Vibrio atypicus sp. nov., isolated from the digestive tract of the Chinese prawn (*Penaeus chinensis* O'sbeck)

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A culture (designated strain HHS02^T) was isolated from Chinese prawn (*Penaeus chinensis*, O'sbeck) and determined to be a member of the genus *Vibrio*. Strain HHS02^T comprised slightly curved, rod-shaped, non-endospore-forming, Gram-negative, catalase-negative, oxidase-positive, O/129-sensitive and facultatively anaerobic cells that were motile by means of a single polar flagellum. Growth of strain HHS02^T occurred in 0.5–7% (w/v) NaCl [optimally in 1–3% (w/v) NaCl] and between pH 7.0 and 10.0 (optimally at pH 8.0–9.0). The strain showed growth between 16 and 30 °C (optimum 20 °C). Analysis using the 16S rRNA, *gapA*, *gyrB*, *mreB*, *pyrH*, *recA* and *topA* gene sequences of the novel isolate revealed that the organism belonged to the genus *Vibrio*, with ~98, 98, 90, 88, 92, 89 and 83% sequence similarity, respectively, with representatives of the genus *Vibrio*. DNA–DNA hybridization experiments indicated that the novel strain was distinct from recognized species of the genus *Vibrio*. The major fatty acid components were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, 38.7%), C_{16:0} (22.9%) and C_{18:1}ω7c (12.5%). The G + C content of the genomic DNA was 44.4 mol%. On the basis of the polyphasic taxonomic evidence presented in this study, it is concluded that strain HHS02^T should be classified as a novel species of the genus *Vibrio*, for which the name *Vibrio atypicus* sp. nov. is proposed. The type strain is HHS02^T (=CGMCC 1.8461^T=LMG 24781^T).

Vibrios are widespread in the aquatic environment (Huq & Colwell, 1995), with some taxa occupying a variety of ecological niches, such as the human and animal digestive tract, the surface of chitinous organisms, most notably copepods, and the coral mucus layer (Gomez-Gil *et al.*, 1998; Suantika *et al.*, 2001; Sawabe *et al.*, 2007a). Conversely, other vibrios constitute serious pathogens of aquatic and terrestrial animals, including humans (Borrego *et al.*, 1996; Verschuere *et al.*, 2000; Riquelme *et al.*, 2001;

Austin & Austin, 2007). The family *Vibrionaceae* includes the genera *Aliivibrio*, *Allomonas*, *Catenococcus*, *Enterovibrio*, *Grimontia*, *Listonella*, *Photobacterium*, *Salinivibrio* and *Vibrio* (Kalina *et al.*, 1984; MacDonell & Colwell, 1985; Sorokin, 1992; Thompson *et al.*, 2002, 2003; Farmer *et al.*, 2005; Urbanczyk *et al.*, 2007). Moreover, there has been a tremendous improvement in the taxonomy of vibrios in the last few years, particularly due to the description of novel species through the application of genomic techniques. Analyses based on phenotypic and molecular data have shown that the genus *Vibrio* is highly heterogeneous (Thompson *et al.*, 2001, 2005). Indeed, there has been a large increase in the number of recognized species of the genus *Vibrio* (Kumar & Nair, 2007; Nam *et al.*, 2007; Sawabe *et al.*, 2007a; Chang *et al.*, 2008; Gomez-Gil *et al.*, 2008; Rameshkumar *et al.*, 2008; Beaz Hidalgo *et al.*, 2009; Yoshizawa *et al.*, 2009) recently.

In a study of the microbiota of the digestive tract of healthy Chinese prawns (*Penaeus chinensis* O'sbeck), 28 cultures were recovered on marine 2216E agar plates (Difco) after incubation at 28 °C for 5 days. Identification of the cultures was achieved using 16S rRNA gene sequences and physiological and biochemical characteristics. Working cultures were maintained at 28 °C on marine 2216E agar

Abbreviations: MLSA, multilocus sequence analysis; O/129, 2,4-diamino-6,7-diisopropyl pteridine phosphate salt.

The GenBank/EMBL/DDBJ accession numbers for the nucleotide sequences of strain HHS02^T reported in this study are FJ009624 (16S rRNA gene), GQ223380 (partial *gapA* gene), GQ223381 (partial *gyrB* gene), GQ223382 (partial *mreB* gene), GQ223383 (partial *pyrH* gene), GQ223384 (partial *recA* gene) and GQ223385 (partial *topA* gene).

Additional maximum-parsimony phylogenetic trees based on 16S rRNA gene sequences (Fig. S1), additional neighbour-joining phylogenetic trees based on partial *gapA*, *gyrB*, *mreB*, *pyrH*, *recA* and *topA* gene sequences (Figs S2–S7), and concatenated sequences (Fig. S8) and a transmission electron micrograph of cells of strain HHS02^T (Fig. S9) are available as supplementary figures with the online version of this paper. A list of primers and the strains and sequence accession numbers used for the MLSA are also available as supplementary tables.

plates and stocks were kept as suspensions in sterile 0.9 % (w/v) saline supplemented with 15 % (v/v) glycerol at -70°C . One of the isolates, designated HHS02^T, did not correspond to any of the taxa included in the study by Farmer *et al.* (2005) and was characterized using a polyphasic approach.

Standard protocols, including those for Gram staining, flagella staining, for the determination of catalase, phosphatase and oxidase activities and for endospore formation, were employed (Smibert & Krieg, 1994). Motility was assessed in a hanging-drop preparation at $\times 1000$ magnification from 12 h cultures in tryptone soy broth (TSB; Oxoid) supplemented with 1 % (w/v) NaCl. Swarming mobility was tested on tryptone agar (1 % tryptone, 1.5 % NaCl and 0.6 % agar). Degradation of casein, starch, gelatin, Tween 80 and urea was assessed on suitably modified marine 2216E agar plates (after Cowan & Steel, 1974). DNA hydrolysis was determined on DNase test agar (Oxoid). Sensitivity to 2,4-diamino-6,7-diisopropyl pteridine phosphate salt (O/129, Sigma), a vibriostatic agent, was determined by using discs impregnated with 150 μg and 10 μg O/129. The reduction of nitrate was assessed in 2216E broth (Difco) supplemented with 0.25 % (w/v) NaNO_3 and incubated at 28°C for 24 h. Determination of the production of H_2S from thiosulphate was performed according to the method of Smibert & Krieg (1994). Other phenotypic and enzymic characterizations of strain HHS02^T involved the use of API 20E, VITEK 2 GN (bioMérieux) and Gram-negative MicroPlates (Biolog) with incubation at 28°C , according to the manufacturers' instructions, except that sterile 1.5 % (w/v) NaCl was used to prepare the inocula. Growth under anaerobic conditions was determined using marine 2216E broth and marine 2216E broth supplemented with 0.25 % (w/v) NaNO_3 at 28°C for 7 days. Growth in 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 13 % (w/v) NaCl was assessed in appropriately modified TSB (Oxoid). Growth in the absence of NaCl was tested with Luria-Bertani broth (LB; Oxoid). The pH range for growth was determined in TSB supplemented with 1 % (w/v) NaCl at pH 4–11; the final pH was adjusted using NaOH and HCl solutions. The inoculated media were incubated at 28°C for up to 24 h. The temperature range for growth was determined by incubating cultures at 4– 37°C using TSB supplemented with 1 % (w/v) NaCl. Growth was determined by an increase in OD_{600} , as measured spectrophotometrically. For quantitative analysis of cellular fatty acid compositions, cell masses of strain HHS02^T and the type strains of related species of the genus *Vibrio* were obtained from marine 2216E broth after incubation for 48 h at 25°C . Fatty acid profiles were determined as described previously (Xie & Yokota, 2003) using the Sherlock System (MIDI).

For pathogenicity determination, overnight cultures in TSB were used to prepare suspensions in 0.85 % (w/v) saline. The cells were counted by use of a haemocytometer slide (improved Neubauer type; Merck) at a magnification of $\times 400$. Rainbow trout of 21 g average weight from

quarantined stocks recognized as disease-free (after Austin & Austin, 1989) were used to assess pathogenicity. The fish were infected by intraperitoneal (i.p.) injection with 100 μl bacterial suspension of 8.8×10^7 c.f.u. ml^{-1} . The fish were maintained in tanks containing aerated static water at 20°C , with a 50 % water change daily. Controls were injected i.p. with 100 μl saline. The infected fish were maintained for 2 weeks.

Genomic DNA was extracted from 24 h cultures on marine 2216E agar plates by standard methods (Ausubel *et al.*, 1995). The G + C content of the chromosomal DNA was determined according to the methods described by Mesbah & Whitman (1989) using reverse-phase HPLC. DNA–DNA hybridization experiments were performed with genomic DNA from strain HHS02^T and the type strains of related species, *Vibrio parahaemolyticus* LMG 2850^T, *Vibrio natriegens* LMG 10935^T, *Vibrio brasiliensis* LMG 20546^T, *Vibrio xuii* LMG 21346^T and *Vibrio nereis* LMG 3895^T, from different *Vibrio* clades (Sawabe *et al.*, 2007b) following a previously described method (Liu & Shao, 2005). Genomic DNA from *Escherichia coli* DH5 α was used as an outgroup sample and salmon sperm DNA was employed as a negative control.

The 16S rRNA gene (corresponding to positions 8–1510 in the *Escherichia coli* numbering system) was amplified and sequenced using bacterial universal primers as described previously (Liu & Shao, 2005). Multilocus sequence analysis (MLSA), based on partial sequences of genes encoding recombination repair protein (*recA*), uridylate kinase (*pyrH*), glyceraldehyde 3-phosphate dehydrogenase (*gapA*), rod-shaping protein gene B subunit (*mreB*), DNA gyrase B subunit (*gyrB*), and topoisomerase I (*topA*), was used for accurate identification and classification of strain HHS02^T. MLSA was performed essentially as described previously by Thompson *et al.* (2005, 2007). The PCR primers used are listed in Supplementary Table S1 (available in IJSEM Online). Primers for the *recA*, *gapA* and *gyrB* genes yielded specific amplicons of 400–500 bp at 61°C . Primers for the *topA* gene yielded a specific amplicon at 65°C . Nested PCR was applied to obtain specific amplicons (445 bp) of the *mreB* gene. The first round of amplification for the *mreB* gene and the specific amplification of the *pyrH* gene were performed as described previously by Thompson *et al.* (2007) and Sawabe *et al.* (2007b). The annealing temperature for the second round of amplification of the *mreB* gene was 65°C . The PCR products were analysed on a 1 % agarose gel with a molecular mass standard for the quantification of the PCR yield. The PCR products that produced a single band on agarose gels were purified by PEG precipitation (Embley, 1991) and sequenced by MWG Biotech. The nucleotide sequences for the MLSA were submitted to GenBank with nucleotide accession numbers GQ223380–GQ223385.

The 16S rRNA, *gapA*, *gyrB*, *mreB*, *pyrH*, *recA* and *topA* gene sequences were aligned and compared with available

sequences in the NCBI GenBank database using BLAST searches. The 16S rRNA gene sequence was also compared with those in the EzTaxon public database (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Phylogenetic analysis based on the individual and concatenated sequences was performed using the MEGA (molecular evolutionary genetics analysis) version 4.0 software package (Tamura *et al.*, 2007) after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining and maximum-parsimony methods were determined by using bootstrap values based on 1000 replications. The GenBank accession numbers of the *gapA*, *gyrB*, *mreB*, *recA* and *topA* gene sequences used to construct the phylogenetic trees are listed in Supplementary Table S2 (available in IJSEM Online).

Searches using the almost-complete 16S rRNA gene sequence (1298 bp) of strain HHS02^T in the NCBI GenBank database and EzTaxon public database placed it among members of the genus *Vibrio*. The closest phylogenetic neighbours were species of the genus *Vibrio* from the *harveyi*, *nereis* and *orientalis* clades (Sawabe *et al.*, 2007b), which showed 98 % 16S rRNA gene sequence similarity with the novel isolate. Neighbour-joining and maximum-parsimony trees based on 16S rRNA gene sequences revealed that strain HHS02^T clustered with the *harveyi* clade (Sawabe

et al., 2007b), but formed a separate branch that was clearly phylogenetically distinct from known species in this clade (Fig. 1; the maximum-parsimony tree is available as Supplementary Fig. S1 in IJSEM Online).

The MLSA allele sequences of *gapA* (442 bp), *gyrB* (390 bp), *mreB* (420 bp), *pyrH* (360 bp), *recA* (461 bp) and *topA* (462 bp) were aligned using CLUSTAL_X and it was found that strain HHS02^T had 83–92 % allele sequence similarities with other species in the genus *Vibrio* (except for *gapA*, which showed 98 % sequence similarity between strain HHS02^T and *Vibrio rotiferianus*). A previous study concluded that species within the genus *Vibrio* share at least 85 % *recA* and *pyrH* gene sequence similarity and form discrete clusters on the basis of *recA* and *pyrH* sequences, with a species cut-off level of >94 % gene sequence similarity (Thompson *et al.*, 2005). Strain HHS02^T showed 89 % *recA* gene sequence similarity with *Vibrio campbellii* and *Vibrio chagasii* and 92 % *pyrH* gene sequence similarity with *Vibrio harveyi*, supporting the view that strain HHS02^T represented a novel species in the genus *Vibrio*. Neighbour-joining and maximum-parsimony trees were constructed for each allele (the neighbour-joining trees based on *gapA*, *gyrB*, *mreB*, *pyrH*, *recA* and *topA* gene sequences are available as Supplementary Figs S2–S7, respectively) and it was found that strain HHS02^T grouped with different species of the genus *Vibrio* within the *orientalis* clade (based on *gyrB*, *mreB* and *pyrH*

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses) constructed after multiple alignment of data. Distances (distance options according to the Kimura two-parameter model) and clustering with neighbour-joining method were performed by using the MEGA (molecular evolutionary genetics analysis) software package version 4.0 (Tamura *et al.*, 2007). Bootstrap values based on 1000 replicates are listed as percentages at branching points. Bar, 0.005 substitution units (K_{nuc}).

gene sequences), *nereis* clade (based on *topA* gene sequences) and *harveyi* clade (based on *gapA* gene sequences). The phylogenetic trees based on *recA* gene sequences showed that the novel species branched deeply with species in different *Vibrio* clades (Sawabe *et al.*, 2007b). Strain HHS02^T clustered with species within the *orientalis* clade (Sawabe *et al.*, 2007b) in the neighbour-joining phylogenetic tree based on the concatenated sequences of the six housekeeping genes (Fig. 2), with (3833 bp; the maximum-parsimony tree is available as Supplementary Fig. S8) or without (2535 bp; results not shown) the 16S rRNA gene. The concatenated tree showed differences from the phylogenetic tree based on 16S rRNA gene sequences.

The results of DNA–DNA hybridization experiments showed that strain HHS02^T had a maximum DNA–DNA relatedness of $53 \pm 3\%$ to *Vibrio parahaemolyticus* LMG 2850^T, which is less than the recommended cut-off level for genomic species of 70% (Wayne *et al.*, 1987). DNA–DNA hybridization values between strain HHS02^T and *Vibrio natriegens* LMG 10935^T, *Vibrio brasiliensis* LMG 20546^T, *Vibrio xuii* LMG 21346^T and *V. nereis* LMG 3895^T, which are representatives from different phylogenetically related *Vibrio* clades (Sawabe *et al.*, 2007b), e.g. the *harveyi* clade, *orientalis* clade and *nereis* clade, were $46 \pm 1\%$, $35 \pm 3\%$, $49 \pm 4\%$ and $35 \pm 4\%$, respectively. The results corroborated those of the MLSA and 16S rRNA gene sequence

analysis and suggested that strain HHS02^T represents a novel species within the genus *Vibrio*. The DNA G+C content of strain HHS02^T was 44.4 mol%, which was well within the range for the genus *Vibrio*, i.e. 38–51 mol% (Farmer *et al.*, 2005).

Consistent with other species of the genus *Vibrio* (Farmer *et al.*, 2005), strain HHS02^T comprised slightly curved bacteria with single polar flagella (a transmission electron micrograph of cells of strain HHS02^T is available as Supplementary Fig. S9). The cells were $0.5\text{--}0.7 \times 1\text{--}1.5\text{ }\mu\text{m}$ in size. Endospores were not observed. Colonies on marine 2216 agar were non-pigmented, non-luminescent, circular, smooth and convex and were 2.0 mm in diameter after incubation at 28 °C for 1 day. Growth occurred in 0.5–7% (w/v) NaCl, and optimally in 1–3% (w/v) NaCl. The pH range for growth was between pH 7.0 and 10.0 (optimally at pH 8.0–9.0). The strain showed growth between 16 and 30 °C (optimum 20 °C). No growth was observed after 3 weeks incubation at 4 °C or 37 °C. Growth occurred under anaerobic conditions in marine broth and in marine broth supplemented with nitrate and the nitrate was reduced to nitrite. The detailed morphological, physiological and biochemical characteristics of strain HHS02^T are given in the species description or are shown in Table 1 and Supplementary Fig. S9 (available in IJSEM Online). Phenotypically, strain HHS02^T could be clearly assigned to

Fig. 2. Phylogenetic tree based on seven concatenated gene loci sequences available from the EMBL database (accession numbers are listed in Supplementary Table S2). The 16S rRNA (1298 bp), *gapA* (442 bp), *gyrB* (390 bp), *mreB* (420 bp), *pyrH* (360 bp), *recA* (461 bp) and *topA* (462 bp) gene sequences were concatenated and the phylogenetic tree was constructed after multiple alignment of data. For details of tree construction, refer to Fig. 1. Bar, 0.02 substitution units (K_{nuc}).

Table 1. Characteristics that differentiate strain HHS02^T from closely related species

Taxa: 1, HHS02^T; 2, *V. parahaemolyticus* LMG 2850^T; 3, *V. alginolyticus* LMG 4409^T; 4, *V. natriegens* LMG 10935^T; 5, *V. harveyi* LMG 4044^T; 6, *V. campbellii* LMG 11216^T; 7, *V. brasiliensis* LMG 20546^T; 8, *V. tubiashii* LMG 10936^T; 9, *V. orientalis* LMG 7897^T; 10, *V. xuii* LMG 21346^T; 11, *V. nereis*; 12, *V. rotiferianus*; 13, *V. mytili*; 14, *V. chagasii*; 15, *V. halitocoli*; 16, *V. hepatarius*; 17, *V. azureus* (data from Yoshizawa *et al.*, 2009). Data in columns 1–10 are from this study; data in columns 11–16 are from Noguerola & Blanch, 2008. +, Positive for ≥90 %; (+), positive for 75–89 %; –, negative for ≤10 %; (–), negative for 25–11 %; v, variable for 26–74 %; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Arginine dihydrolase	–	–	–	–	–	–	+	+	–	+	+	–	+	v	–	ND	ND
Lysine decarboxylase	–	+	+	–	+	+	–	–	+	–	–	+	–	–	–	–	–
Ornithine decarboxylase	–	+	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–
Growth in/at:																	
4 °C	–	–	–	–	–	–	–	–	+	–	(–)	–	–	v	–	+	–
35 °C*	–	+	+	+	+	+	+	–	+	+	+	+	+	–	–	+	+
8 % (w/v) NaCl	–	+	+	+	–	–	–	–	+	+	+	–	+	+	–	+	–
ONPG*	+	–	–	–	–	–	+	+	–	–	–	–	v	–	+	–	ND
Indole*	–	+	+	–	+	+	+	+	+	+	(+)	+	–	+	+	+	+
Citrate utilization*	–	+	+	+	+	–	+	+	+	+	+	–	(+)	+	–	ND	–
Voges–Proskauer reaction	–	–	–	–	–	–	+	–	–	+	–	–	–	ND	–	ND	ND
Acid from:																	
L-Arabinose	–	+	–	+	–	–	–	–	–	+	–	+	+	–	–	–	–
Mannitol*	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	–
Rhamnose	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
Sucrose*	+	–	+	+	–	–	+	+	+	+	+	+	+	–	–	+	–
Catalase	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

*Test useful to differentiate A-, L-, O-*Vibrio* species according to the scheme of Noguerola & Blanch (2008).

the genus *Vibrio* (Alsina & Blanch, 1994) and belonged to the arginine-dihydrolase (A)-, lysine (L)- and ornithine (O)-decarboxylase-negative (A-L-O-) cluster (Noguerola & Blanch, 2008). *V. natriegens* and *V. campbellii*, which were two of the closest phylogenetic neighbours (based on 16S rRNA gene sequence and MLSA) of strain HHS02^T, were in the same cluster. However, most of the phylogenetically related species, i.e. *V. parahaemolyticus*, *V. alginolyticus*, *V. harveyi*, *V. rotiferianus*, *V. mytili*, *V. brasiliensis*, *V. tubiashii*, *V. orientalis*, *V. nereis*, *V. xuii* and *V. diabolicus*, belonged to other phenotypic clusters. The novel isolate was negative for catalase which was different from all other related species of the genus *Vibrio*. In addition, strain HHS02^T showed many other characteristics that clearly distinguished it from its closest phylogenetic neighbours (Table 1) including *V. natriegens* and *V. campbellii*, confirming that strain HHS02^T represents a novel species. Strain HHS02^T was not pathogenic for rainbow trout.

The major fatty acids of strain HHS02^T were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, 38.7 %), C_{16:0} (22.9 %) and C_{18:1}ω7c (12.5 %). The saturated fatty acids C_{12:0}, C_{14:0} and C_{18:0} were detected with compositions of 2.9, 4.0 and 6.2 %, respectively. The hydroxy fatty acids C_{12:0} 3-OH were detected in minor quantities (1.2 %). Summed feature 2 (C_{14:0} 3-OH and/or iso-C_{16:1} I) was detected in a moderate amount (4.3 % total fatty acids). Fatty acids C_{11:0}, iso-C_{12:0}, iso-C_{13:0}, iso-C_{14:0}, iso-C_{15:0}, iso-C_{17:0}, iso-C_{14:0} 3-OH, C_{16:1}ω5c and C_{18:1}ω9c were

also detected (0.2, 0.2, 0.6, 0.5, 0.2, 0.2, 0.4, 0.3 and 0.5 %, respectively). Fatty acid analysis of strain HHS02^T showed a distinct pattern that could be distinguished from those of the type strains of phylogenetically related species. The quantities of fatty acids C_{18:1}ω7c, C_{12:0} 3-OH and C_{14:0} 3-OH/iso-C_{16:1} I in strain HHS02^T were much lower than those of *V. parahaemolyticus* LMG 2850^T, *V. natriegens* LMG 10935^T, *V. brasiliensis* LMG 20546^T, *V. xuii* LMG 21346^T and *V. nereis* LMG 3895^T (Table 2). For other fatty acids, no clear differences were observed between strain HHS02^T and the type strains of related species of the genus *Vibrio* (Table 2).

Based on differential phenotypic properties, the fatty acid profile, the 16S rRNA gene sequence and MLSA and DNA–DNA hybridization results, it is concluded that strain HHS02^T belongs to the genus *Vibrio* and represents a novel species, for which the name *Vibrio atypicus* sp. nov. is proposed.

Description of *Vibrio atypicus* sp. nov.

Vibrio atypicus [a.ty.pi'cus. Gr. pref. a- not; L. adj. *typicus* typical; N.L. masc. adj. *atypicus* atypical, referring to atypical characters (catalase-negative) when compared with other recognized species of the genus *Vibrio*].

Gram-negative, slightly curved cells, approximately 0.5–0.7 µm × 1–1.5 µm in size, motile by single polar flagella. Facultatively anaerobic. No endospores are formed.

Table 2. Total fatty acid contents (%) of strain HHS02^T and related species

Taxa: 1, HHS02^T; 2, *V. parahaemolyticus* LMG 2850^T; 3, *V. natriegens* LMG 10935^T; 4, *V. brasiliensis* LMG 20546^T; 5, *V. xuii* LMG 21346^T; 6, *V. nereis* LMG 3895^T. Fatty acids that were found in trace quantities (<1.00 %) in all the strains tested or were not found in all the strains of each species are not shown. —, Not detected. All strains were grown in marine 2216E broth at 25 °C for 48 h prior to fatty acid analysis.

Fatty acid	1	2	3	4	5	6
C _{12:0}	2.9	2.8	2.8	3.0	3.8	3.5
C _{14:0}	4.0	5.1	6.8	6.2	5.1	7.5
C _{16:0}	22.9	24.5	23.5	24.6	21.0	16.1
C _{17:0}	0.9	2.2	1.5	0.7	1.0	1.2
C _{17:1ω8c}	0.6	1.7	2.3	0.5	0.6	2.7
C _{18:0}	6.2	3.3	4.9	1.7	6.1	1.6
C _{18:1ω7c}	12.5	22.4	13.7	20.0	17.0	21.0
iso-C _{10:0}	—	—	1.3	—	—	—
iso-C _{11:0}	—	—	1.6	—	—	—
iso-C _{15:0} 3-OH	—	—	2.4	—	—	—
iso-C _{16:0}	2.1	1.5	0.8	1.2	2.6	0.9
C _{12:0} 3-OH	1.2	2.0	2.4	2.6	3.1	3.5
Summed features*						
2	4.3	1.4	1.2	1.2	1.3	1.3
3	38.7	28.9	34.1	33.8	35.6	36.7

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 2 consisted of C_{14:0} 3-OH and/or iso-C_{16:1} I; summed feature 3 consisted of C_{16:1ω7c} and/or iso-C_{15:0} 2-OH.

Colonies on marine 2216 agar are non-pigmented, non-luminescent, circular, smooth and convex with an entire edge. Grows on thiosulphate citrate bile salt sucrose (TCBS) agar as bright yellow colonies, does not produce H₂S from thiosulphate and is sensitive to the vibriostatic agent O/129 (at 10 and 150 µg). Growth occurs with 0.5–7 % (w/v) NaCl and optimally in 1–3 % (w/v) NaCl. Grows between pH 7.0 and 10.0 (optimally at pH 8.0–9.0). Grows between 16 and 30 °C (optimum 20 °C). No growth is observed at 4 °C or 37 °C. D-Glucose, sucrose, mannitol (from API 20E) and cellobiose (from VITEK 2 GN) are fermented, but no fermentation of D-sorbitol, maltose, D-mannose, D-tagatose, L-arabinose, L-arabitol, adonitol, L-rhamnose, inositol, melibiose or amygdalin. Positive reactions in tests for oxidase, urease, amylase, gelatinase, caseinase, phosphatase, lipase, nitrate reductase activity, DNase, β-galactosidase, L-proline arylamidase, β-N-acetylglucosaminidase and β-N-acetylgalactosaminidase, but not for swarming mobility, citrate utilization, indole production, catalase, the Voges–Proskauer reaction, elastase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, L-pyrrolydonyl-arylamidase, ala-phe-pro-arylamidase, tyrosine arylamidase, glutamyl arylamidase pNA, β-glucosidase, β-xylosidase, glycine arylamidase, glu-gly arg-arylamidase or α-galactosidase. The major cellular

fatty acids are C_{16:0}, C_{18:1ω7c}, summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH), C_{18:0}, C_{14:0} and C_{12:0}.

The type strain, HHS02^T (=CGMCC 1.8461^T=LMG 24781^T), was isolated from the digestive tract of healthy Chinese prawns from Qingdao, P. R. China. The DNA G + C content of the type strain is 44.4 mol% (determined by HPLC).

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