Vibrio hangzhouensis sp. nov., isolated from sediment of the East China Sea

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Strain CN83^T, a Gram-negative, aerobic, rod-shaped bacterium, was isolated from sediment of the East China Sea. The isolate was catalase- and oxidase-positive and cells were motile by means of polar flagella. The DNA G+C content was 44.9 mol%. The major fatty acids were $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, $C_{16:0}$, $C_{18:1}\omega7c$, $C_{14:0}$ and $C_{12:0}$. 16S rRNA gene sequence analysis showed that strain CN83^T belonged to the genus *Vibrio* and had the highest sequence similarity to *Vibrio agarivorans* (98.4 %) and *Vibrio campbellii* (97.8 %). Phylogenetic analysis revealed that strain CN83^T formed a monophyletic clade adjacent to the type strain of *V. agarivorans*. The DNA–DNA hybridization values of strain CN83^T with *V. agarivorans* DSM 13756^T and *V. campbellii* DSM 19270^T were 44.6 and 25.5 %, respectively. On the basis of the phenotypic and genotypic data, strain CN83^T represents a novel species of the genus *Vibrio*, for which the name *Vibrio hangzhouensis* sp. nov. is proposed. The type strain is CN83^T (=CGMCC 1.7062^T=JCM 15146^T).

Species of *Vibrio* Pacini 1854 are common inhabitants of aquatic environments and are often found associated with various organisms ranging from plankton to animals (Thompson *et al.*, 2004a). Several species are pathogenic for humans and animals (Farmer & Hickman-Brenner, 2006). In 1980, the genus *Vibrio* encompassed nine recognized species (Skerman *et al.*, 1980). Subsequently four species were reclassified to other genera, including *Wolinella succinogenes*, *Listonella anguillarum*, *Salinivibrio costicola* and *Aliivibrio fischeri* (Tanner *et al.*, 1981; MacDonell & Colwell, 1985; Mellado *et al.*, 1996; Urbanczyk *et al.*, 2007). However, more *Vibrio* species were described during the past three decades. At the time of writing, the genus *Vibrio* included 68 species with validly published names (Farmer *et al.*, 2005; Euzéby, 1997). Here we present a polyphasic study describing a novel *Vibrio* strain isolated from sediment of the East China Sea.

The sediment sample was collected by using a multicorer from the East China Sea (120° 34′ 29″ E, 27° 19′ 57″ N) at a depth of 49 m. An approximately 100 mg subsample was suspended in 3 ml sterile seawater and vortexed for 15 min. The dispersed sediment suspension was plated on modified ZoBell agar plates using a tenfold dilution series method at 25 °C for several days (ZoBell, 1941; Huo *et al.*, 2008). Colonies were picked and purified after three subcultures. Purity was confirmed by the uniformity of colony morphology. An isolate that formed non-pigmented colonies was obtained and designated strain CN83^T. The isolate was cultured routinely on marine agar 2216 (MA; Difco) and maintained as a glycerol suspension (30 %, v/v) at −80 °C.

The 16S rRNA, *gyrB*, *gapA*, *rpoD*, *pyrH* and *recA* genes were amplified and analysed as described by Lawrence *et al.*

Abbreviations: *gapA*, glyceraldehyde-3-phosphate dehydrogenase; *gyrB*, DNA gyrase B subunit; *pyrH*, uridylate kinase; *recA*, recombinase A; *rpoD*, RNA polymerase σ^{70}.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CN83^T is EU082035. The accession numbers for the *gyrB*, *gapA*, *rpoD*, *pyrH* and *recA* gene sequences of strain CN83^T and *Vibrio agarivorans* DSM 13756^T are FJ436361–FJ436366, FJ436368, FJ463225, FJ178183 and FJ178184. The accession number for the *rpo*D gene sequence of *Vibrio campbellii* DSM 19270^T is FJ436367.

Tables showing the fatty acid compositions of strain CN83^T (*V. hangzhouensis* sp. nov.) and *V. agarivorans* DSM 13756^T and differential characteristics of strain CN83^T and related *Vibrio* species and phylogenetic trees based on 16S RNA gene sequences using maximum-parsimony and 16S rRNA, *gyrB*, *gapA*, *pyrH*, *rpoD* and *recA* gene sequences using neighbour-joining are available as supplementary material with the online version of this paper.

(1991), Yamamoto & Harayama (1995, 1998), Thompson *et al.* (2004b, 2005) and Xu *et al.* (2007). PCR products were cloned into pMD 19-T vector (TaKaRa) and then sequenced. An almost complete 16S rRNA gene sequence (1475 nt) was obtained and compared with closely related sequences of reference organisms from the FASTA and EzTaxon service (Chun *et al.*, 2007). Sequence data were aligned with CLUSTAL W 1.8 (Thompson *et al.*, 1994). Phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods (Fitch, 1971) with the MEGA 4 program package (Tamura *et al.*, 2007). Evolutionary distances were calculated according to the algorithm of the Kimura two-parameter model (Kimura, 1980) for the neighbour-joining method.

Comparisons of 16S rRNA gene sequences showed that strain CN83^T should be positioned within the genus *Vibrio*, being most closely related to the type strain of *V. agarivorans* (98.4 % similarity); the sequence similarities with respect to type strains of other recognized *Vibrio* species were 94.1–97.8 %. Phylogenetic analysis based on the neighbour-joining method showed that strain CN83^T formed a monophyletic clade adjacent to the type strain of *V. agarivorans* with high bootstrap support (93 %) (Fig. 1). The topologies of the phylogenetic trees built using the maximum-parsimony method also supported the notion that strain CN83^T formed a stable clade with the type strain of *V. agarivorans* (see Supplementary Fig. S1 in IJSEM Online). Analysis of the *gyrB*, *gapA*, *rpoD*, *pyrH* and *recA* genes also supported the phylogenetic position of strain CN83^T within the genus *Vibrio* (Supplementary Fig. S2, in IJSEM Online).

The optimal conditions for growth were determined in PY broth (Shieh *et al.*, 2000) with different NaCl concentrations (0, 0.5, 1, 3, 5, 7.5, 10, 15, 20 and 30 %, w/v). The pH range for growth was determined by adding MES (pH 5.0–6.0),

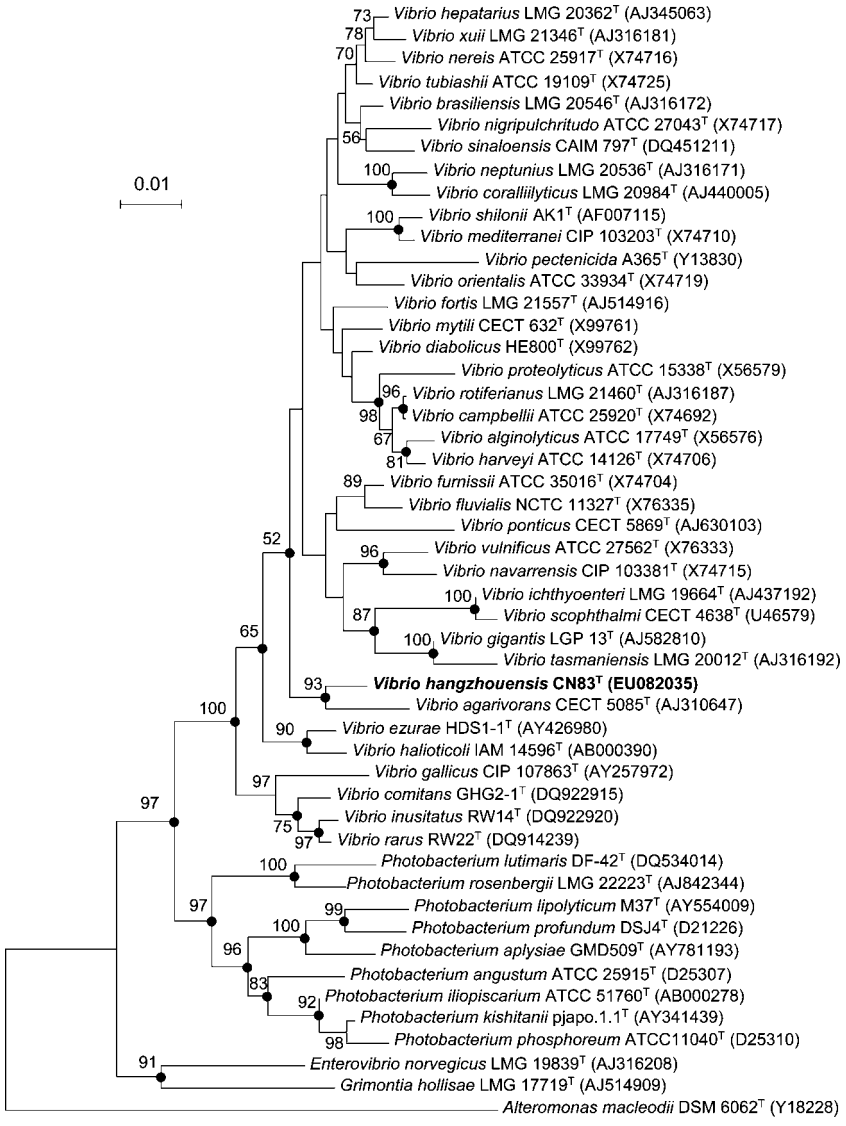

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of the novel isolate and related taxa. Bootstrap values are based on 1000 replicates; only values >50 % are shown. Bar, 0.01 substitutions per nucleotide position. Filled circles indicate that the corresponding nodes were also recovered with bootstrap values >50 % in the maximum-parsimony tree.

PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5) and CAPSO (pH 9.0–10.5) to PY broth at a concentration of 50 mM. The temperature range for growth was determined after 8 h and 2 days incubation at 4, 10, 15, 20, 25, 30, 35, 37, 42, 48 and 55 °C. Cell morphology and motility were examined by optical microscopy (BX40, Olympus) and electron microscopy (S260, Cambridge; JEM-1230, JEOL). The NaCl concentration, pH and temperature ranges for growth of strain CN83^T were 0.5–7.5 % (w/v), pH 6.0–10.0 and 20–37 °C, respectively. Cells of strains CN83^T were Gram-negative rods and motile by means of polar flagella (Fig. 2).

Biochemical tests were performed using the methods described by Macián *et al.* (2001a, b). Single carbon source assimilation tests were performed using the basal medium of Baumann and Baumann (BM medium; Farmer & Hickman-Brenner, 2006). The BM medium contained (l^{-1} distilled water): 1.0 g NH_4Cl, 0.044 g K_2HPO_4, 0.028 g $FeSO_4.7H_2O$, 500 ml artificial seawater, 100 ml Tris/HCl (1 M, pH 7.5). The artificial seawater contained (l^{-1} distilled water): 23.4 g NaCl, 24.6 g $MgSO_4.7H_2O$, 1.5 g KCl and 2.9 g $CaCl_2$. Another basal medium (Kämpfer *et al.*, 1991) was used to confirm assimilation of substrates. The basal medium contained (l^{-1} distilled water): 9.0 g NaCl, 0.5 g $MgSO_4.7H_2O$, 0.1 g $CaCl_2.2H_2O$, 1.74 g K_2HPO_4, 1.36 g KH_2PO_4, 5 g $(NH_4)_2SO_4$, 0.02 g yeast extract (Difco), 0.02 g peptone (Difco), 1 ml vitamin mixture solution, 5 ml mineral mixture solution and 25 mM PIPES, pH 7.2. Acid production was performed using the MOF medium supplemented with 1 % sugars or alcohols (Leifson, 1963). Sensitivity to antimicrobial agents was determined in marine broth 2216 (Difco) containing each antimicrobial agent at 50 mg l^{-1} for at least 3 days. Additional enzyme activities and biochemical characteristics were determined using API 20E, API 20 NE and API ZYM kits at 30 °C as recommended by the manufacturer (bioMérieux). *V. agarivorans* DSM 13756^T and *V. campbellii* DSM 19270^T were used as controls in the tests. Detailed results are given in the species description.

The genomic DNA G+C content was determined by using thermal denaturation (T_m) (Marmur & Doty, 1962) with *Escherichia coli* K-12 DNA as calibration standard. Cellular fatty acid methyl esters obtained from cells grown in MA for 24 h at 30 °C were analysed by using GC/MS (Kuykendall *et al.*, 1988), according to the instructions of the Microbial Identification System (MIDI Inc.). The major fatty acids of strain CN83^T were $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH (34.1 %), $C_{16:0}$ (21.1 %), $C_{18:1}\omega7c$ (18.3 %), $C_{14:0}$ (11.8 %) and $C_{12:0}$ (5.1 %). This profile was different from that of *V. agarivorans* DSM 13756^T (Supplementary Table S1, in IJSEM Online).

DNA–DNA hybridizations were performed by using the thermal denaturation and renaturation method of De Ley *et al.* (1970) as modified by Huß *et al.* (1983), using a Beckman DU 800 Spectrophotometer. The hybridization temperature (70 °C) was calculated from the DNA G+C content with the formula of De Ley *et al.* (1970). The levels of DNA–DNA relatedness of 44.6 and 25.5 % between strain CN83^T and *V. agarivorans* DSM 13756^T and *V. campbellii* DSM 19270^T were significantly below the value of 70 % that is considered to be the threshold for the delineation of species (Wayne *et al.*, 1987). Additionally, strain CN83^T could be differentiated from *V. agarivorans* DSM 13756^T on the basis of several phenotypic characteristics (Table 1 and Supplementary Table S2).

On the basis of the phenotypic and phylogenetic data presented in this study, strain CN83^T represents a novel species within the genus *Vibrio*, for which the name *Vibrio hangzhouensis* sp. nov. is proposed.

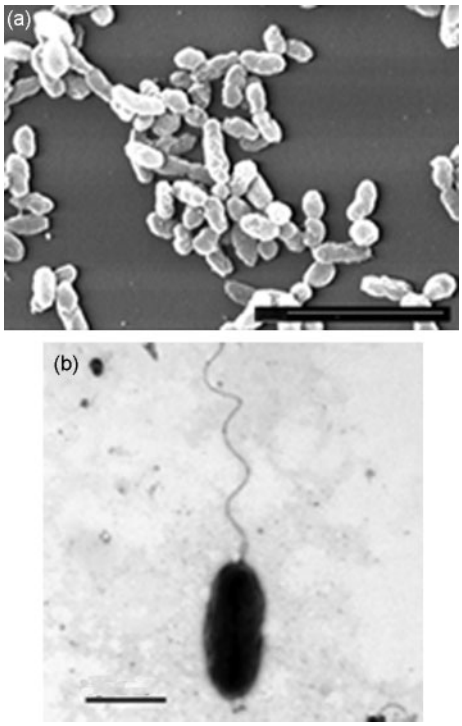

Fig. 2. Electron micrographs of cells of strain CN83^T. (a) Scanning electron micrograph of exponentially growing cells, 0.6–0.8×1.2–2.0 μm in size; (b) transmission electron micrograph showing a slightly ovoid rod with polar flagellum. Bars, 5 μm (a) and 1 μm (b).

Description of *Vibrio hangzhouensis* sp. nov.

Vibrio hangzhouensis (hang.zhou.en′sis. N.L. masc. adj. *hangzhouensis* pertaining to Hangzhou, a city in eastern China, near where the sample from which the type strain was isolated was collected).

Gram-negative rods, motile by means of polar flagella. Cells are straight to slightly curved and rod-shaped (0.5–0.8 μm in width and 1.0–2.0 μm in length) with rounded ends. No endospores are formed. Colonies on MA are

Table 1. Phenotypic characteristics that differentiate strain CN83^T from other related *Vibrio* species

Strains: 1, CN83^T (*V. hangzhouensis* sp. nov.); 2, *V. agarivorans* DSM 13756^T; 3, *V. campbellii* DSM 19270^T. +, Positive; −, negative; w+, weakly positive.

Characteristic	1	2	3
Growth on MacConkey agar	+	−	−
Indole production	+	−	+
Lecithinase	−	−	+
Hydrolysis of:			
Agar	−	+	−
Casein	−	−	+
DNA	−	−	+
Tween 80	−	w+	+
Tyrosine	+	−	+
Utilization of:			
Acetate	+	+	−
L-Alanine	+	−	+
Citrate	+	−	+
D-Galactose	+	+	−
Gluconate	+	−	+
Glycine	+	−	+
L-Histidine	+	−	+
Lactate	+	−	+
Lactose	+	+	−
D-Mannose	+	−	+
L-Ornithine	+	−	+
Propionate	+	−	−
Ribose	+	−	+
Sucrose	+	−	−
Xylose	−	+	−
Acid production from:			
Lactose	+	+	−
D-Mannose	+	−	+
Rhamnose	−	+	−
Ribose	+	−	+
Sucrose	+	−	−
Trehalose	+	−	+
Xylose	−	+	−
Sensitive to:			
Ampicillin	−	+	−
Nalidixic acid	−	+	−
Neomycin	−	+	−
Polymyxin B	−	+	−
API ZYM			
N-Acetyl-β-glucosaminidase	−	+	+
α-Chymotrypsin	−	+	−
Cystine arylamidase	−	+	+
β-Galactosidase	−	−	w+
Lipase (C14)	−	+	w+
Leucine arylamidase	−	+	+
Trypsin	−	+	−
Valine arylamidase	−	+	+

1–2 mm in diameter, smooth and circular, with slightly irregular borders and non-pigmented after 48 h. Growth occurs at NaCl concentrations of 0.5–7.5 % (w/v) with optimum growth with 3.0 %. pH and temperature ranges for growth are pH 6.0–10.0 and 20–37 °C (optimum growth at pH 7.0–8.0 and 30 °C). No growth is detected below 15 or above 42 °C. Growth occurs on MacConkey agar (red colonies). No growth occurs on Cetrimide agar. Positive for oxidase and catalase. Nitrate is reduced to nitrite but not further to N_2O or N_2. Aesculin, gelatin, starch, Tween 20 and tyrosine are hydrolysed. Agar, casein, DNA and Tween 80 are not hydrolysed. H_2S is produced from thiosulfate or L-cysteine. Glucose fermentation, indole production and *o*-nitrophenyl-β-D-galactopyranosidase are positive. Negative for arginine dihydrolase, lecithinase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease and Voges–Proskauer reaction. The follow constitutive enzyme activities are detected in API ZYM tests: acid and alkaline phosphatases, esterase (C4), esterase lipase (C8) and naphthol-AS-β-1-phosphohydrolase. *N*-Acetyl-β-glucosaminidase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α- and β-galactosidases, α- and β-glucosidases, β-glucuronidase, lipase (C14), leucine arylamidase, α-mannosidase, trypsin and valine arylamidase activities are not observed. Chemo-organotrophic. The following compounds are utilized as sole carbon and energy sources: acetate, L-alanine, L-arginine, L-asparagine, L-aspartate, D-cellobiose, citrate, L-cysteine, D-fructose, fumarate, D-galactose, D-gluconate, glucose, L-glutamate, glycerol, glycine, L-glutamine, L-histidine, lactate, lactose, malate, maltose, D-mannitol, D-mannose, L-ornithine, L-proline, propionate, pyruvate, ribose, L-serine, succinate, sucrose and D-trehalose. The following compounds are not utilized as sole carbon and energy sources: adonitol, L-arabinose, ethanol, formate, inositol, L-isoleucine, L-lysine, malonate, L-methionine, L-rhamnose, ribitol, L-sorbitol, sorbose, L-valine, xylitol and xylose. Acid is produced from D-fructose, D-galactose, glucose, lactose, maltose, D-mannitol, D-mannose, ribose, sucrose and trehalose. Susceptible to chloramphenicol, erythromycin, nitrofurantoin, novobiocin and the vibriostatic agent O/129; not susceptible to ampicillin, bacitracin, carbenicillin, cefotaxime, kanamycin, nalidixic acid, neomycin, nystatin, polymyxin B, streptomycin and tetracycline. Principal fatty acids (greater than 5 %) are $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, $C_{16:0}$, $C_{18:1}\omega7c$, $C_{14:0}$ and $C_{12:0}$. The DNA G+C content of the type strain is 44.9 mol% (T_m).

The type strain, CN83^T (=CGMCC 1.7062^T=JCM 15146^T), was isolated from a marine sediment sample from Zhejiang, China.

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