Vibrio kanaloae sp. nov., *Vibrio pomeroyi* sp. nov. and *Vibrio chagasii* sp. nov., from sea water and marine animals

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The taxonomic position of the fluorescent amplified fragment length polymorphism fingerprinting groups A46 (five isolates), A51 (six isolates), A52 (five isolates) and A53 (seven isolates) obtained in a previous study were further analysed through a polyphasic approach. The 23 isolates were phylogenetically related to *Vibrio splendidus*, but DNA–DNA hybridization experiments proved that they belong to three novel species. Chemotaxonomic and phenotypic analyses further disclosed several features that differentiate between the 23 isolates and known *Vibrio* species. The names *Vibrio kanaloae* sp. nov. (type strain LMG 20539^T = CAIM 485^T; EMBL accession no. AJ316193; G + C content 44·7 mol%), *Vibrio pomeroyi* sp. nov. (type strain LMG 20537^T = CAIM 578^T; EMBL accession no. AJ491290; G + C content 44·1 mol%) and *Vibrio chagasii* sp. nov. (type strain LMG 21353^T = CAIM 431^T; EMBL accession no. AJ316199; G + C content 44·6 mol%) are respectively proposed to encompass the five isolates of A46, the six isolates of A51 and the 12 isolates of A52/A53. The three novel species can be distinguished from known *Vibrio* species by several phenotypic features, including utilization and fermentation of various carbon sources, β-galactosidase activity and fatty acid content (particularly of 12 : 0, 14 : 0, 14 : 0 iso and 16 : 0 iso).

INTRODUCTION

Several *Vibrio* species are ubiquitous in aquatic ecosystems and display an extraordinarily high growth rate, which makes them highly successful and dominant, particularly in eutrophic environments (Aiyar *et al.*, 2002; Macián *et al.*, 2000). In this study, we report on the taxonomic analysis of four unidentified groups of vibrios, A46 (five isolates), A51 (six isolates), A52 (seven isolates) and A53 (five isolates), found previously (Thompson *et al.*, 2001). These isolates were phylogenetically related to *Vibrio splendidus*, a ubiquitous luminous marine bacterium that was first described by the early microbial ecologist Beijerinck in 1900. *V. splendidus* strains have consistently been found in association with cultured oysters (*Ostrea edulis*) in the Mediterranean Sea over the years, suggesting a close relationship between the bacterium and the host invertebrate (Macián *et al.*, 2000). *V. splendidus* has also been implicated as an aetiological agent of septicaemia in various species of fish (Austin & Austin, 1999) and as the causative agent of bacillary necrosis of oyster larvae (Sugumar *et al.*, 1998). Our polyphasic taxonomic study, including genomic, phenotypic and chemotaxonomic analyses, revealed that the 23 isolates belong to three novel species, for which we propose the names *Vibrio kanaloae* sp. nov., *Vibrio pomeroyi* sp. nov. and *Vibrio chagasii* sp. nov. *V. kanaloae* was found to be ubizquitous in the aquatic environment, whereas

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Abbreviation: FAFLP, fluorescent amplified fragment length polymorphism.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of strains LMG 20539^T, LMG 21522, LMG 21523, LMG 21353^T, LMG 13237, LMG 21354, LMG 20537^T, LMG 21351 and LMG 21352 are respectively AJ316193, AJ490153, AJ490154, AJ316199, AJ490157, AJ490158, AJ491290, AJ316197 and AJ490152.

Results of repetitive extragenic palindrome-PCR fingerprinting of *V. kanaloae*, *V. pomeroyi*, *V. chagasii* and the most closely related *Vibrio* species and variable features/differentiating characteristics for *V. kanaloae*, *V. pomeroyi* and *V. chagasii* are available as supplementary data in IJSEM Online.

Table 1. Strains studied

Strains prefixed R and VIB are respectively held in the Research Collection and the *Vibrio* Collection at the BCCM/LMG Bacteria Collection.

Strain(s)	Place and date of isolation	Source	16S rDNA accession no. (length, bp)
***Vibrio kanaloae* sp. nov. (FAFLP group A46)**			
LMG 20539^T (=CAIM 485^T=INCO 191^T)	IFREMER (France), 1998	Diseased oyster larvae (*Ostrea edulis*)	AJ316193 (1494)
LMG 21521 (=CAIM 486=R-15012=INCO 192)	IFREMER (France), 1998	Diseased oyster larvae (*O. edulis*)	
LMG 11753 (=CAIM 522=VIB 470)	Hawaii (USA)	Sea water	
LMG 21522 (=CAIM 543=R-15009=STD3-1036)	Dahua (China), 1996	Shrimp (*Penaeus chinensis*)	AJ490153 (488)
LMG 21523 (=CAIM 546=R-15010=STD3-1085)	Feng Cheng (China), 1996	Shrimp (*P. chinensis*)	AJ490154 (454)
***Vibrio pomeroyi* sp. nov. (FAFLP group A51)**			
LMG 20537^T (=CAIM 578^T=INCO 62^T)	Florianópolis (Brazil), 1998	Healthy bivalve larvae (*Nodipecten nodosus*)	AJ491290 (1507)
LMG 19269 (=INCO 175), R-14801 (=INCO 67), R-14802 (=INCO 63)	Florianópolis (Brazil), 1998	Healthy bivalve larvae (*N. nodosus*)	
LMG 21351 (=CAIM 579=R-14805=VIB 575)	Spain, 1988	Turbot (*Scophthalmus maximus*)	AJ316197 (485)
LMG 21352 (=CAIM 580=R-14806=VIB 556)	Spain, 1992	Turbot (*S. maximus*)	AJ190156 (468)
***Vibrio chagasii* sp. nov. (FAFLP groups A52 and 53)**			
LMG 21353^T (=CAIM 431^T=R-3712^T)	Austevoll (Norway), 1997	Gut of turbot larvae (*S. maximus*)	AJ316199 (1435)
LMG 21354 (=CAIM 435=R-3722)	Austevoll (Norway), 1997	Gut of turbot larvae (*S. maximus*)	AJ490158 (887)
R-3765 (=CAIM 443), R-3718 (=CAIM 433), R-3803 (=CAIM 448)	Austevoll (Norway), 1997	Gut of turbot larvae (*S. maximus*)	
LMG 13219 (=CAIM 581=VIB 192=KR104)	Greece, 1991	Rotifer (*Brachionus plicatilis*)	AJ316198 (471)
LMG 13220 (=CAIM 582=VIB 193), LMG 13238 (=CAIM 584=VIB 211)	Greece, 1991	Water	
LMG 13237 (=CAIM 583=VIB 210)	Greece, 1991	Water	AJ490157 (1435)
LMG 13222 (=CAIM 585=VIB 195)	Spain, 1990	*Artemia* sp.	
LMG 13239 (=CAIM 586=VIB 212), LMG 13251 (=CAIM 587=VIB 224)	Greece, 1991	Sea bass (*Dicentrarchus labrax*)	

V. pomeroyi isolates were abundant in cultures of *Nodipecten nodosus* larvae in the south of Brazil. *V. chagasii* isolates were found to be regular inhabitants of rotifer cultures in Greece (Verdonck *et al.*, 1997).

METHODS

Bacterial strains, growth conditions and DNA isolation. Strains used 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 were deposited in the BCCM/LMG Bacteria Collection at Ghent University and in the Collection of Aquacultural Important Micro-organisms (CAIM) of the Centre for Research on Nutrition and Development in Mazatlán, Mexico.

Genotypic analyses. Selective amplification of restriction fragments, using fluorescent amplified fragment length polymorphism (FAFLP), and determination 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 neighbour-joining (Saitou & Nei, 1987), maximum-likelihood and maximum-parsimony methods and stability of the clusters (bootstrap analysis with 1000 replicates) were performed with the software BioNumerics 2.5 (Applied Maths). Repetitive extragenic palindrome-PCR fingerprinting was performed essentially as described previously (Sawabe *et al.*, 2002). DNA–DNA hybridization experiments using photobiotin-labelled DNAs were run under stringent conditions (39 °C) by following the methodology described by Willems *et al.* (2001). Hybridizations were performed on four replicates. Each DNA relatedness value is the mean of reciprocal and non-reciprocal reactions. The G+C content (mol%) of DNA was determined by using HPLC (Mesbah *et al.*, 1989).

Phenotypic analyses. Phenotypic characterization of the isolates was performed using API 20E and API ZYM (bioMérieux) and BIOLOG GN metabolic fingerprinting according to 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; Murray *et al.*, 1994; Thompson *et al.*, 2002; Vandamme *et al.*, 1998). Antibiograms were carried out using disc-diffusion methodology (Acar & Goldstein, 1996) with commercial discs (Oxoid). The inhibition zone of each antibiotic was measured on strains grown on

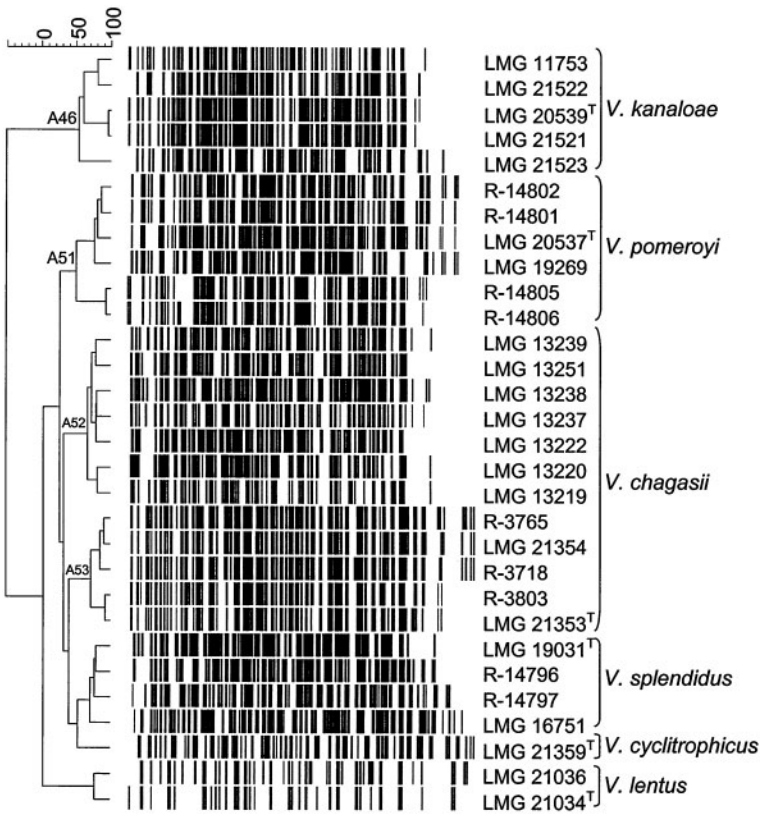

Fig. 1. Dendrogram of the FAFLP patterns of *Vibrio kanaloae* sp. nov. ($n=5$), *Vibrio pomeroyi* sp. nov. ($n=6$) and *Vibrio chagasii* sp. nov. ($n=12$). *V. splendidus*, *V. cyclitrophicus* and *V. lentus* were included as outgroups. A band-based (Dice) cluster analysis (Ward) was used.

Iso-sensitest agar (Oxoid) supplemented with 1·5 % (w/v) NaCl for 24 h at 28 °C. Fatty acid methyl ester analysis was carried out as described by Huys *et al.* (1994). 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 at 28 °C for 24 h. Approximately 50 mg cells was harvested and the fatty acids were isolated, according to the recommendations of the manufacturer, using the Microbial Identification System manual and software, version 3.9 (Microbial ID).

RESULTS AND DISCUSSION

The FAFLP patterns of groups A46, A51 and A52/A53 consisted of 116 ± 19, 126 ± 11 and 119 ± 14 bands, respectively, and were clearly different from those of their closest phylogenetic neighbours (Fig. 1) and from other known *Vibrio* species (Thompson *et al.*, 2001). Isolates of groups A46, A51 and A52/A53 had mutual pairwise similarities of at least 57·2, 72·2 and 60 %, respectively. Representative strains of each FAFLP group were also distinguishable from other closely related *Vibrio* species on the basis of repetitive chromosomal element analysis (available as supplementary data in IJSEM Online).

The 16S rDNA sequences of at least three representative isolates of each FAFLP group were determined (Table 1). FAFLP group A46 (LMG 20539^T, LMG 21522, LMG 21523), FAFLP group A51 (LMG 20537^T, LMG 21351, LMG 21352) and FAFLP groups A52 and A53 (LMG 21353^T, LMG 13237, LMG 21354, LMG 13219) were allocated to the genus *Vibrio* by the FASTA program (Pearson & Lipman, 1988). The 16S rDNA sequence similarity within each FAFLP group was $\geqslant$99 %. Isolates LMG 20539^T, LMG 21522 and LMG 21523 shared 99·5 % 16S rDNA similarity, while LMG 20537^T, LMG 21351 and LMG 21352 shared 99·4 %. Isolates LMG

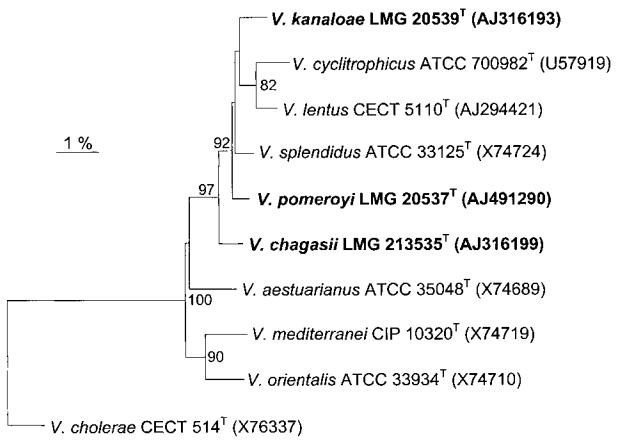

Fig. 2. Phylogenetic tree, with the estimated positions of *Vibrio kanaloae* sp. nov., *Vibrio pomeroyi* sp. nov. and *Vibrio chagasii* sp. nov., produced by using the neighbour-joining method based on almost-complete 16S rDNA sequences. Bootstrap values (>50 %) after 1000 simulations are shown. Bar, 1 % estimated sequence divergence.

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

Strain	G+C content (mol%)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
V. kanaloae sp. nov. (A46)															
1. LMG 20539^T	44·7	(100)													
2. LMG 21523	44·6	89	(100)												
V. pomeroyi sp. nov. (A51)															
3. LMG 20537^T	44·1	64	65	(100)											
4. LMG 21352	44·0	ND	ND	77	(100)										
V. chagasii sp. nov. (A52 and A53)															
5. LMG 21353^T	44·6	49	48	50	49	(100)									
6. LMG 13219	44·5	ND	ND	53	54	72	(100)								
7. LMG 13237	44·4	ND	ND	60	53	74	85	(100)							
V. splendidus															
8. LMG 19031^T	45·0	64	61	44	54	44	47	39	(100)						
9. LMG 16751	44·5	ND	ND	56	62	53	56	54	70	(100)					
10. *V. lentus* LMG 21034^T	45·2	62	60	43	56	45	47	39	62	57	(100)				
11. *V. cyclitrophicus* LMG 21359^T	44·2	65	63	45	60	49	51	42	59	61	58	(100)			
12. *V. orientalis* LMG 7897^T	44·4	ND	ND	36	ND	32	ND	ND	ND	ND	ND	ND	(100)		
13. *V. mediterranei* LMG 11258^T	43·8	ND	ND	27	ND	25	ND	ND	ND	ND	ND	ND	30	(100)	
14. *V. aestuarianus* LMG 7909^T	ND	24	24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	(100)

ND, Not determined.

21353^T and LMG 13237 showed 99·7 % similarity. The similarity between representative isolates of each FAFLP group was at least 97·4 %. Phylogenetic trees based on almost-complete sequences and using neighbour-joining, maximum-likelihood and maximum-parsimony methods were all in agreement and revealed that the three novel *Vibrio* species are closely related to *V. splendidus* (respectively 98·0, 99·1 and 98·5 % similarity), *Vibrio lentus* (97·8, 98·4 and 98·2 %), *Vibrio cyclitrophicus* (97·0, 98·3 and 97·7 %), *Vibrio mediterranei* (95·7, 97·2 and 97·8 %) and *Vibrio orientalis* (96·0, 97·1 and 97·6 %) (Fig. 2). The 16S rDNA similarity of the three novel species towards other *Vibrio* species and other genera of the family *Vibrionaceae* was below 97 and below 93·5 %, respectively.

At least two representative isolates of each FAFLP group were chosen for DNA–DNA hybridization experiments. The isolates of A46, A51 and A52/A53 had 89, 77 and 72 % mutual DNA–DNA relatedness, respectively, but $\leqslant$65 % with other *Vibrio* species. Thus, these results confirm their status as novel species (Table 2). DNA hybridization experiments further confirmed that the three novel species and other *Vibrio* species in the same phylogenetic branch have intermediate DNA–DNA relatedness. Macián *et al.* (2001) have already demonstrated that *V. lentus* and *V. splendidus* have 59 % DNA–DNA relatedness. High DNA–DNA relatedness between different species of the *Vibrio* core group has also been found (Baumann & Baumann, 1977; Dorsch *et al.*, 1992). For instance, strains of *Vibrio harveyi* and *Vibrio campbellii* have up to 74 % DNA–DNA relatedness and very similar phenotypes, but they can be clearly distinguished by genomic fingerprinting techniques such as FAFLP and repetitive extragenic palindrome-PCR (Thompson *et al.*, 2001; B. Gomez-Gil and others, unpublished). *V. cyclitrophicus* (Hedlund & Staley, 2001) was reported to have a G+C content of 39 mol%, but our results clearly show that this bacterium has a G+C content of 44·2 mol%. Measurements of the G+C content of DNA by renaturation methods, like that used by Hedlund & Staley (2001), are prone to errors caused by low-quality DNA (i.e. fragmented DNA and/or DNA contaminated with proteins and/or RNA), which might influence the results significantly (Mesbah *et al.*, 1989).

The three novel *Vibrio* species examined in this study share the main phenotypic and chemotaxonomic features of the genus *Vibrio* (Bertone *et al.*, 1996; Farmer & Hickman-Brenner, 1992; Lambert *et al.*, 1983). The three novel *Vibrio* species had several phenotypic features in common: the 23 isolates were Gram-negative, facultatively anaerobic, catalase- and oxidase-positive and showed prolific growth on thiosulphate/citrate/bile salts (TCBS) agar, forming yellow colonies (except strains LMG 21537^T and LMG 13251, which formed green colonies). Isolates were motile by means of at least one polar flagellum, susceptible to 10 and 150 μg O/129 (strain LMG 21523 was resistant to both concentrations) and did not grow in the absence of NaCl. The predominant fatty acids (accounting for >80 % of the total cellular fatty acid composition) were summed feature 3 (comprising 16:1ω7*c* and/or 15:0 iso 2-OH), 16:0, 18:1ω7*c*, 14:0 and 12:0.

The three novel species fermented D-glucose and mannitol but not inositol or rhamnose. Strains of the novel

species utilized α-D-glucose, dextrin, glycogen, *N*-acetyl-D-glucosamine, D-fructose, maltose, D-trehalose, DL-lactic acid, succinic acid, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, monomethyl succinate, glycyl L-aspartic acid, L-threonine, inosine and glycerol as sole carbon sources. None of the novel species utilized *N*-acetyl-D-galactosamine, adonitol, D-arabitol, i-erythritol, L-fucose, *m*-inositol, α-lactose, D-melibiose, D-raffinose, L-rhamnose, turanose, xylitol, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, malonic acid, L-leucine, L-pyroglutamic acid, DL-carnitine, γ-aminobutyric acid, urocanic acid, phenylethylamine, 2-aminoethanol or 2,3-butanediol. Strains of the three species produced indole, alkaline phosphatase, esterase (C_4), esterase lipase (C_8) and naphthol-AS-BI-phosphohydrolase but not lysine or ornithine decarboxylase, H_2S, urease, cystine arylamidase, α-galactosidase, β-glucuronidase, α- or β-glucosidase, α-mannosidase or α-fucosidase. The three novel species were non-luminescent, reduced nitrate and were methyl-red-positive (A46 isolates were methyl-red-negative). FAFLP groups A51 and A52/A53 were susceptible to polymixin B (300 U), tetracycline (30 μg per disc) and chloramphenicol (30 μg per disc), but were resistant to ampicillin (25 μg per disc). None of the 23 isolates grew on 0 or $\geqslant$10·0 % NaCl or at $\geqslant$35 °C. Additional phenotypical features found to be variable among the three novel *Vibrio* species are available as supplementary data in IJSEM Online.

Genomic and phenotypic evidence presented in this study clearly indicates that the 23 isolates should be accommodated in three novel *Vibrio* species. Although the novel species have the main phenotypic traits of the genus *Vibrio*, several useful differentiating features were found that distinguish them from known *Vibrio* species (available as supplementary material in IJSEM Online).

Description of *Vibrio kanaloae* sp. nov.

Vibrio kanaloae (ka.na.lo′a.e. N.L. gen. n. *kanaloae* of Kanaloa, the Hawaiian god of the sea and seamen).

Cells are slightly curved, 1 μm wide by 2–3 μm long and motile by at least one polar flagellum. They form translucent, convex, non-swarming, smooth, rounded colonies with entire margins; colonies are beige in colour and about 5 mm in diameter on TSA after 48 h incubation at 28 °C. Strains form yellow, translucent, 5–10 mm colonies on TCBS agar. All strains ferment sucrose and arabinose but not sorbitol, melibiose or amygdalin. Cells grow at 4 °C but not in the absence of NaCl. All strains utilize Tween 40, D-mannitol, sucrose, monomethyl succinate, α-ketoglutaric acid, D-alanine, L-alanine, L-ornithine, L-proline, L-serine and L-threonine. None of the strains utilizes cellobiose, D-sorbitol, D-saccharic acid, sebacic acid, succinamic acid, hydroxy-L-proline, L-phenylalanine or DL-α-glycerol phosphate. Strains produce leucine arylamidase, trypsin, tryptophan deaminase, acetoin and gelatinase, but not α-chymotrypsin, α-galactosidase, β-galactosidase or lysine. Arginine dihydrolase activity is variable, but is positive for the type strain. The major fatty acids are summed feature 3 (39·2 ± 0·2 %, comprising 16 : 1ω7*c* and/or 15 : 0 iso 2-OH), 16 : 0 (25·6 ± 1·0 %), 14 : 0 (5·0 ± 0·3 %), 12 : 0 (4·2 ± 0·1 %), 18 : 1ω7*c* (10·2 ± 1·0 %), summed feature 2 (2·1 ± 0·6 %, comprising 14 : 0 3-OH and/or 16 : 1 iso I and/or unidentified fatty acid with equivalent chain-length value of 10·928 and/or 12 : 0 ALDE), 12 : 0 3-OH (3·4 ± 0·1 %), 18 : 0 (1·0 ± 0·1 %) and 16 : 0 3-OH (0·3 ± 0·1 %). Additional phenotypical features are listed as supplementary material in IJSEM Online.

The type strain, strain LMG 20539^T (= CAIM 485^T = INCO 191^T), was isolated from diseased oyster (*Ostrea edulis*) larvae in France. The G + C content of the DNA of the type strain is 44·5 mol%.

Description of *Vibrio pomeroyi* sp. nov.

Vibrio pomeroyi (po.me.roy′i. N.L. gen. n. *pomeroyi* of Pomeroy, in honour of the North American microbial ecologist L. R. Pomeroy).

Cells are slightly curved, 1 μm wide by 2–3 μm long and motile by at least one polar flagellum. They form translucent, convex, non-swarming, smooth, rounded colonies with entire margins; colonies are beige in colour and about 3 mm in diameter on TSA after 48 h incubation at 28 °C. Strains (except LMG 20537^T) form yellow, translucent colonies on TCBS agar. Cells grow at 4 °C but not in the absence of NaCl. All strains utilize D-galactose, cellobiose, monomethyl succinate, sucrose, glycyl L-glutamic acid, L-serine, L-threonine, inosine, uridine and thymidine. None of the strains utilizes α-cyclodextrin, gentiobiose, α-D-lactose lactulose, putrescine, formic acid, D-glucuronic acid, α-hydroxybutyric acid, α-ketoglutaric acid, quinic acid, D-saccharic acid, sebacic acid, succinamic acid, glucuronamide, L-histidine, hydroxy-L-proline, L-leucine, L-phenylalanine, L-pyroglutamic acid, D-serine, DL-carnitine or DL-α-glycerol phosphate. Strains produce β-galactosidase and acid phosphatase, but not lipase (C_{14}), tryptophan deaminase or valine arylamidase. Arginine dihydrolase activity is variable, but is positive for the type strain. The major fatty acids are summed feature 3 (32·9 ± 1·6 %), 16 : 0 (29·2 ± 1·7 %), 14 : 0 (10·5 ± 0·4 %), 12 : 0 (8·9 ± 1·2 %), 18 : 1ω7*c* (7·6 ± 1·8 %), summed feature 2 (4·1 ± 0·6 %), 12 : 0 3-OH (3·9 ± 0·6 %), 18 : 0 (1·6 ± 0·2 %) and 16 : 0 3-OH (0·7 ± 0·1 %). Additional phenotypic features are available as supplementary material in IJSEM Online.

The type strain, LMG 20537^T (= CAIM 578^T = INCO 62^T), was isolated from bivalve (*Nodopecten nodosus*) larvae in southern Brazil. The G + C content of the DNA of the type strain is 44·1 mol%.

Description of *Vibrio chagasii* sp. nov.

Vibrio chagasii (cha.ga′si.i. N.L. gen. n. *chagasii* of Chagas, in honour of the Brazilian physician and microbiologist C. Chagas).

Cells are slightly curved, 1 μm wide by 2–3 μm long and motile by means of at least one polar flagellum. They form opaque, convex, non-swarming, smooth, rounded colonies with entire margins; colonies are dark beige in colour and 3–4 mm in diameter on TSA after 48 h incubation at 28 °C. Does not grow in the absence of NaCl. All strains (except LMG 13251) form green, transparent colonies on TCBS agar. All strains utilize Tweens 40 and 80, cellobiose, L-alanine, D-mannitol, psicose and α-ketoglutaric acid as sole carbon sources. None of the strains utilizes L-arabinose, methyl β-D-glucoside, α-cyclodextrin, gentiobiose, α-D-lactose, lactulose, putrescine, β-hydroxybutyric acid, S-saccharic acid, sebacic acid, L-ornithine or L-phenylalanine. Strains do not produce tryptophan deaminase The most abundant fatty acids are summed feature 3 (38·4±3·5 %), 16 : 0 (22·4±3·9 %), 18 : 1ω7*c* (9·7±1·6 %), 14 : 0 (7·2±3·5 %), 16 : 0 iso (5·2±2·6 %), 12 : 0 (3·8±2·0 %), summed feature 2 (3·3±1·3 %), 12 : 0 3-OH (2·7±1·4 %), 18 : 0 (1·1±0·5 %), 14 : 0 iso (1·1±0·7 %), 15 : 0 (0·6±0·3 %), 17 : 0 (0·5±0·3 %) and 14 : 0 iso 3-OH (0·5±0·3 %). Additional phenotypic features are available as supplementary material in IJSEM Online.

The type strain, LMG 21353^{T} (=CAIM 431^{T}=R-3712^{T}), was isolated from the gut of turbot larvae (*Scophthalmus maximus*) in Norway. The G+C content of the DNA of the type strain is 44·5 mol%.

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REFERENCES

Acar, J. F. & Goldstein, F. W. (1996). Disc susceptibility test. In *Antibiotics in Laboratory Medicine*, 4th edn, pp. 1–51. Edited by V. Lorian. Baltimore: Williams & Wilkins.

Aiyar, S. E., Gaal, T. & Gourse, R. L. (2002). rRNA promoter activity in the fast-growing bacterium *Vibrio natriegens*. *J Bacteriol* **184**, 1349–1358.

Baumann, P. & Baumann, L. (1977). Biology of the marine enterobacteria: genera *Beneckea* and *Photobacterium*. *Annu Rev Microbiol* **31**, 39–61.

Baumann, P., Furniss, A. L. & Lee, J. V. (1984). Genus I. *Vibrio* Pacini 1854, 411^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 518–538. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Bertone, S., Giacomini, M., Ruggiero, C., Piccarolo, C. & Calegari, L. (1996). Automated systems for identification of heterotrophic marine bacteria on the basis of their fatty acid composition. *Appl Environ Microbiol* **62**, 2122–2132.

Dorsch, M., Lane, D. & Stackebrandt, E. (1992). Towards a phylogeny of the genus *Vibrio* based on 16S rRNA sequences. *Int J Syst Bacteriol* **42**, 58–63.

Farmer, J. J., III & Hickman-Brenner, F. W. (1992). The genera *Vibrio* and *Photobacterium*. In *The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, and Applications*, 2nd edn, pp. 2952–3011. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. Berlin: Springer.

Hedlund, B. P. & Staley, J. T. (2001). *Vibrio cyclotrophicus* sp. nov., a polycyclic aromatic hydrocarbon (PAH)-degrading marine bacterium. *Int J Syst Evol Microbiol* **51**, 61–66.

Huys, G., Vancanneyt, M., Coopman, R., Janssen, P., Falsen, E., Altwegg, M. & Kersters, K. (1994). Cellular fatty acid composition as a chemotaxonomic marker for the differentiation of phenospecies and hybridization groups in the genus *Aeromonas*. *Int J Syst Bacteriol* **44**, 651–658.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Lambert, M. A., Hickman-Brenner, F. W., Farmer, J. J., III & Moss, C. W. (1983). Differentiation of *Vibrionaceae* species by their cellular fatty acid composition. *Int J Syst Bacteriol* **33**, 777–792.

Macián, M. C., Garay, E., González-Candelas, F., Pujalte, M. J. & Aznar, R. (2000). Ribotyping of *Vibrio* populations associated with cultured oysters (*Ostrea edulis*). *Syst Appl Microbiol* **23**, 409–417.

Macián, M. C., Ludwig, W., Aznar, R., Grimont, P. A. D., Schleifer, K. H., Garay, E. & Pujalte, M. J. (2001). *Vibrio lentus* sp. nov., isolated from Mediterranean oysters. *Int J Syst Evol Microbiol* **51**, 1449–1456.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Murray, R. G. E., Doetsch, R. N. & Robinow, C. F. (1994). Determinative and cytological light microscopy. In *Methods for General and Molecular Bacteriology*, pp. 21–41. Edited by P. Gerhardt. Washington, DC: American Society for Microbiology.

Pearson, W. R. & Lipman, D. J. (1988). Improved tools for biological sequence comparison. *Proc Natl Acad Sci U S A* **85**, 2444–2448.

Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.

Pujalte M.-J., Ortigosa, M., Urdaci, M.-C., Garay, E. & Grimont, P. A. D. (1993). *Vibrio mytili* sp. nov., from mussels. *Int J Syst Bacteriol* **43**, 358–362.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sawabe, T., Thompson, F. L., Heyrman, J. & 7 other authors (2002). Fluorescent amplified fragment length polymorphism and repetitive extragenic palindrome-PCR fingerprinting reveal host-specific genetic diversity of *Vibrio halioticoli*-like strains isolated from the gut of Japanese abalone. *Appl Environ Microbiol* **68**, 4140–4144.

Sugumar, G., Nakai, T., Hirata, Y., Matsubara, D. & Muroga, K. (1998). *Vibrio splendidus* biovar II as the causative agent of bacillary necrosis of Japanese oyster *Crassostrea gigas* larvae. *Dis Aquat Organ* **33**, 111–118.

Thompson, F. L., Hoste, B., Vandemeulebroecke, K. & Swings, J. (2001). Genomic diversity amongst *Vibrio* isolates from different sources determined by fluorescent amplified fragment length polymorphism. *Syst Appl Microbiol* **24**, 520–538.

Thompson, F. L., Hoste, B., Vandemeulebroecke, K., Engelbeen, K., Denys, R. & Swings, J. (2002). *Vibrio trachuri* Iwamoto *et al.* 1995 is a junior synonym of *Vibrio harveyi* (Johnson and Shunk 1936) Baumann *et al.* 1981. *Int J Syst Evol Microbiol* **52**, 973–976.

Vandamme, P., Segers, P., Ryll, M. & 8 other authors (1998). *Pelistega europaea* gen. nov., sp. nov., a bacterium associated with respiratory disease in pigeons: taxonomic structure and phylogenetic allocation. *Int J Syst Bacteriol* **48**, 431–440.

Verdonck, L., Grisez, L., Sweetman, E., Minkoff, G., Sorgeloos, P., Ollevier, F. & Swings, J. (1997). Vibrios associated with routine productions of *Brachionus plicatilis*. *Aquaculture* **149**, 203–214.

Willems, A., Doignon-Bourcier, F., Goris, J., Coopman, R., de Lajudie, P., De Vos, P. & Gillis, M. (2001). DNA–DNA hybridization study of *Bradyrhizobium* strains. *Int J Syst Evol Microbiol* **51**, 1315–1322.