Vibrio communis sp. nov., isolated from the marine animals *Mussismilia hispida*, *Phyllogorgia dilatata*, *Palythoa caribaeorum*, *Palythoa variabilis* and *Litopenaeus vannamei*

Luciane A. Chimetto,[1,2,3] Ilse Cleenwerck,[3] Nelson Alves, Jr,[2] Bruno Sergio Silva,[2] Marcelo Brocchi,[1] Anne Willems,[4] Paul De Vos[3,4] and Fabiano L. Thompson[2]

Correspondence
Fabiano L. Thompson
fabiano.thompson@biologia.ufrj.br

[1]Department of Genetics, Evolution and Bioagents, Biology Institute, State University of Campinas (UNICAMP), Campinas, Brazil

[2]Department of Genetics, Institute of Biology, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

[3]BCCM/LMG Bacteria Collection, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium

[4]Laboratory of Microbiology, Faculty of Sciences, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium

Eight *Vibrio* isolates originating from the marine corals *Mussismilia hispida* and *Phyllogorgia dilatata* and the zoanthids *Palythoa caribaeorum* and *Palythoa variabilis* in Brazil and the Pacific white shrimp (*Litopenaeus vannamei*) in Ecuador were studied by means of a polyphasic approach. The novel isolates formed a tight monophyletic group in the genus *Vibrio* and were closely related to species of the *Vibrio harveyi* group, to which they showed more than 99 % 16S rRNA gene sequence similarity. Analysis based on concatenated sequences of the following seven genes, 16S rRNA, *gyrB*, *recA*, *rpoA*, *topA*, *pyrH* and *mreB* (5633 bp in length), showed clear separation between the isolates and species of the *V. harveyi* group. Amplified fragment length polymorphism (AFLP) analysis, performed previously, revealed that a representative isolate of this group, LMG 20370, was clearly separate from known *Vibrio* species (it belonged to the so-called AFLP cluster A31). DNA–DNA hybridization (DDH) experiments with representative isolates and type strains of the *V. harveyi* species group revealed high DDH between the novel isolates (more than 74 %) and less than 70 % DDH towards type strains of related *Vibrio* species, proving the novel species status of the isolates. Phenotypically, the novel species belongs to the arginine dihydrolase (A)-negative, lysine decarboxylase (L)-positive and ornithine decarboxylase (O)-positive (A−/L+/O+) cluster reported previously. Most species of the *V. harveyi* group (i.e. *Vibrio rotiferianus*, *V. harveyi*, *V. parahaemolyticus* and *V. alginolyticus*) also belong to this A−/L+/O+ cluster. However, several phenotypic features can be used for the identification of the novel species. In contrast to its closest phylogenetic neighbours, the novel species exhibits esterase (C4) and *N*-acetyl-β-glucosaminidase activities, but it does not produce acetoin, does not use citrate, α-ketoglutaric acid or propionic acid and does not ferment melibiose. The novel species can also be differentiated on the basis of the presence of the fatty acids $C_{17:0}$, $C_{17:1}\omega8c$, iso-$C_{17:0}$ and iso-$C_{13:0}$ and the absence of the fatty acid $C_{18:0}$. The name *Vibrio communis* sp. nov. is proposed for this taxon. Strain R-40496^T (=LMG 25430^T =CAIM 1816^T) is the type strain.

Abbreviations: AFLP, amplified fragment length polymorphism; DDH, DNA–DNA hybridization; MLSA, multilocus sequence analysis.

The GenBank/EMBL/DDBJ accession numbers for the sequences of strains of *Vibrio communis* sp. nov. determined in this study are GU078670–GU078676 and AJ345066 (16S rRNA gene), GU078680–GU078684 (*gyrB*), GU078693–GU078696, EU717055 and EF596446 (*recA*), GU078697–GU078703 and AJ842625 (*rpoA*), GU078704–GU078710 (*topA*), GU078691, GU078692, EU251617, EU251620, EU251630, EU251638, EU716913 and EF596375 (*pyrH*) and GU078685–GU078690 (*mreB*), as detailed in Supplementary Table S1.

Five supplementary tables and eight supplementary figures are available with the online version of this paper.

The family *Vibrionaceae* belongs to the *Gammaproteobacteria* and at the time of writing includes six genera: *Vibrio* (Baumann & Schubert, 1984), *Photobacterium* (Baumann & Baumann, 1984), *Salinivibrio* (Mellado *et al.*, 1996), *Grimontia* (Thompson *et al.*, 2003), *Enterovibrio* (Thompson *et al.*, 2002b) and *Aliivibrio* (Urbanczyk *et al.*, 2007). The family includes over 115 formally described species (http://www.vibriobiology.net/). Vibrios are common inhabitants of aquatic environments, especially the ocean, and they are known to live either freely or associated as symbionts with aquatic animals in marine or estuarine waters or as parasites of fish, crustaceans and molluscs (Thompson *et al.*, 2004). Vibrios appear to have a key role in the health of corals (Rosenberg *et al.*, 2007). They may provide protection against pathogens or contribute to nitrogen fixation within the holobiont (Ritchie, 2006; Olson *et al.*, 2009). *Vibrio harveyi*, *V. campbellii*, *V. rotiferianus*, *V. alginolyticus*, *V. parahaemolyticus*, *V. natriegens* and *V. mytili* are members of the *Vibrio* core group (Dorsch *et al.*, 1992), later called the Harveyi clade (Sawabe *et al.*, 2007b). Recently, *Vibrio azureus* was included in this group (Yoshizawa *et al.*, 2009). Bacteria of this clade have been found associated with coral disease, as in yellow band disease, which is one of the most significant coral diseases of the tropics (Cervino *et al.*, 2008). Members of the *Vibrio* core group have been described as closely related by 16S rRNA gene sequence analysis and, in some cases, are indistinguishable by more than 100 phenotypic features. For instance, several isolates phenotypically identified as *V. harveyi* were in fact shown to be members of *V. campbellii* by means of molecular identification and DNA–DNA hybridization (DDH) (Gomez-Gil *et al.*, 2004). Multilocus sequence analysis (MLSA) has become an important methodology for studying the taxonomy of *Vibrio*. It has greatly improved species identification and classification (Thompson *et al.*, 2005, 2007; Sawabe *et al.*, 2007b) and is now widely applied (Sawabe *et al.*, 2007a; Gomez-Gil *et al.*, 2008; Rameshkumar *et al.*, 2008; Beaz Hidalgo *et al.*, 2009; Yoshizawa *et al.*, 2009; Xu *et al.*, 2009; Wang *et al.*, 2010).

In a recent study aimed at the taxonomic characterization of vibrios associated with corals in Brazil, several isolates potentially belonging to a novel species were obtained (Chimetto *et al.*, 2008, 2009; Alves *et al.*, 2010). These isolates formed a large, tight group (named L1 or *V. harveyi*-like) on the basis of *pyrH* gene sequences and were closely related to strain LMG 20370. This strain was reported in a previous study as a separated group, amplified fragment length polymorphism (AFLP) cluster A31, within the *Vibrio* group (Thompson *et al.*, 2001b). In the present study, a detailed polyphasic taxonomic analysis was performed in order to determine the exact taxonomic position of a representative group of eight novel isolates, including LMG 20370 and seven Brazilian isolates.

The eight isolates originated from different places over a period of time (Table 1) and were obtained as described previously (Chimetto *et al.*, 2008, 2009). Sequences of genes encoding the 16S rRNA, recombination repair protein (*recA*), topoisomerase I (*topA*), actin-like cytoskeleton protein (*mreB*), RNA polymerase alpha subunit (*rpoA*), DNA gyrase B subunit (*gyrB*) and uridylate kinase (*pyrH*) were obtained as described previously (Thompson *et al.*, 2001b, 2007; Sawabe *et al.*, 2007b). Briefly, PCR products were purified with the enzyme Exosap according to the instructions of the manufacturer (GE Health Care). Subsequently, 5 µl purified PCR products was mixed with 4 µl ET Terminator Mix (GE Health Care), 0.6 µl sequencing primers (20 µmol l^{-1}) and 0.4 µl Milli-Q water. The thermal program consisted of 30 cycles of 20 s at 95 °C, 15 s at 50 °C and 1 min at 60 °C. Purification of the sequencing products was done by adding 1 µl ammonium acetate (7.5 mol l^{-1}) and 27.5 µl absolute ethanol to each product, incubating the mixture in the dark for 30 min and subsequently centrifuging at 20 800 ***g*** for 75 min at 4 °C. After this, the supernatant was removed and 100 µl 70 % ethanol was added. A last centrifugation step was performed at 3700 r.p.m. for 45 min at 4 °C. Separation of the DNA fragments was performed using the MegaBace 1000 system (GE Health Care). Voltage and time of injection were 3 kV and 80 s. Electrophoresis was performed at 9 kV for 100 min at 44 °C. Raw sequence data were transferred to ChromasPro version 1.34 (Technelysium Pty Ltd), where consensus sequences were determined. Sequences were aligned using CLUSTAL W. Pairwise similarity was calculated with the BioNumerics 4.5 software package (Applied Maths), using an open gap penalty of 100 % and a unit gap penalty of 0 %. Similarity matrices and phylogenetic trees were constructed using the MEGA software version 4.0 (Tamura *et al.*, 2007). Trees were drawn using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Eck & Dayhoff, 1966) methods. The robustness of each topology was checked by 1000 bootstrap replications (Felsenstein, 1985). The gene sequence data obtained in this study are also available through the Taxvibrio website (http://www.taxvibrio.lncc.br/). The GenBank accession numbers for the 16S rRNA, *gyrB*, *recA*, *rpoA*, *topA*, *pyrH* and *mreB* gene sequences obtained in this study are listed in Supplementary Table S1, available in IJSEM Online. DDH experiments were performed using the microplate method as described in detail previously (Ezaki *et al.*, 1989; Willems *et al.*, 2001). The hybridization temperature was 40 °C in the presence of 50 % formamide. Reciprocal reactions were performed for every DNA pair and their variation was within the limits of this method (Goris *et al.*, 1998). DDH experiments were performed in four replicates. The G+C content of DNA was determined by the HPLC method as described previously (Mesbah *et al.*, 1989). Analysis of fatty acid methyl esters was carried out as described by Huys *et al.* (1994). For fatty acid analysis, cells were grown on TSA (Difco) supplemented with 1.5 % NaCl for 24 h at 28 °C. Catalase activity was determined by adding young cells to a drop of 3 % H_2O_2 solution and observation of bubble production. Oxidase activity was tested using 1 % *N*,*N*,*N*′,*N*′-tetramethyl *p*-phenylenediamine (Kovács, 1956). Phenotypic characterization was performed using API ZYM and API 20E strips (bioMérieux) and the Biolog GN2 metabolic fingerprinting kit as described previously (Thompson *et al.*, 2001a, 2002a).

Table 1. Isolates of *Vibrio communis* sp. nov.

Isolate	Source	Locality	Year
R-40496^T (=LMG 25430^T =CAIM 1816^T)	Mucus of apparently healthy *Mussismilia hispida*	Grande beach in São Sebastião Channel, São Paulo, Brazil	2005
R-40498 (=LMG 25431)	As above	As above	2005
R-40501	Mucus of apparently healthy *Palythoa caribaeorum*	Preta beach in São Sebastião Channel, São Paulo, Brazil	2005
R-40504	Mucus of apparently healthy *Palythoa variabilis*	Portinho beach in São Sebastião Channel, São Paulo, Brazil	2005
R-40506 (=LMG 25432)	Mucus of apparently healthy *Mussismilia hispida*	As above	2006
R-40900	As above	Abrolhos Bank, Bahia, Brazil	2007
R-40901 (=LMG 25433)	Mucus of apparently healthy *Phyllogorgia dilatata*	As above	2007
LMG 20370	Digestive gland of white shrimp (*Litopenaeus vannamei*)	CENAIM (Ecuador)	2000

The following type strains of phylogenetically closely related *Vibrio* species were included in the phenotypic analyses: *V. rotiferianus* LMG 21460^T, *V. harveyi* LMG 4044^T, *V. parahaemolyticus* LMG 2850^T, *V. alginolyticus* LMG 4409^T, *V. campbellii* LMG 11216^T, *V. natriegens* LMG 10935^T and *V. azureus* LMG 25266^T. The temperature range for growth was determined by incubation on tryptone soy agar (TSA; Oxoid) supplemented with 2 % NaCl (w/v) at 0–42 °C. Growth at 0–10 % (w/v) NaCl was assessed by incubation on TSA (Oxoid) supplemented with NaCl for 72 h at 28 °C.

Phylogenetic analyses based on the 16S rRNA gene sequence classified the eight isolates in a tight monophyletic group in the genus *Vibrio* (Fig. 1). The eight isolates R-40496^T, R-40498, R-40501, R-40504, R-40508, R-40900, R-40901 and LMG 20370 showed more than 99.8 % mutual 16S rRNA gene sequence similarity. They were most closely related to species of the *V. harveyi* group (Reichelt & Baumann, 1973), also called the *Vibrio* core group (Dorsch *et al.*, 1992) or the Harveyi clade (Sawabe *et al.*, 2007b). Phylogenetic analysis with the maximum-parsimony method produced results congruent to those obtained with the neighbour-joining method (Supplementary Fig. S1). The 16S rRNA gene sequence similarity of the isolates towards their closest phylogenetic neighbours varied between 99.5 % for *V. rotiferianus* LMG 21460^T and 98.8 % for *V. mytili* CECT 632^T (Supplementary Table S2). Trees based on housekeeping genes (*gyrB*, *recA*, *rpoA*, *topA*, *pyrH* and *mreB*) confirmed the tight grouping of the novel isolates (Supplementary Figs S2–S7). Similarities between the novel isolates were 97.4–98.9 % for *gyrB*, 98.3–99.6 % for *recA*, 99.4–100 % for *rpoA*, 97.0–99.5 % for *topA*, 98.5–99.8 % for *pyrH* and 95.5–97.7 % for *mreB*. Similarities between representative strain R-40496^T and the type strains of the phylogenetically closest species of the *V. harveyi* group were 83.9–91.7 % for *gyrB*, 89.0–98.3 % for *recA*, 95.8–98.3 % for *rpoA*, 80.7–90.7 % for *topA*, 87.5–96.3 % for *pyrH* and 86.4–96.5 % for *mreB* (Supplementary Table S2). These data indicate that the isolates belong to a novel species in the *V. harveyi* group, since the *gyrB*, *rpoA*, *topA* and *pyrH* gene sequences have high phylogenetic resolution for species identification within the genus *Vibrio* (Thompson *et al.*, 2005, 2007). MLSA of concatenated 16S rRNA, *rpoA* and *pyrH* gene sequences confirmed the tight grouping of the eight isolates (Fig. 2) and a similar result was obtained by MLSA of concatenated 16S rRNA, *gyrB*, *recA*, *rpoA*, *topA*, *pyrH* and *mreB* gene sequences (Supplementary Fig. S8). The MLSA data confirmed that the eight isolates belong to a novel species within the genus *Vibrio*.

DDH experiments were performed with representative strains of the novel group and the type strains of the closest phylogenetic neighbours in order to prove that the isolates belong to a novel species (Supplementary Table S3). The representative strains showed less than 70 % DDH with *V. harveyi* LMG 4044^T, *V. campbellii* LMG 11216^T, *V. rotiferianus* LMG 21460^T, *V. parahaemolyticus* LMG 2850^T, *V. alginolyticus* LMG 4409^T, *V. natriegens* LMG 10935^T and *V. azureus* LMG 25266^T. DDH between the novel isolates varied between 74 and 86 %. DDH between LMG 20370 and *V. harveyi* LMG 4044^T was 66 %, whereas DDH between LMG 20370 and R-40496^T was 79 %. Clearly, the DDH data prove that the eight new isolates represent a novel species.

Phenotypically, the eight isolates are assigned to the genus *Vibrio* (Alsina & Blanch, 1994) and belong to the arginine dihydrolase (A)-negative, lysine decarboxylase (L)-positive and ornithine decarboxylase (O)-positive (A−/L+/O+) cluster (Noguerola & Blanch, 2008). Based on 16S rRNA gene sequence comparisons and MLSA data, most of the phylogenetically related species, i.e. *V. rotiferianus*, *V. harveyi*, *V. parahaemolyticus* and *V. alginolyticus*, belong in the same phenotypic cluster (A−/L+/O+). However, *V. campbellii* and *V. natriegens* belong to the A−/L−/O− cluster and *V. mytili* belongs to the A+/L−/O− cluster according to Noguerola & Blanch (2008). Several phenotypic features can be used to differentiate the novel species from its closest neighbours (Table 2). In contrast to its closest neighbours, the novel species exhibits esterase (C4) and *N*-acetyl-β-glucosaminidase activities, but it does not produce acetoin and does not utilize citrate, α-ketoglutaric

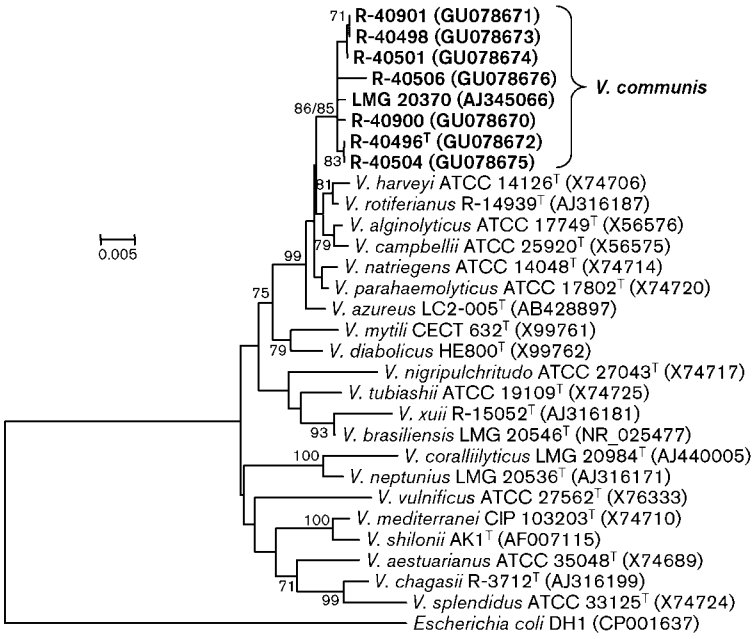

Fig. 1. Neighbour-joining phylogenetic tree showing the phylogenetic position of strains of *Vibrio communis* sp. nov. based on 16S rRNA gene sequences (1470 bp). The optimal tree with the sum of branch lengths=0.28450859 is shown. Evolutionary distances were computed using the Jukes–Cantor method. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (pairwise deletion option). Bootstrap values (⩾70 %) based on 1000 repetitions are shown. The bootstrap value for the *V. communis* branch was also computed by the maximum-parsimony method (neighbour-joining/maximum-parsimony). The sequence of *Escherichia coli* DH1 was used as an outgroup. Bar, 0.5 % estimated sequence divergence.

acid or propionic acid. It also does not ferment melibiose, but it ferments amygdalin and has weak growth on 8 % NaCl. Yellow colonies are observed on thiosulfate-citrate-bile salts-sucrose (TCBS) agar and beige, translucent colonies on marine agar. Phenotypic variation was observed among the isolates (Supplementary Table S4), suggesting a good representation of the phenotype of the novel species. The novel isolates can also be differentiated from their neighbours on the basis of the presence of the fatty acids $C_{17:0}$, $C_{17:1}\omega8c$, iso-$C_{17:0}$ and iso-$C_{13:0}$ and the absence of the fatty acid $C_{18:0}$, which is normally present in other species of the *V. harveyi* group (Supplementary Table S5).

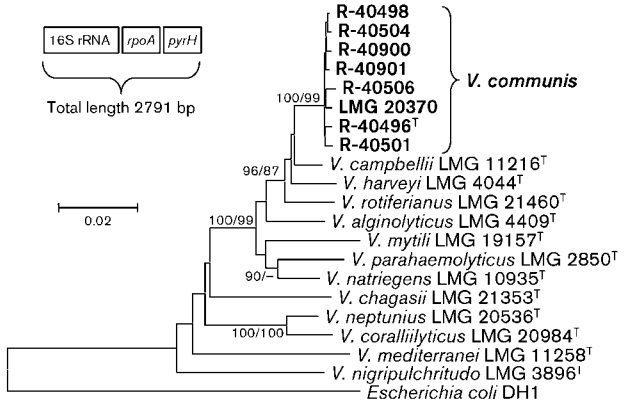

Fig. 2. Neighbour-joining phylogenetic tree showing the phylogenetic position of strains of *V. communis* sp. nov. based on concatenated 16S rRNA (1470 bp), *rpoA* (790 bp) and *pyrH* (531 bp) gene sequences. Evolutionary distances were computed using the Jukes–Cantor method. Phylogenetic analyses were conducted in MEGA4. Bootstrap values (>70 %) based on 1000 repetitions are shown. Numbers at nodes denote bootstrap values derived from the neighbour-joining/maximum-parsimony methods; –, <70 %. The sequence of *Escherichia coli* DH1 was used as an outgroup. Bar, 2 % estimated sequence divergence.

Based on the polyphasic analysis presented in this study, we propose to classify the eight isolates in the novel species *Vibrio communis* sp. nov. The novel species can be differentiated from its phylogenetic neighbours by means of AFLP (Thompson *et al.*, 2001b), MLSA, DDH and several phenotypic and chemotaxonomic tests.

Description of *Vibrio communis* sp. nov.

Vibrio communis (com.mu'nis. L. masc. adj. *communis* common, widespread, referring to the frequent isolation of the species in the marine environment).

Cells are Gram-negative, motile bacilli, 1 μm wide and 2.5–3.5 μm long. Catalase- and oxidase-positive. Forms translucent, convex, smooth-rounded colonies with entire margins, beige in colour and 1 mm in diameter after 1 day of incubation at 28 °C on TSA. Prolific growth occurs at 15–37 °C and at 0.5–6.0 % (w/v) NaCl. Weak growth is observed in the presence of 8 % NaCl. No growth is observed in the absence of NaCl or at or above 9 % NaCl, at 7 °C or below or at 42 °C or above. Yellow colonies are observed on the selective medium TCBS. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, *N*-acetyl-β-glucosaminidase, α-glucosidase (except R-40498), lysine decarboxylase and ornithine decarboxylase (except R-40900) enzyme activities, production of indole and acetoin (Voges–Proskauer), fermentation of glucose, mannitol, sucrose and amygdalin, gelatinase diffusion

Table 2. Differential phenotypic characteristics of strains of *V. communis* sp. nov. and related species of the genus *Vibrio*

Strains: 1, *V. communis* sp. nov. (eight strains); 2, *V. rotiferianus* LMG 21460^T; 3, *V. harveyi* LMG 4044^T; 4, *V. parahaemolyticus* LMG 2850^T; 5, *V alginolyticus* LMG 4409^T; 6, *V campbellii* LMG 11216^T; 7, *V natriegens* LMG 10935^T; 8, *V. azureus* LMG 25266^T. +, Positive; −, negative; w, weakly positive. Data were obtained in this study. Species are assigned to phenotypic clusters according to Noguerola & Blanch (2008); characteristics in bold are useful to differentiate A−/L+/O+ species according to Noguerola & Blanch (2008).

Characteristic	A−/L+/O+					A−/L−/O−		
	1	2	3	4	5	6	7	8
Growth in NaCl at:								
8 % (w/v)	w	−	−	+	+	w	+	−
10 % (w/v)	−	−	−	−	+	−	−	−
Production of:								
Indole	+	+	+	+	+	+	−	−
Acetoin	+	−	−	−	+	−	−	+
Enzyme activities								
Esterase (C4)	+	+	−	+	+	+	−	−
Lipase (C14)	+	−	+	−	−	+	+	−
***β*-Galactosidase**	+	−	+	−	−	−	+	−
N-Acetyl-*β*-glucosaminidase	+	−	−	+	+	+	−	+
α-Chymotrypsin	+	+	+	−	−	+	−	−
Tryptophan deaminase	−	+	−	−	−	−	+	−
Urea	−	+	−	−	−	−	+	−
Utilization of:								
L-Arabinose	−	+	−	+	−	w	+	−
Citrate	−	−	+	−	−	−	+	−
cis-Aconitic acid	−	−	+	w	+	−	+	−
α-Ketoglutaric acid	−	−	+	−	+	−	+	−
Propionic acid	−	−	+	−	+	−	+	−
L-Glutamic acid	+	−	+	w	+	+	+	w
Sucrose	+	+	+	−	+	−	−	−
Glycerol	+	−	−	+	+	+	−	−
L-Alanine	+	w	+	−	+	−	−	−
L-Alanyl glycine	+	−	+	−	+	+	−	w
DL-α-Glycerol phosphate	+	−	−	+	+	+	−	−
Fermentation of:								
Amygdalin	+	+	−	−	−	+	−	+
Melibiose	−	+	+	−	−	−	+	−

(except R-40504) and nitrate reduction to nitrite; weakly positive for *β*-glucosidase activity (negative for R-40496^T, R-40498, R-40900 and R-40901). Negative for cystine arylamidase, α-galactosidase, *β*-glucuronidase, α-mannosidase, α-fucosidase, arginine dihydrolase, urease and tryptophan deaminase enzyme activities, citrate utilization (except R-40498), H_2S production, fermentation of inositol, sorbitol, rhamnose, melibiose and arabinose and reduction of nitrate to dinitrogen gas. All known strains utilize α-cyclodextrin, dextrin, glycogen, Tweens 40 and 80, *N*-acetyl-D-glucosamine, cellobiose, D-fructose, α-D-glucose, maltose, D-mannitol, D-mannose, psicose, sucrose, trehalose, methyl pyruvate, D-gluconic acid, DL-lactic acid, alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-threonine, inosine, uridine, thymidine, glycerol, DL-α-glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate. None of the known strains utilizes *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, i-erythritol, L-fucose, *myo*-inositol, lactose, lactose lactulose, melibiose, raffinose, L-rhamnose, turanose, xylitol, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, *β*-hydroxybutyric acid, *γ*-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketoglutaric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinamic acid, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, DL-carnitine, *γ*-aminobutyric acid, phenylethylamine, putrescine, 2-aminoethanol or 2,3-butanediol. Utilization of the following compounds is variable (results in parentheses are from the type strain): D-galactose (−), gentiobiose (+), methyl *β*-D-glucoside (−), D-sorbitol (−), monomethyl succinate (−), α-hydroxybutyric acid (+),

α-ketobutyric acid (+), succinic acid (+), bromosuccinic acid (+), glucuronamide (+), D-alanine (−), hydroxy-L-proline (−), L-proline (+), D-serine (−), L-serine (+) and urocanic acid (+) (Supplementary Table S4). The fatty acid profiles of the eight known isolates are similar. The main cellular fatty acids are summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{14:0}$ (Supplementary Table S5). The following fatty acids are present in small amounts: summed feature 2 ($C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I, an unidentified fatty acid with an equivalent chain-length of 10.928 and/or $C_{12:0}$ ALDE), $C_{12:0}$ 3-OH, $C_{12:0}$, iso-$C_{16:0}$, $C_{17:0}$, $C_{17:1}\omega8c$, iso-$C_{17:0}$ and iso-$C_{13:0}$. The DNA G+C content of the type strain is 45.8 mol%; the range for the eight known strains is 45.2–46.0 mol%.

The type strain, R-40496^T (=LMG 25430^T =CAIM 1816^T), was isolated from mucus of the endemic coral *Mussismilia hispida* in the São Sebastião channel, SP, Brazil. Other strains are detailed in Table 1.

Acknowledgements

The authors acknowledge grants from FAPERJ, FAPESP, CNPq and IFS. L. A. C. acknowledges a PhD scholarship provided by CNPq. The BCCM/LMG Bacteria Collection is supported by the Federal Public Planning Service-Science Policy, Belgium. We thank Katrien Engelbeen (LMG/BCCM), Stefanie Van Trappen (LMG/BCCM), Alvaro Migotto (CEBIMAR-USP), Bruno Gomez-Gil and Cristiane C. Thompson for technical assistance and valuable comments.

References

Alsina, M. & Blanch, A. R. (1994). A set of keys for biochemical identification of environmental *Vibrio* species. *J Appl Bacteriol* **76**, 79–85.

Alves, N., Jr, Silva, B. S. O., Mala Neto, O. S., Moura, R. L., Francini-Filho, R. B., Castro, C. B., Paranhos, R., Bitner-Mathé, B. C., Kruger, R. H. & other authors (2010). Diversity and pathogenic potential of vibrios isolated from Abrolhos Bank corals. *Environ Microbiol Rep* **2**, 90–95.

Baumann, P. & Baumann, L. (1984). Genus II. *Photobacterium* Beijerinck 1889, 401^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 539–545. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Baumann, P. & Schubert, R. H. W. (1984). Family II. *Vibrionaceae* Véron 1965, 5245^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 516–517. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Beaz Hidalgo, R., Cleenwerck, I., Balboa, S., Prado, S., De Vos, P. & Romalde, J. L. (2009). *Vibrio breoganii* sp. nov., a non-motile, alginolytic, marine bacterium within the *Vibrio halioticoli* clade. *Int J Syst Evol Microbiol* **59**, 1589–1594.

Cervino, J. M., Thompson, F. L., Gomez-Gil, B., Lorence, E. A., Goreau, T. J., Hayes, R. L., Winiarski-Cervino, K. B., Smith, G. W., Hughen, K. & Bartels, E. (2008). The *Vibrio* core group induces yellow band disease in Caribbean and Indo-Pacific reef-building corals. *J Appl Microbiol* **105**, 1658–1671.

Chimetto, L. A., Brocchi, M., Thompson, C. C., Martins, R. C. R., Ramos, H. B. & Thompson, F. L. (2008). Vibrios dominate as culturable nitrogen-fixing bacteria of Brazilian coral *Mussismilia hispida*. *Syst Appl Microbiol* **31**, 312–319.

Chimetto, L. A., Brocchi, M., Gondo, M., Thompson, C. C., Gomez-Gil, B. & Thompson, F. L. (2009). Genomic diversity of vibrios associated with the Brazilian coral *Mussismilia hispida* and its sympatric zoanthids (*Palythoa caribaeorum*, *Palythoa variabilis* and *Zoanthus solanderi*). *J Appl Microbiol* **106**, 1818–1826.

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

Eck, R. V. & Dayhoff, M. O. (1966). *Atlas of Protein Sequence and Structure*. Silver Springs, MD: National Biomedical Research Foundation.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Farmer, J. J., III, Janda, J. M., Brenner, F. W., Cameron, D. N. & Birkhead, K. M. (2005). Genus I. *Vibrio* Pacini 1854, 411^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2B, pp. 494–546. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Gomez-Gil, B., Soto-Rodríguez, S., García-Gasca, A., Roque, A., Vazquez-Juarez, R., Thompson, F. L. & Swings, J. (2004). Molecular identification of *Vibrio harveyi*-related isolates associated with diseased aquatic organisms. *Microbiology* **150**, 1769–1777.

Gomez-Gil, B., Fajer-Avila, E., Pascual, J., Macián, M. C., Pujalte, M. J., Garay, E. & Roque, A. (2008). *Vibrio sinaloensis* sp. nov., isolated from the spotted rose snapper, *Lutjanus guttatus* Steindachner, 1869. *Int J Syst Evol Microbiol* **58**, 1621–1624.

Goris, J., Suzuki, K., De Vos, P., Nakase, T. & Kersters, K. (1998). Evaluation of a microplate DNA-DNA hybridization method compared with the initial renaturation method. *Can J Microbiol* **44**, 1148–1153.

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.

Kovács, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* **178**, 703.

Mellado, E., Moore, E. R. B., Nieto, J. J. & Ventosa, A. (1996). Analysis of 16S rRNA gene sequences of *Vibrio costicola* strains: description of *Salinivibrio costicola* gen. nov., comb. nov. *Int J Syst Bacteriol* **46**, 817–821.

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.

Noguerola, I. & Blanch, A. R. (2008). Identification of *Vibrio* spp. with a set of dichotomous keys. *J Appl Microbiol* **105**, 175–185.

Olson, N. D., Ainsworth, T. D., Gates, R. D. & Takabayashi, M. (2009). Diazotrophic bacteria associated with Hawaiian *Montipora* corals: diversity and abundance in correlation with symbiotic dinoflagellates. *J Exp Mar Biol Ecol* **371**, 140–146.

Rameshkumar, N., Fukui, Y., Sawabe, T. & Nair, S. (2008). *Vibrio porteresiae* sp. nov., a diazotrophic bacterium isolated from a mangrove-associated wild rice (*Porteresia coarctata* Tateoka). *Int J Syst Evol Microbiol* **58**, 1608–1615.

Reichelt, J. L. & Baumann, P. (1973). Taxonomy of the marine, luminous bacteria. *Arch Mikrobiol* **94**, 283–330.

Ritchie, K. B. (2006). Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Mar Ecol Prog Ser* **322**, 1–14.

Rosenberg, E., Koren, O., Reshef, L., Efrony, R. & Zilber-Rosenberg, I. (2007). The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* **5**, 355–362.

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

Sawabe, T., Fujimura, Y., Niwa, K. & Aono, H. (2007a). *Vibrio comitans* sp. nov., *Vibrio rarus* sp. nov. and *Vibrio inusitatus* sp. nov., from the gut of the abalones *Haliotis discus discus*, *H. gigantea*, *H. madaka* and *H. rufescens*. *Int J Syst Evol Microbiol* **57**, 916–922.

Sawabe, T., Kita-Tsukamoto, K. & Thompson, F. L. (2007b). Inferring the evolutionary history of vibrios by means of multilocus sequence analysis. *J Bacteriol* **189**, 7932–7936.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Thompson, F. L., Hoste, B., Thompson, C. C., Huys, G. & Swings, J. (2001a). The coral bleaching *Vibrio shiloi* Kushmaro et al. 2001 is a later synonym of *Vibrio mediterranei* Pujalte and Garay 1986. *Syst Appl Microbiol* **24**, 516–519.

Thompson, F. L., Hoste, B., Vandemeulebroecke, K. & Swings, J. (2001b). 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. (2002a). *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.

Thompson, F. L., Hoste, B., Thompson, C. C., Goris, J., Gomez-Gil, B., Huys, L., De Vos, P. & Swings, J. (2002b). *Enterovibrio norvegicus* gen. nov., sp nov., isolated from the gut of turbot (*Scophthalmus maximus*) larvae: a new member of the family *Vibrionaceae*. *Int J Syst Evol Microbiol* **52**, 2015–2022.

Thompson, F. L., Hoste, B., Vandemeulebroecke, K. & Swings, J. (2003). Reclassification of *Vibrio hollisae* as *Grimontia hollisae* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **53**, 1615–1617.

Thompson, F. L., Iida, T. & Swings, J. (2004). Biodiversity of vibrios. *Microbiol Mol Biol Rev* **68**, 403–431.

Thompson, F. L., Gevers, D., Thompson, C. C., Dawyndt, P., Naser, S., Hoste, B., Munn, C. B. & Swings, J. (2005). Phylogeny and molecular identification of vibrios based on multilocus sequence analysis. *Appl Environ Microbiol* **71**, 5107–5115.

Thompson, F. L., Gomez-Gil, B., Vasconcelos, A. T. R. & Sawabe, T. (2007). Multilocus sequence analysis reveals that *Vibrio harveyi* and *V. campbellii* are distinct species. *Appl Environ Microbiol* **73**, 4279–4285.

Urbanczyk, H., Ast, J. C., Higgins, M. J., Carson, J. & Dunlap, P. V. (2007). Reclassification of *Vibrio fischeri*, *Vibrio logei*, *Vibrio salmonicida* and *Vibrio wodanis* as *Aliivibrio fischeri* gen. nov., comb. nov., *Aliivibrio logei* comb. nov., *Aliivibrio salmonicida* comb. nov. and *Aliivibrio wodanis* comb. nov. *Int J Syst Evol Microbiol* **57**, 2823–2829.

Wang, Y., Zhang, X.-H., Yu, M., Wang, H. & Austin, B. (2010). *Vibrio atypicus* sp. nov., isolated from the digestive tract of the Chinese prawn (*Penaeus chinensis*, O'sbeck). *Int J Syst Evol Microbiol* **60**, 2517–2523.

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.

Xu, X.-W., Wu, Y.-H., Wang, C.-S., Oren, A. & Wu, M. (2009). *Vibrio hangzhouensis* sp. nov., isolated from sediment of the East China Sea. *Int J Syst Evol Microbiol* **59**, 2099–2103.

Yoshizawa, S., Wada, M., Kita-Tsukamoto, K., Ikemoto, E., Yokota, A. & Kogure, K. (2009). *Vibrio azureus* sp. nov., a luminous marine bacterium isolated from seawater. *Int J Syst Evol Microbiol* **59**, 1645–1649.