Vibrio fortis sp. nov. and *Vibrio hepatarius* sp. nov., isolated from aquatic animals and the marine environment

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In this study, the taxonomic positions of 19 *Vibrio* isolates disclosed in a previous study were evaluated. Phylogenetic analysis based on 16S rDNA sequences partitioned these isolates into groups that were closely related (98·8–99·1 % similarity) to *Vibrio pelagius* and *Vibrio xuii*, respectively. DNA–DNA hybridization experiments further showed that these groups had <70 % similarity to other *Vibrio* species. Two novel *Vibrio* species are proposed to accommodate these groups: *Vibrio fortis* sp. nov. (type strain, LMG 21557^T = CAIM 629^T) and *Vibrio hepatarius* sp. nov. (type strain, LMG 20362^T = CAIM 693^T). The DNA G+C content of both novel species is 45·6 mol%. Useful phenotypic features for discriminating *V. fortis* and *V. hepatarius* from other *Vibrio* species include production of indole and acetoin, utilization of cellobiose, fermentation of amygdalin, melibiose and mannitol, β-galactosidase and tryptophan deaminase activities and fatty acid composition.

INTRODUCTION

Vibrios are among the most abundant culturable microbes in aquatic environments (Heidelberg *et al.*, 2002a). A recent study of the bacterioplankton of Chesapeake Bay showed that *Vibrio* and *Photobacterium* species comprised up to 4 % (2×10^8 cells l^{-1}) of total bacteria (Heidelberg *et al.*, 2002a). High *Vibrio* and *Photobacterium* numbers ($4{\cdot}3\times10^6$ mm^{-2}) were also reported to be attached to the external surface of zooplankton. It was concluded that a close partnership exists between these bacteria and zooplankton (Heidelberg *et al.*, 2002b; Lipp *et al.*, 2002). Vibrios also belong to the normal microflora of the shrimp *Litopenaeus vannamei* (Vandenberghe *et al.*, 1999). Moss *et al.* (2000) reported that *Vibrio* and *Aeromonas* species comprised up to 85 % [about 10^9 c.f.u. (g gut tissue)$^{-1}$] of bacterial flora in the gut of this shrimp, whereas Gomez-Gil *et al.* (1998) found a high abundance of vibrios (10^5 c.f.u. g^{-1} and 10^4 c.f.u. ml^{-1}, respectively) in the hepatopancreas and haemolymph of healthy *L. vannamei*. Certain *Vibrio* strains have been reported to be potential probiotics for this shrimp (Gomez-Gil *et al.*, 1998, 2000, 2002). Use of probiotics, i.e. live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host, has been reported to reduce the need for medication (e.g. antibiotics and pesticides) and water exchange, which are used massively in intensive shrimp-rearing (Verschuere *et al.*, 2000).

We have demonstrated that the genus *Vibrio* harbours a wealth of diverse genomes and represents cosmopolitan and endemic species that are yet to be described (Thompson *et al.*, 2001). The exact ecological role of several of these groups is unknown at present. In this study, we report the taxonomic characterization of FAFLP (fluorescent amplified fragment length polymorphism) clusters A9, A26 and A60 that were disclosed in a former study (Thompson *et al.*, 2001). Group A9 ($n=8$) was found to be ubiquitous in the marine environment and was associated with both diseased and healthy aquatic animals. Group A26 consisted of three isolates that originated from the hepatopancreas of wild healthy adults of *L. vannamei* from Ecuador. Recent results suggest that these isolates may have probiotic properties for *L. vannamei* under culture conditions (M. Gullian & J. Rodriguez, unpublished data). A representative *Vibrio* strain, LMG 20362^T, showed high levels of colonization in the hepatopancreas of *L. vannamei* and out-competed and excluded the shrimp pathogen *Vibrio harveyi*. Additionally,

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Abbreviations: FAFLP, fluorescent amplified fragment length polymorphism; FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of strains LMG 21557^T and LMG 20362^T are AJ514916 and AJ345063, respectively.

Tables of variable features among strains of *V. fortis* and *V. hepatarius* are available as supplementary material in IJSEM Online.

this strain seems to enhance the health and weight of shrimps (M. Gullian & J. Rodriguez, unpublished data). Group A60 ($n=8$) was restricted to cultures of bivalve larvae (*Nodipecten nodosus*) in south Brazil. We propose to accommodate isolates of groups A9 and A60 in a novel *Vibrio* species, *Vibrio fortis* sp. nov., and isolates of group A26 in another novel species, *Vibrio hepatarius* sp. nov.

METHODS

Bacterial strains, growth conditions and DNA isolation. Strains characterized in this study are listed in Table 1. Strains were grown aerobically on tryptone soy agar (TSA; Oxoid) supplemented with 2 % (w/v) NaCl for 24 h at 28 °C. DNA was extracted by following the methodology described by Pitcher *et al.* (1989). All strains included in this study have been deposited in the BCCM/LMG Bacteria Collection at Ghent University, Belgium, and in the CAIM collection of the Centre for Research on Nutrition and Development (CIAD) in Mazatlán, Mexico.

Genotypic analyses. Determination of almost-complete 16S rDNA sequences was accomplished essentially as described previously (Thompson *et al.*, 2001). Alignment of 16S rDNA sequences, distance estimations (Jukes & Cantor, 1969), clustering by the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood and maximum-parsimony methods and calculation of cluster stability (bootstrap analysis with 1000 replicates) were performed with BioNumerics 2.5 software (Applied Maths). DNA–DNA hybridization experiments with photobiotin-labelled DNA were run under stringent conditions (39 °C) following the methodology described by Willems *et al.* (2001). Hybridizations were performed in four replicates. DNA binding values are means of reciprocal and non-reciprocal reactions. The DNA G+C content (mol%) was determined by HPLC (Mesbah *et al.*, 1989).

Phenotypic analyses. Phenotypic characterization of the isolates was performed by using API 20E and API ZYM kits (bioMérieux) and Biolog GN metabolic fingerprinting, following the instructions of the manufacturers with slight modifications (Thompson *et al.*, 2002). Classical phenotypic tests were performed as described previously (Baumann *et al.*, 1984; Farmer & Hickman-Brenner, 1992; Vandamme *et al.*, 1998; Thompson *et al.*, 2002). Antibiograms were determined by using the disc-diffusion method (Acar & Goldstein, 1996) with commercial discs (Oxoid). The inhibition zone of each antibiotic was measured with strains grown on Iso-Sensitest agar (Oxoid) supplemented with 1·5 % (w/v) NaCl for 24 h at 28 °C. Fatty acid methyl ester (FAME) analysis was carried out as described by Huys *et al.* (2001). Isolates were grown on Trypticase Soy Broth (Becton Dickinson) supplemented with 1·5 % (w/v) Bacto agar (Becton Dickinson) and 1·5 % (w/v) NaCl.

RESULTS AND DISCUSSION

The phylogenetic positions of five strains representative of *V. fortis*, LMG 21557^T (GenBank/EMBL accession no. AJ514916), LMG 21558 (AJ514913), LMG 20547 (AJ316202), LMG 21566 (AJ514917) and LMG 21562 (AJ514915), from FAFLP groups A9 and A60, were analysed by means of their 16S rDNA sequences. These isolates had nearly identical 16S rDNA sequences (Fig. 1) and their closest neighbour was *Vibrio pelagius* CECT 4202^T (AJ293802) with 98·8 % sequence similarity. When the *V. pelagius* sequence of Ruimi *et al.* (1994) was used, i.e. X74722, similarity between the five novel isolates and *V. pelagius* dropped to

Table 1. List of strains used in this study

Strain	Source
Vibrio fortis (FAFLP cluster A9):	
LMG 21557^T (=CAIM 629^T=R-15032^T=STD3-1247^T)	CENAIM (Ecuador), 1996; shrimp larvae (*Litopenaeus vannamei*)
LMG 21558 (=CAIM 45=R-15033=STD3-931)	Sinaloa (Mexico), 1994: sea water
LMG 21556 (=CAIM 628=R-15031=INCO 297); LMG 21560 (=CAIM 631=R-15035=INCO 305)	LCMM Florianópolis (Brazil), 1999: healthy bivalve larvae (*Nodipecten nodosus*)
LMG 21559 (=CAIM 630=R-15034=VIB 839)	MPL (Tasmania), 1990s: Atlantic salmon (*Salmo salar*)
LMG 21561 (=CAIM 632=R-15036=INCO 257)	LCMM Florianópolis (Brazil), 1999: diseased bivalve larvae (*Nodipecten nodosus*)
LMG 21562 (=CAIM 633=R-15037=INCO 31); LMG 21563 (=CAIM 634=R-15038=INCO 35)	Guernsey Sea Farm (UK), 1998: diseased oyster larvae (*Crassostrea gigas*)
(FAFLP cluster A60):	
LMG 20547 (=CAIM 635=INCO 318); LMG 21564 (=CAIM 637=R-14861=INCO 336); LMG 21565 (=CAIM 638=R-14862=INCO 409); LMG 21566 (=CAIM 639=R-14863=INCO 386); LMG 21567 (=CAIM 640=R-14865=INCO 312); LMG 21568 (=CAIM 641=R-14866=INCO 311); LMG 21569 (=CAIM 642=R-14867=INCO 406); LMG 21570 (=CAIM 643=R-14868=INCO 384)	LCMM Florianópolis (Brazil), 1999: bivalve larvae (*Nodipecten nodosus*)
V. hepatarius (FAFLP cluster A26):	
LMG 20362^T (=CAIM 693^T=1P^T=P62^T); LMG 20366 (=CAIM 694=5P); LMG 20371 (=CAIM 695=2C)	Manglaralto (Ecuador), 2000: hepatopancreas of wild adult *P. vannamei*

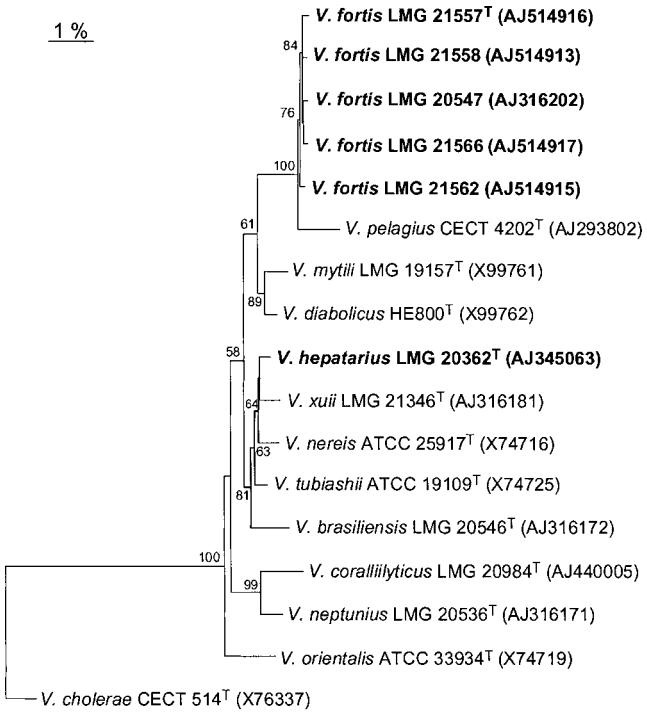

Fig. 1. Phylogenetic tree showing the positions of *V. fortis* and *V. hepatarius*, estimated by using the neighbour-joining method and based on almost-complete 16S rDNA sequences. Bootstrap values (>50 %) after 1000 simulations are shown. Bar, 1 % estimated sequence divergence.

97·4 %. Macián *et al.* (2000) have already suggested that the latter GenBank/EMBL entry is most probably a sequence of *Vibrio natriegens*, rather than *V. pelagius*. Other phylogenetic neighbours of *V. fortis*, with a maximum of 97·8 % similarity, are shown in Fig. 1. *Vibrio cholerae*, *Vibrio mimicus* and *Vibrio metschnikovii* were the most distant phylogenetic relatives of *V. fortis* within the genus *Vibrio*, with 92·5–92·9 % similarity. *V. hepatarius* LMG 20362^T was most closely related to the recently described species *Vibrio xuii* (99·1 % similarity) (Thompson *et al.*, 2003) and to *Vibrio tubiashii* and *Vibrio nereis* (98·6–99·0 %) (Fig. 1). *V. hepatarius* was also related to *Vibrio mytili*, *Vibrio diabolicus* and *Vibrio orientalis* (98·0–98·2 %) and its most distant relatives within the genus *Vibrio* were *V. mimicus* and *Vibrio salmonicida* (92·9 %).

Results of DNA–DNA hybridization experiments are summarized in Table 2. We chose four representative isolates from cluster A9, as this group was found to be very heterogeneous by FAFLP analysis, and two representative isolates from group A60, which was a very tight FAFLP cluster (Thompson *et al.*, 2001). These six *V. fortis* isolates formed a single novel species with at least 70 % DNA–DNA similarity, and a maximum of 66 % similarity towards *V. pelagius*. *V. hepatarius* LMG 20362^T had a maximum of 66 % similarity towards *V. orientalis*. Both novel species had <45 % DNA–DNA similarity to the recently described species *Vibrio coralliilyticus* (Ben-Haim *et al.*, 2003) and to *Vibrio neptunius*, *Vibrio brasiliensis* and *V. xuii* (Thompson *et al.*, 2003). These results corroborate

Table 2. DNA–DNA binding values and DNA G+C contents of *Vibrio* strains examined

Taxa: 1, *V. fortis* LMG 21557^T; 2, *V. fortis* LMG 21558; 3, *V. fortis* LMG 21562; 4, *V. fortis* LMG 21561; 5, *V. fortis* LMG 20547; 6, *V. fortis* LMG 21565; 7, *V. pelagius* LMG 3897^T; 8, *V. coralliilyticus* LMG 20984^T; 9, *V. neptunius* LMG 20536^T; 10, *V. brasiliensis* LMG 20546^T; 11, *V. xuii* LMG 21346^T; 12, *V. hepatarius* LMG 20362^T; 13, *V. diabolicus* LMG 19805^T; 14, *V. tubiashii* LMG 10936^T; 15, *V. nereis* LMG 3895^T; 16, *V. orientalis* LMG 7897^T; 17, *V. mytili* LMG 19157^T.

Taxon	G+C content (mol%)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	45·6	100																
2	45·3	95	100															
3	45·6	78	85	100														
4	45·8	70		81	100													
5	45·9	78	89	100	83	100												
6	45·9	71		91	85	98	100											
7	45·7	58		65	66	66	65	100										
8	45·6	34	40	33		30			100									
9	46·0	30	37	30		27				100								
10	45·9	35	44	34		34			39	40	100							
11	46·6	30	38	29		27			34	32	39	100						
12	45·5	23				25			40	34	42	30	100					
13	45·6	24				25							28	100				
14	44·8	20				23							29	24	100			
15	45·9	23				26							30	32	26	100		
16	45·6	23				26							66	27	30	32	100	
17	44·6	20				22							22	33	20	26	22	100

those of our previous FAFLP fingerprinting experiments, which suggested that groups A9, A26 and A60 were novel species within the genus *Vibrio* (Thompson *et al.*, 2001).

Both novel *Vibrio* species represented by the 19 isolates examined in this study shared the main phenotypic and chemotaxonomic features of the genus *Vibrio* (Lambert *et al.*, 1983; Farmer & Hickman-Brenner, 1992; Bertone *et al.*, 1996). They were facultatively anaerobic, oxidase-positive and showed prolific growth on thiosulfate/citrate/bile salts/sucrose agar (TCBS). Isolates were slightly curved rods, motile, susceptible to vibriostatic agent O/129 (except for LMG 21568 and LMG 21559) and their growth was stimulated by NaCl. In spite of their similarity to other *Vibrio* species, the two novel species showed several differentiating phenotypic features (Table 3). We therefore propose to accommodate A9 and A60 isolates in a novel species, *V. fortis* sp. nov., and A26 isolates in another, *V. hepatarius* sp. nov. Because several phylogenetic neighbours of *V. fortis* and *V. hepatarius* (i.e. *V. neptunius*, *V. brasiliensis*, *V. xuii* and *V. coralliilyticus*) were analysed phenotypically and described by using the same methodologies as in this study, we assume that these results are largely comparable. Similar phenotypic methodologies have also been applied to the description of *V. diabolicus* (Raguénès *et al.*, 1997), *V. mytili* (Pujalte *et al.*, 1993) and the two novel species in this study, suggesting that comparisons may be reliable.

Description of *Vibrio fortis* sp. nov.

Vibrio fortis (for′tis. L. adj. *fortis* strong).

Cells are slightly curved, 1 μm in width and 3 μm in length. They form translucent to opaque, low convex, non-swarming, smooth-rounded colonies with entire margins that are beige in colour and about 4 mm in diameter on TSA after 48 h incubation at 28 °C. Strains form yellow and/or green, translucent, smooth-rounded colonies of 4–5 mm diameter on TCBS. All strains have a facultatively anaerobic metabolism and ferment glucose and mannitol,

Table 3. Features that diffentiate *V. fortis* and *V. hepatarius* from closely related *Vibrio* species

Taxa: 1, *V. fortis* ($n=16$); 2, *V. hepatarius* ($n=3$); 3, *V. neptunius* (Thompson *et al.*, 2003); 4, *V. brasiliensis* (Thompson *et al.*, 2003); 5, *V. xuii* (Thompson *et al.*, 2003); 6, *V. pelagius* (Alsina & Blanch, 1994); 7, *V. coralliilyticus* (Ben-Haim *et al.*, 2003); 8, *V. diabolicus* (Raguénès *et al.*, 1997); 9, *V. mytili* (Pujalte *et al.*, 1993); 10, *V. nereis* (Alsina & Blanch, 1994); 11, *V. orientalis* (Alsina & Blanch, 1994); 12, *V. tubiashii* (Alsina & Blanch, 1994). Fatty acid data are given as mean ± SD (%). Abbreviations: +, positive; −, negative; V, variable feature (followed by type strain feature); ND, no data available. Utilization of gentiobiose, fermentation of amygdalin and melibiose, enzyme activities and fatty acid profiles of known *Vibrio* species are from our own database.

Feature	1	2	3	4	5	6	7	8	9	10	11	12
Production of:												
Indole	+	+	+	+	+	−	+	+	−	V	+	+
Acetoin	V+	+	+	+	+	−	−	−	−	−	−	−
Utilization of:												
Cellobiose	+	+	−	+	+	−	−	−	+	−	+	+
D-Galactose	+	V+	−	+	−	+	+	+	+	−	+	+
Gentiobiose	+	V−	−	+	V	V	−	−	+	−	ND	−
Growth on 10 % (w/v) NaCl	V−	+	−	−	+	−	−	ND	+	+	+	V
Nitrate reduction	V+	+	+	+	+	+	+	+	+	+	+	+
Fermentation of:												
Mannitol	+	+	−	+	+	+	−	+	+	−	+	+
Amygdalin	V+	+	−	+	+	−	−	−	+	−	−	−
Melibiose	V+	−	−	−	−	−	−	−	−	−	−	V
Enzyme activity:												
β-Galactosidase	V+	−	−	+	−	+	+	−	+	−	−	+
Tryptophan deaminase	+	V+	+	−	+	−	−	+	+	−	−	−
FAME composition:												
iso-$C_{14:0}$	2·7±1·5	1·3±1·1	0·2±0·1	3·3±0·4	1·2±0·1	2·5	0·5	0·2	0·0	0·2	0·6	0·0
iso-$C_{14:0}$ 3-OH	1·1±0·6	0·8±0·6	0·1±0·1	1·3±0·2	0·9±0·1	1·0	0·3	0·3	0·3	0·3	0·0	0·0
$C_{16:0}$	18±7	20·0±2·8	18·0±0·8	11·3±0·3	12·5±0·6	12	15	14·4	18·8	12·9	27·6	17·3
iso-$C_{16:0}$	8·4±4·3	4·8±3·9	0·5±0·1	10·5±0·6	5·5±0·4	7·5	0·8	1·7	1·5	1·1	0·0	0·0
$C_{17:0}$	0·5±0·9	0·3±0·1	2·3±0·2	0·6±0·1	0·5±0·1	0·5	2·5	1·6	0·0	1·9	0·0	0·1
$C_{17:1}\omega 8c$	0·7±1·9	0·2±0·2	2·1±0·1	0·7±0·1	0·5	0·5	1·8	2·5	0·0	4·6	0·0	0·1
$C_{17:1}\omega 6c$	0·1±0·1	0·0	1·2±0·1	0·3±0·1	0·2	0·0	0·6	0·7	0·0	1·3	0·0	0·0
$C_{18:1}\omega 7c$	11·9±2·9	15·5±0·5	17·8±1·6	17·3±0·3	21·0±2·4	23	18·2	17·4	19·9	22·6	4·3	25·4

but not inositol, sorbitol or rhamnose. Growth occurs at 4–35 °C and in media that contain 1–8 % (w/v) NaCl. Prolific growth occurs at 28 °C in media that contain 2·5 % (w/v) NaCl. The following features are positive for all strains: oxidase, catalase, β-galactosidase and tryptophan deaminase activities and indole production. All strains utilize dextrin, glycogen, Tween 40, Tween 80, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, maltose, D-mannitol, D-mannose, psicose, D-sorbitol, sucrose, D-trehalose, methyl pyruvate, monomethyl succinate, D-gluconic acid, DL-lactic acid, succinic acid, L-alanine, L-alanylglycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-proline, L-serine, L-threonine, inosine, thymidine and glycerol as sole carbon sources. None of the strains can utilize adonitol, D-arabitol, *i*-erythritol, L-fucose, *m*-inositol, L-rhamnose, xylitol, citric acid, formic acid, D-galactonic acidolactone, D-glucuronic acid, α-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, malonic acid, D-saccharic acid, sebacic acid, succinamic acid, glucuronamide, alaninamide, hydroxy L-proline, L-phenylalanine, L-pyroglutamic acid, DL-carnitine, urocanic acid, phenylethylamine, 2-aminoethanol, 2,3-butanediol or glucose 1-phosphate as sole carbon sources. The following tests are negative for all strains: arginine dihydrolase, lysine and ornithine decarboxylase activities, H_2S production and urease activity. The most abundant fatty acids are summed feature 3 (37·5±2·9 %; comprises $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), $C_{16:0}$ (18·0±7·0 %), $C_{18:1}\omega7c$ (11·9±2·9 %), iso-$C_{16:0}$ (8·4±4·3 %), $C_{14:0}$ (5·3±1·4 %), $C_{12:0}$ (3·6±1·2 %), iso-$C_{14:0}$ (2·7±1·5 %), summed feature 2 (2·4±0·4 %; comprises $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I and/or unidentified fatty acid with equivalent chain-length value of 10·928 and/or $C_{12:0}$ ALDE), $C_{12:0}$ 3-OH (1·5±0·4 %), $C_{18:0}$ (1·2±0·6 %), iso-$C_{14:0}$ 3-OH (1·1±0·6 %), $C_{17:1}\omega8c$ (0·7±1·9 %), unknown 12·484 (0·6±0·2 %), iso-$C_{18:0}$ (0·6±0·3 %), $C_{15:0}$ (0·6±1·2 %), $C_{17:0}$ (0·5±0·9 %), iso-$C_{12:0}$ (0·4±0·2 %), summed feature 7 (0·4±0·2 %; comprises $C_{19:1}\omega6c$ and/or unidentified fatty acid with equivalent chain-length value of 18·846), iso-$C_{12:0}$ 3-OH (0·3±0·2 %), $C_{15:1}\omega8c$ (0·3±0·9 %), $C_{20:1}\omega7c$ (0·2±0·1 %), $C_{16:1}\omega5c$ (0·2±0·1 %), $C_{16:1}\omega7c$ alcohol (0·2±0·1 %), unknown 11·799 (0·2±0·1 %), $C_{18:1}\omega5c$ (0·1±0·1 %), $C_{11:0}$ 3-OH (0·1±0·5 %), $C_{10:0}$ 3-OH (0·1±0·4 %), $C_{12:0}$ 2-OH (0·1±0·0 %), $C_{16:0}$ 3-OH (0·1±0·3 %), 11-methyl $C_{18:1}\omega7c$ (0·1±0·1 %), anteiso-$C_{17:0}$ (0·1±0·3 %), $C_{13:0}$ (0·1±0·2 %) and $C_{17:1}\omega6c$ (0·1±0·1 %). All strains are susceptible to tetracycline (30 μg) and chloramphenicol (30 μg), but are moderately resistant to polymyxin (300 U). Additional phenotypic features are listed in Supplementary Table A in IJSEM Online. 16S rDNA sequences of strains LMG 21557^T, LMG 21558, LMG 20547, LMG 21566 and LMG 21562 are deposited in GenBank/EMBL under accession numbers AJ514916, AJ514913, AJ316202, AJ514917 and AJ514915, respectively. DNA G+C content of the type strain is 45·6 mol%.

The type strain of this species, LMG 21557^T (=CAIM 629^T), was isolated from the white shrimp *Litopenaeus vannamei* in Ecuador.

Description of *Vibrio hepatarius* sp. nov.

Vibrio hepatarius (he.pa.ta′ri.us. L. masc. adj. *hepatarius* of or belonging to the liver).

Cells are slightly curved, 1 μm in width and 2–3 μm in length. They form translucent, convex, non-swarming, smooth-rounded colonies with entire margins that are beige in colour and about 6 mm in diameter on TSA after 48 h incubation at 28 °C. Strains form yellow, translucent, smooth-rounded colonies of 6 mm diameter on TCBS. All strains have a facultatively anaerobic metabolism and ferment glucose, mannitol, sucrose and amygdalin, but not inositol, rhamnose, melibiose or arabinose. Growth occurs at 4–35 °C and in media that contain 0–8 % (w/v) NaCl. Prolific growth occurs at 28 °C in media that contain 2·5 % (w/v) NaCl. The following tests are positive for all strains: oxidase and catalase activities, indole and acetoin production and NO_3 reduction. All strains utilize dextrin, *N*-acetyl-D-glucosamine, cellobiose, D-fructose, α-D-glucose, maltose, psicose, sucrose, D-trehalose, methyl pyruvate, inosine and glycerol as sole carbon sources. None of the strains can utilize glycogen, Tween 40, *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, *i*-erythritol, L-fucose, D-galactose, gentiobiose, *m*-inositol, α-lactose, α-D-lactose lactulose, D-melibiose, D-raffinose, L-rhamnose, turanose, xylitol, monomethyl succinate, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acidolactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, L-aspartic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, DL-α-glycerol phosphate, glucose 1-phosphate or glucose 6-phosphate as sole carbon sources. The following tests are negative for all strains: β-galactosidase, lysine and ornithine decarboxylases, H_2S production and urease. The most abundant fatty acids are summed feature 3 (28·4±15·2 %; comprises $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), $C_{16:0}$ (14·6±9·6 %), iso-$C_{15:0}$ (13·2±22·1 %), $C_{18:1}\omega7c$ (10·3±9·9 %), $C_{14:0}$ (5·4±1·4 %), iso-$C_{16:0}$ (4·7±2·7 %), iso-$C_{13:0}$ (3·7±5·4 %), iso-$C_{17:0}$ (2·5±3·7 %), $C_{12:0}$ (2·2±1·9 %), summed feature 2 (2·2±0·3 %; comprises $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I and/or unidentified fatty acid with equivalent chain-length value of 10·928 and/or $C_{12:0}$ ALDE), iso-$C_{14:0}$ (2·0±1·4 %), anteiso-$C_{15:0}$ (1·9±2·9 %), $C_{12:0}$ 3-OH (1·1±1·0 %), iso-$C_{17:1}\omega5c$ (1·1±1·9 %), iso-$C_{17:1}\omega10c$ (0·8±1·4 %), unknown 12·484 (0·5±0·5 %), iso-$C_{14:0}$

3-OH (0·5±0·6 %), anteiso-$C_{17:0}$ (0·5±0·6 %), anteiso-$C_{13:0}$ (0·5±0·8 %), $C_{16:1}\omega7c$ alcohol (0·4±0·6 %), $C_{15:0}$ (0·4±0·4 %), $C_{17:0}$ (0·2±0·2 %) and $C_{17:1}\omega8c$ (0·1±0·2 %). All strains are susceptible to the vibriostatic agent O/129 (10 and 150 μg), polymyxin (300 U), tetracycline (30 μg) and chloramphenicol (30 μg), but are moderately resistant to ampicillin (25 μg). Additional phenotypic features are listed in Supplementary Table B in IJSEM Online. The 16S rDNA sequence of strain LMG 20362^T is deposited in GenBank/EMBL under accession number AJ345063. DNA G+C content of the type strain is 45·5 mol%.

The type strain of this species, LMG 20362^T (=CAIM 693^T), was isolated from the white shrimp *Litopenaeus vannamei* in Ecuador.

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