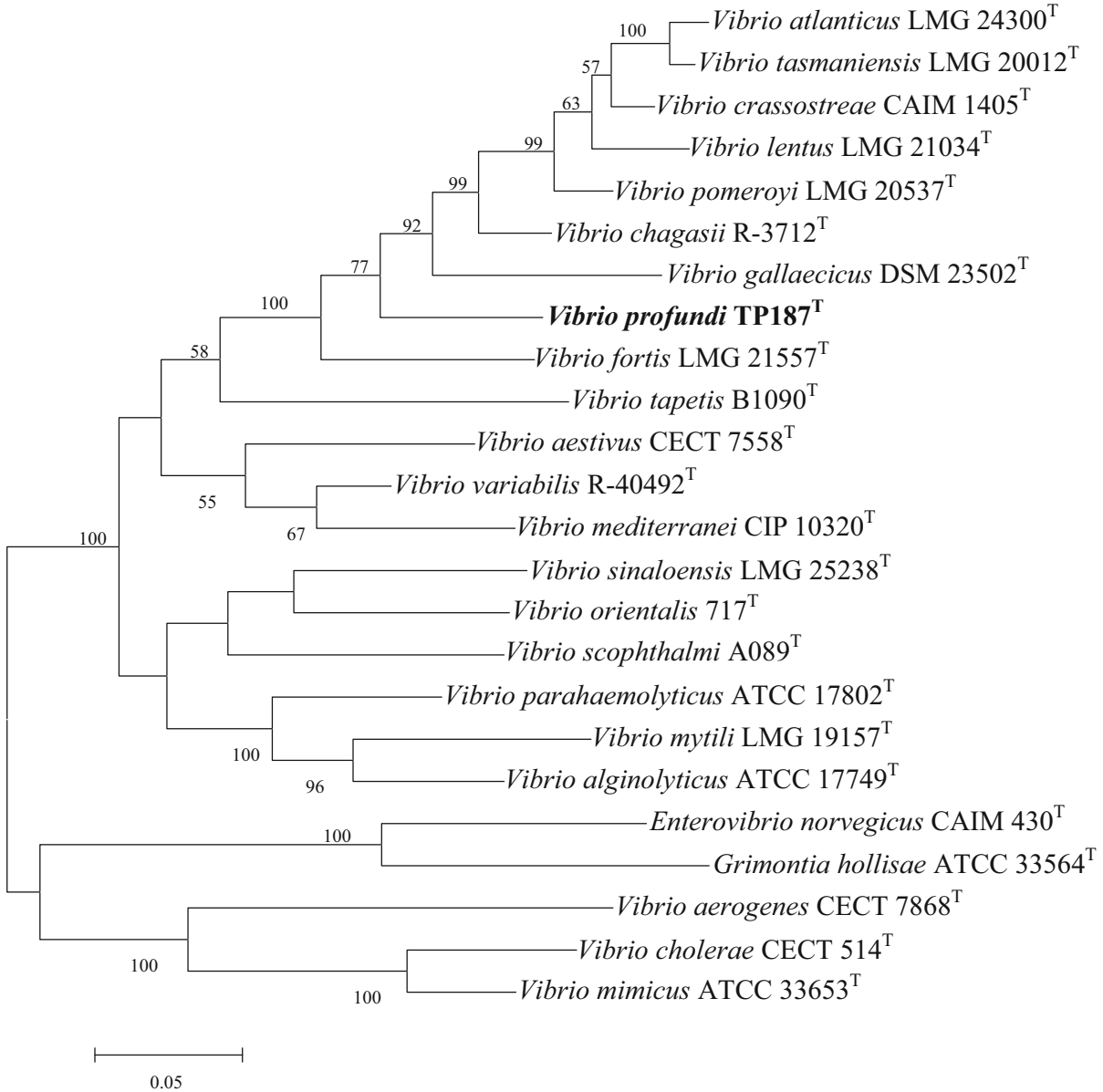

Fig. 2 Maximum-likelihood tree using multilocus sequence analysis (MLSA) based on the concatenated partial sequences of *recA* (665 bp), *pyrH* (316 bp), *gapA* (767 bp), *rpoD* (883 bp) and *topA* (677 bp) of strain 187^T and representatives of the genus *Vibrio* and other genera. *Enterovibrio norvegicus* CAIM 430^T and *Grimontia hollisae* ATCC 33564^T were used as outgroup. Bootstrap values (> 50%) are shown at the nodes. Bar, 0.05 substitutions per nucleotide position

Table 2 Complete genome characteristics, *is*DDH and OrthoANI analysis of TP187^T and two closely related type strains of genus *Vibrio*

Characteristics	TP187^T	LMG 21353^T	DSM 23502^T
Accession numbers	RZIS00000000	SZQG00000000	SZXU00000000
Contigs	25	141	62
Total length (bp)	5189300	5295939	4999218
N50 (bp)	488354	80136	316420
N90 (bp)	112194	26261	220519
CDS number	4719	4903	4470
GC content (mol%)	43.74	44.04	41.18
*is*DDH (%) with TP187T	100	22.40	21.60
OrthoANI value (%) with TP187^T	100	77.50	76.62

Strains: TP187^T; *Vibrio chagasii* LMG 21353^T; *Vibrio gallaecicus* DSM 23502^T (all data from this study)

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Authors' contributions D-C Zhang designed the project and took samples. N-X Zhang performed lab work, N-X Zhang and N–H Qiao analysed data and wrote the manuscript. D-C Zhang revised the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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