Reclassification of *Vibrio hollisae* as *Grimontia hollisae* gen. nov., comb. nov.

Fabiano L. Thompson,[1,2] Bart Hoste,[2] Katrien Vandemeulebroecke[2] and Jean Swings[1,2]

Correspondence
Fabiano L. Thompson
Fabiano.Thompson@ugent.be

[1]Laboratory for Microbiology, Ghent University, K. L. Ledeganckstraat 35, Ghent 9000, Belgium

[2]BCCM™/LMG Bacteria Collection, Laboratory for Microbiology, Ghent University, K. L. Ledeganckstraat 35, Ghent 9000, Belgium

The taxonomic positions of three representative strains of *Vibrio hollisae* (LMG 17719^T, LMG 21416 and LMG 21538) were investigated by means of 16S rDNA sequences and phenotypic data. *V. hollisae* strains (GenBank/EMBL accession nos AJ514909–AJ514911) shared 99·5 % 16S rDNA sequence similarity, but had only 94·6 % similarity to their closest phylogenetic neighbour, *Enterovibrio norvegicus*. 16S rDNA sequence similarity of *V. hollisae* and *Vibrio cholerae* was only 91 %. These results suggest that *V. hollisae* should be placed into a novel genus, for which the name *Grimontia* gen. nov. is proposed.

Vibrio hollisae was first described by Hickman *et al.* (1982). This organism produces a number of toxins and also invades host epithelial cells (Miliotis *et al.*, 1995), causing both gastroenteritis and septicaemia (Abbott & Janda, 1994). Phenotypically, *V. hollisae* strains are atypical of the genus *Vibrio*, as they are arginine dihydrolase-, lysine and ornithine decarboxylase-negative and cannot grow on thiosulphate/citrate/bile salts/sucrose (TCBS) agar. FAFLP (fluorescent amplified fragment length polymorphism) fingerprinting revealed that *V. hollisae* is quite different from other vibrios, as it remained unclustered when 506 *Vibrio* strains were grouped by FAFLP pattern similarity (Thompson *et al.*, 2001). Dorsch *et al.* (1992) analysed the phylogenetic positions of several *Vibrio* species by means of 16S rRNA gene sequences and concluded that *V. hollisae* should be considered as a novel genus, although these authors highlighted the need for more representative 16S rRNA gene sequences of diverse *Vibrio* branches to determine whether *V. hollisae* is actually a novel genus. In a comprehensive phylogenetic study of the families *Vibrionaceae*, *Aeromonadaceae* and *Plesiomonadaceae* based on 16S rRNA gene sequences, Ruimy *et al.* (1994) showed that *V. hollisae* in fact lies at the outskirts of the genus *Vibrio*, being as closely related to this genus as to the genera *Photobacterium* and *Salinivibrio*. Analyses of *toxR* and *gyrB* gene sequences have pointed out the very low similarity between *V. hollisae* and other *Vibrio* species (Osorio & Klose, 2000; Vuddhakul *et al.*, 2000). More recently, on the basis of 16S rDNA sequence analysis, we found that *V. hollisae* branched between *Photobacterium* species and a newly described genus, *Enterovibrio*, rather than with other vibrios (Thompson *et al.*, 2002).

In this study, we further analysed the phylogenetic positions of three representative strains of *V. hollisae* (LMG 17719^T, LMG 21416 and LMG 21538) that were associated with cases of gastroenteritis in the USA. Strains LMG 17719^T (=ATCC 33564^T) and LMG 21416 (=ATCC 33565=JCM 1284) were analysed in detail in the original description of *V. hollisae* (Hickman *et al.*, 1982), while strain LMG 21538 (=CIP 104354) was isolated and studied by Carnahan *et al.* (1994). 16S rDNA sequence analysis was performed as described previously (Thompson *et al.*, 2001). 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. Cells (approx. 50 mg) were harvested and fatty acids were isolated, following the recommendations of the manufacturer, by using the Microbial Identification system and software package, version 3.9 (MIDI).

The results of our phylogenetic analysis are depicted in Fig. 1. The three *V. hollisae* strains shared 99·5 % 16S rDNA sequence similarity, but had only 94·6 % similarity to their closest phylogenetic neighbour, *Enterovibrio norvegicus*. A maximum-parsimony tree gave a very similar branching pattern to the neighbour-joining tree shown in Fig. 1. 16S rDNA sequence similarity of *V. hollisae* to the genera *Photobacterium* and *Salinivibrio* was respectively 93 and 91·2 % whereas similarity between *V. hollisae* and *V. cholerae*

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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 17719^T, LMG 21416 and LMG 21538 are AJ514909–AJ514911.

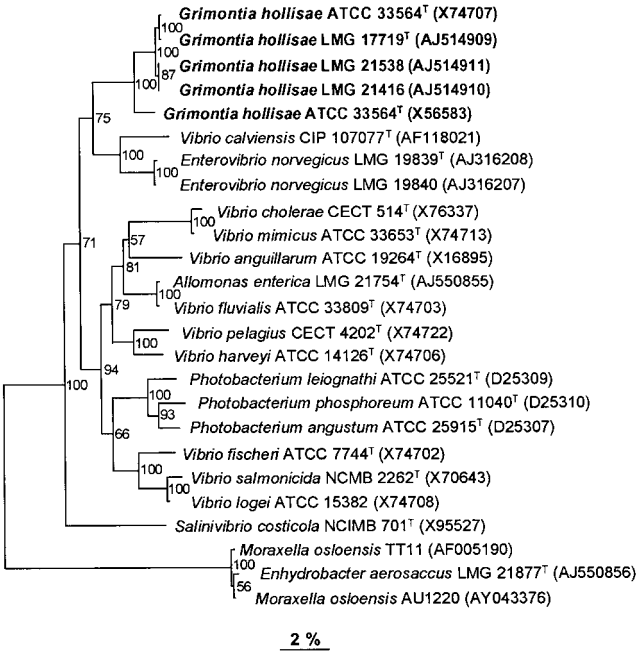

Fig. 1. Phylogenetic tree with estimated positions of *V. hollisae*, *Allomonas enterica* and *Enhydrobacter aerosaccus* by using the neighbour-joining method, based on almost-complete 16S rDNA sequences (positions 10–1415). Bootstrap percentages after 1000 simulations are shown. Bar, 2 % estimated sequence divergence.

was only 90·8 %, clearly indicating that *V. hollisae* belongs to a different genus within the family *Vibrionaceae*.

In our phylogenetic analyses we included representative 16S rDNA sequences of all six genera currently assigned to the family *Vibrionaceae*, i.e. *Allomonas*, *Enhydrobacter*, *Listonella*, *Photobacterium*, *Salinivibrio* and *Vibrio* [see outline of *Bergey's Manual of Systematic Bacteriology* (2002) at http://dx.doi.org/10·1007/bergeysoutline200210]. We also included representative sequences of the newly described genus *Enterovibrio* (Thompson *et al.*, 2002). 16S rDNA sequences of *Allomonas enterica* LMG 21754^T (GenBank/EMBL accession no. AJ550855, 1468 bp) and *Enhydrobacter aerosaccus* LMG 21877^T (AJ550856, 1484 bp) were determined in the course of this study. The genera *Allomonas* (Kalina *et al.*, 1984) and *Enhydrobacter* (Staley *et al.*, 1987) were allocated tentatively to the family *Vibrionaceae* based on phenotypic features, but so far there had been no confirmation of their phylogenetic positions based on 16S rDNA sequence data (Farmer, 1986; Farmer & Hickman-Brenner, 1992). According to our 16S rDNA data, *A. enterica* and *E. aerosaccus* are highly related (with almost 100 % 16S rDNA sequence similarity) to *Vibrio fluvialis* (X74703) and *Moraxella osloensis* (X74897, AF005191 and AY043376), respectively (Pettersson *et al.*, 1998; Coenye *et al.*, 2002). Further experiments with a more comprehensive collection of reference strains of *Allomonas* and *Enhydrobacter* are needed to clarify the taxonomic affiliations of these genera.

The three 16S rDNA sequences of *V. hollisae* determined in the present study (GenBank accession nos AJ514909–AJ514911) were highly related (99·3 %) to sequence X74707, determined by Ruimy *et al.* (1994) for the type strain of *V. hollisae*. The four above-mentioned sequences were ~4 % different from sequence X56583, proposed by Dorsch *et al.* (1992). Mellado *et al.* (1996) had already highlighted the need for an explanation of such a difference in sequence data. For their comparisons, Mellado *et al.* (1996) used the sequence of Dorsch *et al.* (1992), although our results show clearly that the most accurate sequence is in fact that of Ruimy *et al.* (1994) or any of the three sequences determined in this study.

V. hollisae can be also differentiated from other members of the family *Vibrionaceae* by several phenotypic features (Table 1). *V. hollisae* has higher levels of the fatty acids $C_{18:1}\omega9c$ and $C_{16:1}\omega9c$ and does not grow on TCBS, as most vibrios can. *V. hollisae* is arginine dihydrolase-negative and nitrate reduction-positive, by which it differs from the genera *Enterovibrio*, *Photobacterium* and *Salinivibrio*.

On the basis of the phylogenetic and phenotypic data presented here, we propose to accommodate *V. hollisae* in a novel genus, *Grimontia* gen. nov. The type species of the novel genus is *Grimontia hollisae* gen. nov., comb. nov.

Description of *Grimontia* gen. nov.

Grimontia (Gri.mon′ti.a. N.L. gen. n. *Grimontia* of Grimont, after the French microbiologist P. A. D. Grimont).

Table 1. Features that differentiate *Grimontia* gen. nov. from other genera of the family *Vibrionaceae*

Taxa: 1, *Grimontia*; 2, *Enterovibrio* (Thompson *et al.*, 2002); 3, *Photobacterium* (Alsina & Blanch, 1994); 4, *Salinivibrio* (Mellado *et al.*, 1996); 5, *Vibrio* (Alsina & Blanch, 1994). Fatty acid data (%) are from our own database. TCBS medium was obtained from Oxoid.

Feature	1	2	3	4	5
Growth on/in:					
TCBS	−	+	+	+	+*
12 % NaCl	−	−	−	+	−
Production of:					
Acetoin	−	−	+/−	+	−*
Indole	+	+	−	−	+/−
Nitrate reduction	+	−	+/−	−	+*
Arginine dihydrolase	−	+	+	+	+/−
Fatty acids:					
$C_{18:1}\omega9c$	4–6	3	0	<1	0–5
$C_{16:1}\omega9c$	4	4	0	1–2	0–2

*Over 85 % of species show this feature.

Cells are Gram-negative, motile by a polar flagellum and oxidase-positive. Strains have a DNA G+C content of 48·5–51·0 mol%. Most abundant fatty acids are summed feature 3 (31±1 %; comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), $C_{18:1}\omega7c$ (23±2 %), $C_{16:0}$ (14±1 %), $C_{12:0}$ (5±1 %), $C_{18:1}\omega9c$ (5±1 %), summed feature 2 (5±2 %; comprising $C_{14:0}$ 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), $C_{16:1}\omega9c$ (4±0 %), $C_{12:0}$ 3-OH (4±2 %), $C_{14:0}$ (3±0 %) and $C_{18:0}$ (2±1 %). Chemoheterotrophic, mesophilic and moderately halophilic. Strains are negative for Voges–Proskauer reaction, arginine dihydrolase, lysine and ornithine decarboxylase, but indole production and nitrate reduction are positive. 16S rDNA sequences of *Grimontia* strains have typical signatures at positions 970–971 (TC instead of AG) and 1107–1108 (CG instead of AA), which differ from those of other members of the family *Vibrionaceae*.

The type species is *Grimontia hollisae* (LMG 17719^T; GenBank/EMBL accession no. AJ514909, DNA G+C content is 48·5 mol%). Description of *Grimontia hollisae* gen. nov., comb. nov. is based on the original description of Hickman *et al.* (1982).

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