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Vibrio neptunius sp. nov., *Vibrio brasiliensis* sp. nov. and *Vibrio xuii* sp. nov., isolated from the marine aquaculture environment (bivalves, fish, rotifers and shrimps)

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The fluorescent amplified fragment length polymorphism (FAFLP) groups A5 (21 isolates), A8 (6 isolates) and A23 (3 isolates) distinguished in an earlier paper (Thompson *et al.*, *Syst Appl Microbiol* 24, 520–538, 2001) were examined in more depth. These three groups were phylogenetically related to *Vibrio tubiashii*, but DNA–DNA hybridization experiments proved that the three AFLP groups are in fact novel species. Chemotaxonomic and phenotypic analyses further revealed several differences among the 30 isolates and known *Vibrio* species. It is proposed to accommodate these isolates in three novel species, namely *Vibrio neptunius* (type strain LMG 20536^T; EMBL accession no. AJ316171; G+C content of the type strain 46·0 mol%), *Vibrio brasiliensis* (type strain LMG 20546^T; EMBL accession no. AJ316172; G+C content of the type strain 45·9 mol%) and *Vibrio xuii* (type strain LMG 21346^T; EMBL accession no. AJ316181; G+C content of the type strain 46·6 mol%). These species can be differentiated on the basis of phenotypic features, including fatty acid composition (particularly 14 : 0 iso, 14 : 0 iso 3-OH, 16 : 0 iso, 16 : 0, 17 : 0 and 17 : 1ω8*c*), enzyme activities and utilization and fermentation of various carbon sources.

INTRODUCTION

It is well recognized that bacteria play a pivotal role in the cycling of dissolved and particulate organic matter in aquatic ecosystems (Sherr & Sherr, 2000). There has been increasing evidence that bacteria also fuel food webs in marine aquaculture systems and influence the health of cultured marine organisms (Hansen & Olafsen, 1999; Thompson *et al.*, 2002a). Vibrios are highly abundant in aquatic ecosystems, particularly in eutrophic environments, accounting for up to 14–45 % (i.e. 10^4–10^5 cells ml^{-1}) of the culturable microbiota (Eilers *et al.*, 2000; Suantika *et al.*, 2001). Moreover, vibrios are present in large numbers in a successful recirculating system for rotifers (Suantika *et al.*, 2001) and are also part of the normal flora of penaeid

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Abbreviations: FAFLP, fluorescent amplified fragment length polymorphism; FAME, fatty acid methyl ester; TCBS, thiosulphate/citrate/bile salts/sucrose; TSA, tryptone soy agar.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of LMG strains 20536^T, 20546^T, 21346^T, 20613, 20010 and 21347 are respectively AJ316171, AJ316172, AJ316181 and AJ490150–AJ490152.

Additional phenotypic features of the three novel species are listed as supplementary material in IJSEM Online (http://ijs.sgmjournals.org/).

shrimps (Gomez-Gil *et al.*, 1998). Certain *Vibrio* strains stimulate reproduction and ameliorate growth rates of molluscs and rotifers and protect *Artemia* against bacterial infections, whereas other *Vibrio* strains constitute serious pathogens or potential pathogens for the same organisms (Riquelme *et al.*, 2001; Verschuere *et al.*, 2000).

Recently, we surveyed the genomic diversity of 506 strains of the *Vibrionaceae* by means of the fluorescent amplified fragment length polymorphism (FAFLP) technique (Thompson *et al.*, 2001). Many isolates from the aquaculture environment possess genomes that differ from currently known *Vibrio* species and are thus potentially novel species. In the present study, we describe additional genomic and phenotypic characteristics of a subset of 30 isolates distributed in the FAFLP groups A5, A8 and A23. FAFLP cluster A5 represented mainly the dominant culturable bacterial microflora of a recirculating system for rotifers (Suantika *et al.*, 2001). Group A8 was abundant in cultures of larvae of the bivalve *Nodipecten nodosus* at Florianópolis, in southern Brazil, whereas group A23 was found to be ubiquitous and in association with cultured shrimps in China and Ecuador and in cultures of *N. nodosus* larvae in Brazil.

METHODS

Bacterial strains, growth conditions and DNA isolation. Strains used in this study are described 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 following the method described by Pitcher *et al.* (1989). All strains included in this study have been deposited in the BCCM/LMG Bacteria Collection at Ghent University and in the CAIM collection of the Centre for Research on Nutrition and Development (CIAD) in Mazatlán, Mexico.

Genotypic analyses. Selective amplification of restriction fragments (FAFLP) and sequencing of almost complete 16S rDNA sequences were accomplished essentially as described previously (Thompson *et al.*, 2001). Alignment of the 16S rDNA sequences, distance estimations (Jukes & Cantor, 1969), clustering by the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood and maximum-parsimony methods and analysis of the stability of clusters (bootstrap analysis with 1000 replicates) were performed with the software BioNumerics 2.5 (Applied Maths). DNA–DNA hybridization experiments using photobiotin-labelled DNAs were run under stringent conditions (39 °C) following the method of Willems *et al.* (2001). The G+C content of DNA was determined by HPLC (Mesbah *et al.*, 1989).

Phenotypic characterization. Biochemical characterization of the isolates was performed using API 20E and API ZYM test strips (bioMérieux) and metabolic fingerprinting was carried out by means of Biolog GN2 microtitre plates. Preparations were done according to the manufacturers' instructions, with slight modifications (Thompson *et al.*, 2002b). Classical bacteriological tests were performed as described previously (Baumann *et al.*, 1984; Farmer & Hickman-Brenner, 1992; Thompson *et al.*, 2002b; Vandamme *et al.*, 1998). Antibiograms were carried out using the disc-diffusion method (Acar & Goldstein, 1996) with commercial discs (Oxoid). The inhibition zone of each antibiotic was measured for 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.* (1994). Isolates were grown on trypticase soy broth (Becton Dickinson) supplemented with

Table 1. Strains included in this study

Abbreviations: LMG, BCCM/LMG Bacteria Collection, Ghent, Belgium; LCMM, Laboratory for Culture of Marine Molluscs, Florianópolis, Brazil; CENAIM, Center for Marine and Aquaculture Research, Guayaquil, Ecuador; ARC, Artemia Reference Center, Ghent, Belgium; CAIM, Collection of Aquacultural Important Micro-organisms, Mazatlán, Mexico.

Strain(s)	Location and date of isolation	Source
***Vibrio neptunius* sp. nov. (FAFLP group A5)**		
LMG 20536^T (=CAIM 532^T =INCO 17^T)	LCMM, 1998	Bivalve larvae (*Nodipecten nodosus*)
LMG 20610	ARC, 1999	Culture water of rotifers
LMG 20611, R-15119, R-15120, R-15121	ARC, 1999	Rotifer in recirculation system (*Brachionus plicatilis*)
LMG 20612	ARC, 1996	Gut of turbot larvae (*Scophthalmus maximus*)
LMG 20613, R-15113, R-15116, R-15117	ARC, 1999	Rotifer in recirculation system (*B. plicatilis*)
LMG 20614, R- 15118, R-15108, R-15111, R-15112	ARC, 1999	Rotifer in recirculation system (*B. plicatilis*)
LMG 20615	LCCM, 1998	Diseased bivalve larvae (*N. nodosus*)
R-1575, R-1579, R-1592	ARC, 1997	Gut of turbot larvae (*S. maximus*)
R-15123	ARC, 1999	Healthy rotifer (*B. plicatilis*)
***Vibrio brasiliensis* sp. nov. (FAFLP group A8)**		
LMG 20546^T (=CAIM 495^T =INCO 317^T), LMG 20010 (=INCO 320), R-15002, R-15003, R-15004, R-15005	LCMM, 1999	Bivalve larvae (*N. nodosus*)
***Vibrio xuii* sp. nov. (FAFLP group A23)**		
LMG 21346^T (=CAIM 467^T =STD3-1071^T)	Dahua (China), 1995	Shrimp culture water
LMG 21347 (=CAIM 568 =STD3-1204)	CENAIM, 1995	White shrimp (*Litopenaeus vannamei*)
LMG 20011 (=INCO 167)	LCMM, 1998	Bivalve larvae (*N. nodosus*)

1·5 % (w/v) Bacto agar (Becton Dickinson) and 1·5 % (w/v) NaCl at 28 °C for 24 h. Approximately 50 mg cells was harvested and the fatty acids were isolated following the recommendations of the manufacturer using the Microbial Identification System manual and software, version 3.9 (Microbial ID).

RESULTS AND DISCUSSION

The 30 *Vibrio* isolates formed three groups by FAFLP fingerprinting analysis. FAFLP groups A5, A8 and A23 had complex band patterns, respectively consisting of 126 ± 14, 115 ± 7 and 83 ± 14 bands (50–536 bp) (Fig. 1). The three FAFLP groups were clearly different from known *Vibrio* species (Thompson *et al.*, 2001), suggesting that they represent novel species. Isolates of group A5 shared at least 75 % pairwise pattern similarity and showed less than 71 % pairwise pattern similarity towards other *Vibrio* species. Surprisingly, these strains, which were isolated over a 4-year period and from different places, showed remarkable genome resemblance. For instance, strains LMG 20536^T, isolated in 1998 at Florianópolis island (Brazil), and LMG 20612, isolated in 1996 at the ARC (Belgium), had 87·5 % pattern similarity. Some strains, e.g. pairs LMG 20614 and R-15108 and R-15111 and R-15112, clustered at the reproducibility level of FAFLP (i.e. $\geqslant$88 % pattern similarity) and were thus indistinguishable by FAFLP. Isolates of FAFLP groups A8 and A23 respectively showed mutual similarities of at least 82 and 62 % and similarity levels below 73 and 54 % towards other *Vibrio* species.

The value of AFLP in determining genome divergence and species delineation for other bacterial genera, e.g. *Agrobacterium* and *Xanthomonas*, has also been appreciated (Mougel *et al.*, 2002; Rademaker *et al.*, 2000). Mougel *et al.* (2002) calculated that strains belonging to the same species of *Agrobacterium* would have about 86 % FAFLP band pattern similarity, while Rademaker *et al.* (2000) found about 65 % AFLP pattern similarity between strains of the same species.

The 16S rDNA sequences of two representative isolates of each FAFLP group were determined and were allocated to the genus *Vibrio* by the FASTA program. Isolates LMG 20536^T (EMBL accession no. AJ316171; 1468 bp) and LMG 20613 (AJ490150, 681 bp) had 99·9 % 16S rDNA similarity, whereas LMG 20546^T (AJ316172; 1504 bp) and LMG 20010 (AJ490151, 467 bp) had 99·3 % similarity. Strains LMG 21346^T (AJ316181; 1435 bp) and LMG 21347 (AJ490152, 1123 bp) had 99·2 % similarity. Clustering obtained by the neighbour-joining, maximum-likelihood and maximum-parsimony methods was in agreement and the closest phylogenetic neighbours of the three novel *Vibrio* species were *Vibrio tubiashii* (98–98·8 %), *Vibrio nereis* (97·6–98·8 %), *Vibrio coralliilyticus* (96·8–98·5 %), *Vibrio mytili* (96·8–98·2 %) and *Vibrio diabolicus* (97·1–98·1 %) (Fig. 2). *V. coralliilyticus* and LMG 20536^T were closely related, having 98·2 % 16S rDNA similarity, and so were LMG 20546^T and LMG 21346^T (98·4 %). Strain LMG 20536^T had 97·2 % 16S rDNA similarity towards strains

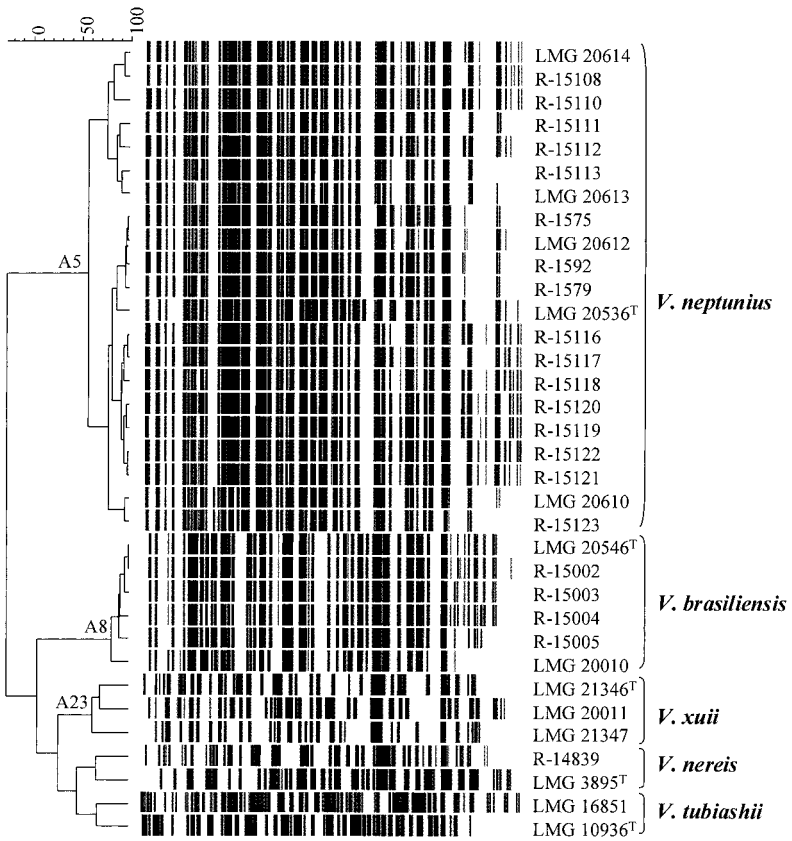

Fig. 1. Dendrogram of FAFLP patterns of 30 marine aquaculture *Vibrio* isolates. *V. tubiashii* and *V. nereis* were included as outgroups. A band-based (Dice) cluster analysis (Ward) was used.

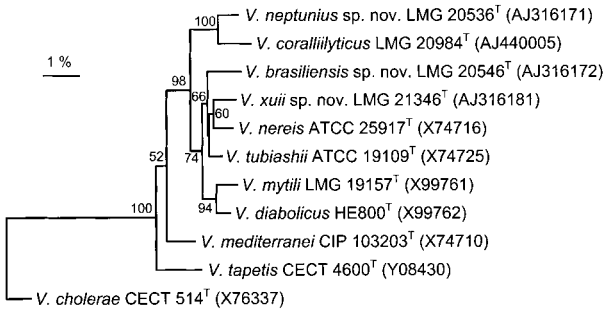

Fig. 2. Phylogenetic tree with the estimated positions of *Vibrio neptunius* sp. nov., *Vibrio brasiliensis* sp. nov. and *Vibrio xuii* sp. nov., using the neighbour-joining method based on the almost complete 16S rDNA sequences. Bootstrap analyses were made with 1000 cycles; values greater than 50 % are indicated on the branching nodes. Bar, 1 % estimated sequence divergence.

LMG 20546^T and LMG 21346^T. Similarity levels of the three proposed novel species towards other genera of the family *Vibrionaceae* were below 95 %.

Two representative isolates of each FAFLP group were chosen for DNA–DNA hybridization experiments. The levels of DNA relatedness within each FAFLP group were $\geqslant$93 %, but less than 67 % towards other phylogenetic related *Vibrio* species (Table 2). DNA–DNA hybridizations confirmed the FAFLP grouping and also revealed other interesting relationships. For instance, FAFLP group A5 was found to be highly related (64–66 %) to the recently described coral-pathogenic species *V. coralliilyticus* (Ben-Haim *et al.*, 2003); *V. coralliilyticus* belongs to FAFLP clusters A1–A3 (Thompson *et al.*, 2001).

The 30 *Vibrio* isolates examined in this study had the main phenotypic and chemotaxonomic features of the genus *Vibrio* (Bertone *et al.*, 1996; Farmer & Hickman-Brenner, 1992; Lambert *et al.*, 1983). They were slightly curved rods, Gram-negative, oxidase- and catalase-positive and motile by means of at least one polar flagellum. The major fatty acids were summed feature 3 (comprising 16 : 1ω7*c* and/or 15 : 0 iso 2-OH), 16 : 0, 18 : 1ω7*c* and 14 : 0, accounting for $\geqslant$68 % of the total fatty acids (Table 3). These facultatively anaerobic isolates grew on thiosulphate/citrate/bile salts/sucrose (TCBS) agar, forming yellow colonies, but they did not grow without NaCl or in presence of the vibriostatic agent O/129 at 10 or 150 μg per disc (except LMG 21346^T). Prolific growth occurred in media containing 2·5 % (w/v) NaCl at 28 °C. None of the isolates fermented inositol, sorbitol, rhamnose or melibiose. All isolates utilized dextrin, *N*-acetyl-D-glucosamine, D-fructose, α-D-glucose, maltose, D-mannose, psicose, D-trehalose, DL-lactic acid, succinic acid, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, L-serine, inosine, uridine and thymidine as sole carbon sources. None of the isolates utilized adonitol, D-arabitol, *i*-erythritol, L-fucose, *m*-inositol, α-lactose, α-D-lactose lactulose, D-melibiose, D-raffinose, L-rhamnose, xylitol, *cis*-aconitic acid (except LMG 21346^T), citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, itaconic acid, α-ketovaleric acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, succinamic acid, glucuronamide, L-histidine, L-leucine, L-pyroglutamic acid, DL-carnitine, urocanic acid or phenyl ethylamine. None of the isolates was luminescent, but they reduced nitrate and were Voges–Proskauer and methyl red positive. The 30 isolates produced indole, alkaline phosphatase, esterase

Table 2. DNA–DNA similarity and DNA G+C content of marine aquaculture *Vibrio* isolates and related *Vibrio* species

Strain	G+C content (mol%)	DNA–DNA hybridization with DNA from:											
		1	2	3	4	5	6	7	8	9	10	11	12
1. *V. mediterranei* LMG 11258^T	43·8	100											
2. *V. mytili* LMG 19157^T	44·6	21	100										
3. *V. diabolicus* LMG 19805^T	45·6	22	37	100									
4. *V. coralliilyticus* LMG 20984^T	46·2	19	22	23	100								
5. *V. nereis* LMG 3895^T	45·9	25	30	34	32	100							
6. *V. tubiashii* LMG 10936^T	44·8	22	35	25	30	34	100						
V. neptunius sp. nov.													
7. LMG 20536^T	46·0	20	25	26	64	34	34	100					
8. LMG 20613	45·3	24	27	27	66	37	38	93	100				
V. brasiliensis sp. nov.													
9. LMG 20546^T	45·9	17	20	22	25	32	34	32	29	100			
10. LMG 20010	45·9	16	21	22	26	32	34	32	28	100	100		
V. xuii sp. nov.													
11. LMG 21346^T	46·6	ND	ND	ND	ND	50	31	ND	ND	ND	ND	100	
12. LMG 21347	47·1	16	28	26	24	41	27	29	26	25	26	94	100

ND, Not done.

Table 3. Fatty acid compositions of the novel *Vibrio* species

Values are percentages (means ± SD) of total fatty acids. Only values above 0·1 % are included.

Fatty acid	*V. neptunius*	*V. brasiliensis*	*V. xuii*
12 : 0	1·9 ± 0·3	1·4 ± 0·2	3·2 ± 0·0
14 : 0	5·5 ± 0·6	4·6 ± 0·1	3·5 ± 0·3
15 : 0	1·7 ± 0·3	1·0 ± 0·1	0·9 ± 0·1
16 : 0	18·0 ± 0·8	11·3 ± 0·3	12·5 ± 0·6
17 : 0	2·3 ± 0·2	0·6 ± 0·1	0·5 ± 0·1
13 : 0 iso	1·0 ± 0·0	1·0 ± 0·0	–
14 : 0 iso	0·2 ± 0·1	3·3 ± 0·4	1·2 ± 0·1
15 : 0 iso	1·2 ± 0·3	1·8 ± 0·1	1·1 ± 0·1
16 : 0 iso	0·5 ± 0·1	10·5 ± 0·6	5·5 ± 0·4
17 : 0 iso	1·5 ± 0·1	1·4 ± 0·1	–
18 : 0 iso	–	1·1 ± 0·0	–
12 : 0 3-OH	2·1 ± 0·6	1·5 ± 0·3	1·4 ± 0·0
14 : 0 iso 3-OH	0·1 ± 0·1	1·3 ± 0·2	0·9 ± 0·1
16 : 1ω7*c* alcohol	0·9 ± 0·3	0·3 ± 0·0	–
17 : 1ω6*c*	1·2 ± 0·1	0·3 ± 0·1	0·2 ± 0·0
17 : 1ω8*c*	2·1 ± 0·1	0·7 ± 0·1	–
18 : 1ω7*c*	17·8 ± 1·6	17·3 ± 0·3	21·0 ± 2·4
11-methyl 18 : 1ω7*c*	0·6 ± 0·3	–	–
Summed feature 2*	2·4 ± 0·3	1·8 ± 0·3	2·6 ± 0·0
Summed feature 3*	35·7 ± 0·9	34·7 ± 1·0	38·7 ± 1·5

*Summed feature 2: one or more of 14 : 0 3-OH, 16 : 1 iso I, an unidentified fatty acid with equivalent chain-length of 10·928 and/or 12 : 0 ALDE. Summed feature 3: 16 : 1ω7*c* and/or 15 : 0 iso 2-OH.

(C4), esterase lipase (C8), lipase (C14), leucine arylamidase and valine arylamidase (except LMG 20613), but they did not produce urease, H_2S, lysine or ornithine decarboxylases, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase or α-fucosidase. The 30 isolates were sensitive to chloramphenicol (30 μg per disc) (except LMG 21346^T), tetracycline (30 μg per disc) and polymyxin B (300 U) and resistant to kanamycin (30 μg per disc).

We propose to accommodate the 30 *Vibrio* isolates examined in the present study in three novel species, *Vibrio neptunius* sp. nov., *Vibrio brasiliensis* sp. nov. and *Vibrio xuii* sp. nov. The three novel *Vibrio* species can be differentiated from each other and from other *Vibrio* species by a number of phenotypic features (Table 4). Quantitative and qualitative differences were detected in the fatty acid compositions of these novel species. Of special interest were the fatty acids 14 : 0 iso, 14 : 0 iso 3-OH and 16 : 0 iso, which appeared at a higher concentration in group A8, and the fatty acids 16 : 0, 17 : 0 and 17 : 1ω8*c*, which were present at a higher concentration in group A5.

Description of *Vibrio neptunius* sp. nov.

Vibrio neptunius (nep.tu′ni.us. L. masc. adj. *neptunius* of Neptune, the Roman god of the sea).

Cells are 1 μm wide and 2·3–3 μm long. Forms translucent, convex, non-swarming, smooth-rounded colonies with entire margins, beige in colour and about 3 mm in diameter on TSA after 48 h incubation at 28 °C; colonies are yellow, umbonate, round, entire, smooth, shiny and transparent and 2–3 mm in size on TCBS after 24 h at 28 °C. No growth in the absence of NaCl or in the presence of ⩾ 8·0 % (w/v) NaCl. No growth at 4 or ⩾40 °C. Strains are facultatively anaerobic and ferment D-glucose and sucrose. None of the strains ferments mannitol or amygdalin. All strains utilize citrate, glycogen, D-mannose, methyl β-D-glucoside, sucrose, D-serine, L-threonine, glucose 1-phosphate and glucose 6-phosphate as sole carbon sources. None of the strains utilizes Tween 80, *N*-acetyl-D-galactosamine, L-arabinose, cellobiose, D-galactose, gentiobiose, D-mannitol, D-sorbitol, turanose, monomethyl succinate, D-gluconic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, hydroxy-L-proline, L-phenylalanine, DL-carnitine, γ-aminobutyric acid, putrescine or 2, 3-butanediol as a sole carbon source. Strains produce gelatinase, tryptophan deaminase, trypsin and *N*-acetyl-β-glucosaminidase, but they do not produce cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase or α-glucosidase. Arginine dihydrolase is variable, but positive for the type strain. The major fatty acids are summed feature 3 (35·7 ± 0·9 %), 16 : 0 (18·0 ± 0·8 %) and 18 : 1ω7*c* (17·8 ± 1·6 %) (Table 3). Strains are resistant to ampicillin (25 μg per disc). Additional phenotypic features are listed as supplementary material in IJSEM Online (http://ijs.sgmjournals.org/). The type strain of this species is LMG 20536^T (=CAIM 532^T), isolated from larvae of the bivalve *Nodipecten nodosus* in the south of Brazil. The G + C content of the type strain is 46·0 mol%.

Description of *Vibrio brasiliensis* sp. nov.

Vibrio brasiliensis (bra.si.li.en′sis. N.L. masc. adj. *brasiliensis* from Brazil).

Cells are 1 μm wide and 2·5–3 μm long. Forms translucent, convex, smooth-rounded colonies with entire margins, beige in colour and 2·5–3 mm in size on TSA after 48 h incubation at 28 °C. Colonies are yellow, umbonate, wavy, shiny, translucent, round with scalloped margins and about 3 mm in size on TCBS after 24 h incubation at 28 °C. No growth in the absence of NaCl or in the presence of ⩾8·0 % NaCl. No growth at 4 or ⩾45 °C. Facultatively anaerobic and ferments D-glucose, sucrose, mannitol and amygdalin. None of the strains ferments arabinose. All strains utilize α-cyclodextrin, glycogen, cellobiose, gentiobiose, D-galactose, gentiobiose, α-D-glucose, D-mannitol, methyl β-D-glucoside, sucrose, methyl pyruvate, β-hydroxybutyric acid, bromosuccinic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-ornithine, L-proline, D-serine, L-threonine and glycerol as sole carbon sources. None of the strains utilizes *N*-acetyl-D-galactosamine, adonitol, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, α-ketovaleric acid, alaninamide, L-phenylalanine, 2-aminoethanol, 2,3-butanediol,

Table 4. Features useful in differentiating *V. neptunius*, *V. brasiliensis* and *V. xuii* spp. nov. from closely related *Vibrio* species

Species are identified as: 1, *V. neptunius* sp. nov. ($n=21$); 2, *V. brasiliensis* sp. nov. ($n=6$); 3. *V. xuii* sp. nov. ($n=3$); 4, *Vibrio aestuarianus*; 5, *Vibrio anguillarum*; 6, *Vibrio cyclitrophicus*; 7, *V. coralliilyticus*; 8, *V. diabolicus*; 9, *Vibrio diazotrophicus*; 10, *Vibrio fluvialis*; 11, *Vibrio lentus*; 12, *V. mediterranei*; 13, *V. mytili*; 14, *V. nereis*; 15, *Vibrio splendidus*; 16, *V. tubiashii*. Phenotypic data for reference species were obtained from Ben-Haim *et al.* (2003); Baumann *et al.* (1984); Farmer & Hickman-Brenner (1992); Hedlund & Staley (2001); Macián *et al.* (2001); Pujalte *et al.* (1993) and Raguénès *et al.* (1997). Fatty acid profiles of known *Vibrio* species (type strains) are from our own database. ND, No data; V, variable.

Feature	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Utilization of:																
Cellobiose	−	+	+	+	+	+	−	−	+	V	+	+	+	−	V	+
D-Galactose	−	+	−	+	V	+	+	+	+	+	+	+	+	−	V	+
Gentiobiose	−	+	V	+	+	ND	−	−	+	+	ND	V	+	−	−	−
β-Hydroxybutyric acid	−	+	+	−	−	−	−	ND	−	+	ND	−	−	+	V	V
Growth on 8 % (w/v) NaCl	−	−	+	V	−	+	−	ND	+	V	ND	V	+	+	V	V
Fermentation of:																
Mannitol	−	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+
Amygdalin	−	+	+	+	+	ND	−	−	+	+	ND	+	+	−	+	−
Melibiose	−	−	−	−	−	ND	−	−	−	−	+	V	−	−	+	V
Enzyme activity:																
Gelatinase	+	+	−	+	+	+	+	+	−	+	+	V	−	V	+	−
β-Galactosidase	−	+	−	+	+	−	+	−	+	V	ND	ND	+	−	V	+
N-Acetyl-β-glucosaminidase	+	−	−	+	+	ND	−	−	−	+	ND	+	+	ND	−	+
FAME composition:*																
14 : 0 iso	0·2±0·1	3·3±0·4	1·2±0·1	0·3	0·0	0·0	0·5	0·2	0·3	1·4	0·0	1·8	0·0	0·2	0·0	0·0
14 : 0 iso 3-OH	0·1±0·1	1·3±0·2	0·9±0·1	0·2	0·0	0·0	0·3	0·3	0·3	0·8	0·0	0·5	0·3	0·3	0·0	0·0
16 : 0	18·0±0·8	11·3±0·3	12·5±0·6	23·2	28·6	30·5	15	14·4	24·5	16·1	24·7	10·9	18·8	12·9	20·8	17·3
16 : 0 iso	0·5±0·1	10·5±0·6	5·5±0·4	2·2	0·4	0·0	0·8	1·7	2·2	4·9	0·0	4·3	1·5	1·1	0·0	0·0
17 : 0	2·3±0·2	0·6±0·1	0·5±0·1	0·3	0·0	0·1	2·5	1·6	0·3	0·7	0·0	0·0	0·0	1·9	0·0	0·1
17 : 1ω8*c*	2·1±0·1	0·7±0·1	0·5	0·5	0·0	0·0	1·8	2·5	0·3	0·9	0·0	0·2	0·0	4·6	0·0	0·1
17 : 1ω6*c*	1·2±0·1	0·3±0·1	0·2	0·0	0·0	0·0	0·6	0·7	0·0	0·2	0·0	0·0	0·0	1·3	0·0	0·0
18 : 1ω7*c*	17·8±1·6	17·3±0·3	21·0±2·4	15·9	12·5	7·5	18·2	17·4	16·5	16·8	8·7	17·2	19·9	22·6	12·0	25·4

*Means ± SD as percentages of total fatty acids.

DL-α-glycerol phosphate, glucose 1-phosphate or glucose 6-phosphate as a sole carbon source. All strains produce arginine dihydrolase, β-galactosidase and gelatinase. None of the strains produces trypsin, acid phosphatase, α-glucosidase or *N*-acetyl-β-glucosaminidase. The most abundant fatty acids are summed feature 3 (34·7±1·0 %), 18 : 1ω7*c* (17·3±0·3 %), 16 : 0 (11·3±0·3 %) and 16 : 0 iso (10·5±0·6 %) (Table 3). Additional phenotypic features are listed online in the supplementary material. Isolated from larvae of the bivalve *N. nodosus* in the south of Brazil. The type strain is strain LMG 20546^T (=CAIM 495^T). The G+C content of the type strain is 45·9 mol%.

Description of *Vibrio xuii* sp. nov.

Vibrio xuii (xu′i.i. N.L. gen. n. *xuii* of Xu, in honour of the microbiologist H. Xu).

Cells are 1 μm wide and 2–3 μm long. Forms translucent, convex, smooth-rounded colonies with entire margins, beige in colour and 3–4 mm in size on TSA after 48 h incubation at 28 °C. Colonies are yellow, convex, round, entire, shiny, translucent and about 2 mm in size on TCBS after 24 h incubation at 28 °C. No growth in the absence of NaCl or in the presence of ⩾10·0 % NaCl. No growth at 4 or ⩾45 °C. Facultatively anaerobic organism that ferments glucose, mannitol, sucrose, amygdalin and arabinose. Utilizes α-cyclodextrin, Tweens 40 and 80, *N*-acetyl-D-galactosamine, L-arabinose, cellobiose, D-mannitol, D-mannose, D-sorbitol, sucrose, methyl pyruvate, monomethyl succinate, acetic acid, D-gluconic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketoglutaric acid, D-alanine, glycyl L-glutamic acid, L-proline, L-threonine, 2,3-butanediol, glycerol and DL-α-glycerol phosphate as sole carbon sources. Does not utilize D-galactose, gentiobiose, methyl β-D-glucoside, D-raffinose, α-ketobutyric acid, propionic acid, D-serine, quinic acid, sebacic acid, hydroxy-L-proline, 2-aminoethanol or glucose 1-phosphate as a sole carbon source. Produces arginine dihydrolase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and tryptophan deaminase. Does not produce cystine arylamidase, trypsin,

β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase or gelatinase. The major fatty acids are summed feature 3 ($38{\cdot}7 \pm 1{\cdot}5$ %), 18 : 1ω7*c* ($21{\cdot}0 \pm 2{\cdot}4$ %) and 16 : 0 ($12{\cdot}5 \pm 0{\cdot}6$ %) (Table 3). Sensitive to ampicillin (25 μg per disc). Additional phenotypic features are listed online in the supplementary material. The type strain, LMG 21346^T (=CAIM 467^T), was isolated from shrimp culture water in China. The G+C content of the type strain is 46·6 mol%.

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