NOTE

Vibrio trachuri Iwamoto *et al.* 1995 is a junior synonym of *Vibrio harveyi* (Johnson and Shunk 1936) Baumann *et al.* 1981

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The taxonomic position of *Vibrio trachuri* was examined through a polyphasic approach using 16S rDNA sequencing, fluorescent amplified fragment length polymorphisms (FAFLP), DNA–DNA hybridization experiments, G+C content of DNA and phenotypical tests. Phylogenetic analysis showed that *Vibrio harveyi* is the closest neighbour of *V. trachuri*, sharing about 98·8% similarity in the 16S rDNA gene. Moreover, numerical analysis of the FAFLP patterns revealed that both species have highly related genomes, sharing 55% pattern similarity. DNA–DNA hybridization experiments and G+C content measurements reinforced these results, since *V. trachuri* and *V. harveyi* had at least 74% DNA similarity and 44·5–45·2 mol% G+C. Phenotypical features of both species were also very similar, except that *V. trachuri* utilized itaconic acid, whereas *V. harveyi* did not. Therefore, it is proposed that the species *V. trachuri* should be reclassified as *V. harveyi*.

Keywords: *Vibrio trachuri*, *Vibrio harveyi*, fish pathogen, FAFLP

Vibrios are readily isolated from a wide range of marine and estuarine environments, including rearing systems (Vandenberghe *et al.*, 1999). Although some vibrios have been found to be symbiotic (e.g. *Vibrio halioticoli* and the abalone *Haliotis hannai hannai*) or probiotic (e.g. *Vibrio alginolyticus* and the shrimp *Litopenaeus vannamei*), they are mostly known as pathogenic, particularly the species *Listonella anguillarum* and *Vibrio harveyi*. Actually, there have been numerous reports on the pathogenicity of *V. harveyi* for a wide range of fish and shellfish (Austin & Austin. 1999). *V. harveyi* is a well characterized bacterial species and has a clearly defined taxonomic position (Baumann *et al.*, 1980; Dorsch *et al.*, 1992; Johnson & Shunk, 1936; Pedersen *et al.*, 1998). It belongs to the core group in the genus and its closest phylogenetic neighbours are *V. alginolyticus* and *Vibrio campbellii* (Dorsch *et al.*, 1992). An AFLP analysis of 36 *V. harveyi* strains revealed a high genotypic heterogeneity within this species and *Vibrio carchariae* was considered its later synonym (Pedersen *et al.*, 1998).

Three *Vibrio trachuri* strains were isolated from diseased cultured Japanese horse mackerel (*Trachurus japonicus*) at Uchiura Bay, Numazu, Japan, in the 1990s. Based mainly on the relatively low DNA homology values found and on its differential phenotypical features with *V. harveyi*, *L. anguillarum* and *Vibrio parahaemolyticus*, it was proposed as a new species (Iwamoto *et al.*, 1995), although the disease symptoms in fish and phenotypical characteristics of *V. trachuri* and *V. harveyi* were quite similar (Austin & Austin, 1999; Iwamoto *et al.*, 1995).

The 12 strains used in this study are listed in Table 1. Bacterial DNAs used in this study were isolated following the technique of Pitcher *et al.* (1989). Concentration and purity of the DNAs were estimated measuring optical densities at 260, 234 and 280 nm in a Uvicom 941+spectrophotometer (Kontron Instruments). DNA integrity was verified on a 1% agarose gel in 1× TAE buffer (40 mM Tris/acetate, 1 mM

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Abbreviation: FAFLP, fluorescent amplified fragment length polymorphisms.

The GenBank accession numbers for the 16S rDNA sequences of *V. trachuri* LMG 19643^T and LMG 19714 (partial sequence) are AJ312382 and AJ312383, respectively.

Table 1. List of strains used in this study

LMG, BCCM/LMG Bacteria Collection, Laboratory for Microbiology, Ghent University, Ghent, Belgium; ATCC, American Type Culture Collection, Manassas, VA, USA; CIP, Institut Pasteur Collection, Paris, France.

Species and strain designation	Place and date of isolation	Source
V. harveyi		
LMG 4044^T (ATCC 14126^T)	Massachusettes (USA), 1935	Dead amphipod (*Talorchestia* sp.)
LMG 7890 (ATCC 35084); formerly *V. carchariae*	Baltimore (USA)	Brown shark (*Carcharhinus plumbeus*)
LMG 11226 (R-14953)	Hawaii (USA)	Sea water
R-14946 (VIB 646), R-14947 (VIB 665)	Denmark, 1993	Water from tanks with sharks
R-14948 (VIB 644)	Japan	Diseased milkfish (*Chanus chanus*)
R-14949 (VIB 811)	Spain, 1986	Sea bass (*Dicentrarchus labrax*)
R-14950 (VIB 23)	Greece, 1991	Sea bream (*Sparus aurata*)
R-14951 (VIB 22)	Greece, 1991	Sea bass (*Dicentrarchus labrax*)
R-14952 (VIB 568)	Spain, 1990	Turbot (*Scophthalmus maximus*)
V. trachuri		
LMG 19643^T (CIP 104774^T)	Numazu (Japan)	Japanese horse mackerel (*Trachurus japonicus*)
LMG 19714 (T9216)		

EDTA, pH 8·0). 16S rDNA sequence and fluorescent amplified fragment length polymorphisms (FAFLP) analyses were done following the protocols described previously (Thompson *et al*., 2001). The G+C content of the genomic DNA and RNA content were determined by HPLC methodology (Tamaoka & Komagata, 1984). DNA–DNA hybridization was performed using the microplate technique with photobiotin-labelled DNA at 36 °C for 3 h as described by Willems *et al*. (2001). The temperature for optimal renaturation (T_{or}) was calculated as [(0·51 × G+C content)+47] −36. To perform the hybridization experiments in stringent conditions, a temperature of 36 °C was chosen. Phenotypical characterization of the strains was performed using the commercial kits API 20E (bioMérieux) and BIOLOG GN microplates, following the instructions of the manufacturers with slight modifications. For API 20E tests, strains were grown on Marine Agar (Difco) for 24 h at 25 °C. Subsequently, cells were resuspended in a 1·5% NaCl solution. These suspensions, which had a standard opacity equivalent to McFarland 3, were inoculated into the API 20E strips which were incubated for 48 h at 25 °C. For BIOLOG GN metabolic fingerprinting, strains were grown on Blood Agar (BIOLOG) for 24 h at 28 °C. Subsequently, cells were resuspended in inoculating fluid (1·5% NaCl, 0·03% Pluronic F-68, 0·01% Gellan Gum) and cell densities were photometrically standardized between 0·28 and 0·30 OD_{590}. The wells of the BIOLOG GN microplates were inoculated with the cell suspension and the microplates were incubated for 24 h at 28 °C. Changes in colour were measured using a spectrophotometer (BIOLOG) at 550 and 750 nm.

The 16S rDNA analysis revealed that the closest phylogenetic neighbours of *V. trachuri* LMG 19643^T (accession no. AJ312382) are *V. harveyi* LMG 4044^T (X74706) and *V. alginolyticus* (X56576) with 98·8 and 98·2% similarity, respectively. To confirm the authenticity of *V. trachuri* LMG 19714 used in this study, a partial 16S rDNA sequence (933 bp) was determined (AJ312383). This partial sequence was compared with the one from *V. trachuri* LMG 19643^T (AJ312382) and they were found to be identical.

Furthermore, numerical analysis of the FAFLP band patterns (114±17 bands) of ten *V. harveyi* and two *V. trachuri* strains revealed that both species have similar genomes (55% pattern similarity) and formed a separated cluster which was distinguishable from all closest phylogenetic neighbours (Fig. 1). The clusters representing the closest phylogenetic neighbours of *V. trachuri* presented in Fig. 1 were described in a previous study (Thompson *et al*., 2001) and were included as outgroups to show the cut-off level for species delineation. Strains clustering at 45% FAFLP pattern similarity should be considered to belong to the same species (Thompson *et al*., 2001). It is important to highlight that FAFLP is a fingerprinting technique which provides discrimination of strains beyond the species level. Recently, Jiang *et al*. (2000) have successfully used AFLP to discriminate *Vibrio cholerae* serogroups O1 and O139. The two *V. trachuri* strains LMG 19643^T and LMG 19714 showed indistinguishable patterns, suggesting the occurrence of a particular clone, highly pathogenic for *Trachurus japonicus* (Iwamoto *et al*., 1995), clearly allocated to the species *V. harveyi*. Strains R-14946 and R-14949 were investigated in a previous study and were found in the so-called cluster 2 (Pedersen *et al*., 1998), but they could not be assigned to any of the species included in that study. Our results demonstrated that these strains belong to *V. harveyi*.

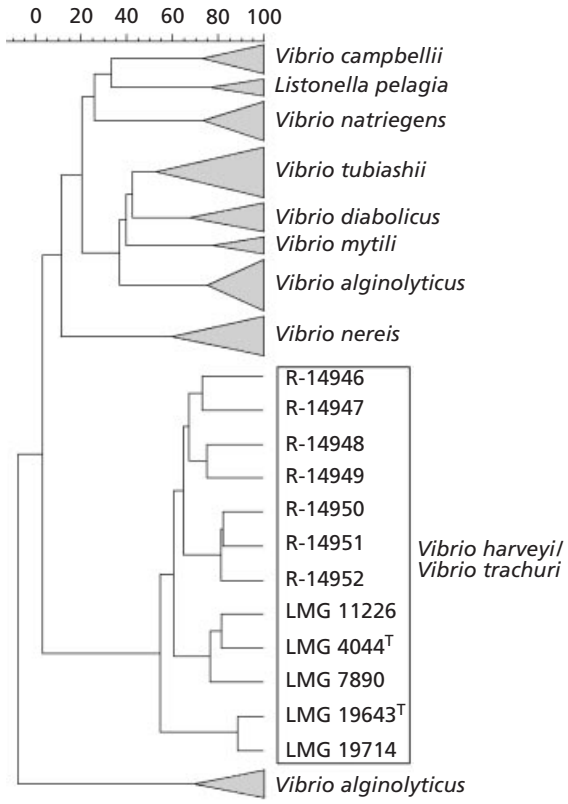

Fig. 1. Dendrogram of the FAFLP patterns of *V. harveyi* and *V. trachuri* strains and their closest phylogenetic neighbours. A band-based (Dice) cluster analysis (Ward) was used. The threshold for cluster delineation was 45 %. *V. harveyi* and *V. trachuri* strains represent the FAFLP cluster A36 found in a previous study (Thompson *et al.*, 2001). Patterns consisted of 114 ± 17 bands.

The G+C content of DNA and the DNA–DNA hybridization results are presented in Table 2. *V. harveyi* and *V. trachuri* had a G+C content of 44·9–45·2 mol% and 44·5–44·6 mol%, respectively. DNA hybridization performed with pure and high-molecular-mass DNA, revealed that internal DNA similarity between *V. harveyi* strains LMG 4044^T and LMG 7890 and *V. trachuri* strains LMG 19643^T and LMG 19714 was 98 and 103 % DNA similarity, respectively. However, DNA similarity between the two type strains (LMG 4044^T and LMG 19643^T) was 80 %. This finding contrasts with the results of Iwamoto *et al.* (1995), since they obtained only 40 % DNA–DNA similarity between the type strains of *V. trachuri* and *V. harveyi*. A reasonable explanation would be that the quality of the DNA of the *V. harveyi* strain used for the DNA–DNA hybridization experiments was not optimal, since it has been documented that protein and RNA contamination and DNA fragmentation are major factors influencing the performance of such experiments (Goris *et al.*, 1998). Because reciprocal values of the DNA–DNA hybridization experiments performed previously have not been shown (Iwamoto *et al.*, 1995), it is difficult to have any evidence about the quality of the DNAs used.

Table 3. Phenotypical features tested by BIOLOG

+, All strains positive; −, all strains negative. Numbers indicate positive strains in each species.

Utilization of:	*V. harveyi* (*n* = 10)	*V. trachuri* (*n* = 2)
Adonitol	1	−
D-Mannose	8	1
D-Melibiose	1	−
β-Methyl-D-glucoside	3	1
D-Sorbitol	3	+
cis-Aconitic acid	5	1
α-Hydroxybutyric acid	1	−
Itaconic acid	−	+
Glucuromide	3	1
Glycerol	8	+
D,L-α-Glycerol phosphate	9	+

The phenotypical features observed for *V. harveyi* and *V. trachuri* strains based on BIOLOG GN analysis are presented in the Table 3. All tests were not discriminatory between *V. harveyi* and *V. trachuri*, except utilization of itaconic acid, indicating that both species have similar phenotypes. In addition, several tests were found to be variable among strains of both species

Table 2. DNA similarity and G+C content (mol%) of *V. harveyi* and *V. trachuri*

Species	DNA similarity values with strains:				G+C (mol%)
	LMG 4044^T	LMG 7890	LMG 19643^T	LMG 19714	
V. harveyi					
LMG 4044^T	100				45·2
LMG 7890	98	100			44·9
V. trachuri					
LMG 19643^T	80	82	100		44·5
LMG 19714	79	74	103	100	44·6

(Table 3). Arginine dihydrolase and gelatinase activity were positive for both *V. harveyi* LMG 4044^T and *V. trachuri* LMG 19643^T, but negative for *V. trachuri* LMG 19714, whereas, tryptophan deaminase activity was found to be positive for *V. trachuri* LMG 19714, but negative for *V. harveyi* LMG 4044^T and *V. trachuri* LMG 19643^T. Besides, some results on the phenotype of *V. trachuri* obtained in this study are in contrast with those of Iwamoto *et al.* (1995). It was reported that arginine dihydrolase, ornithine decarboxylase and citrate utilization were negative for *V. trachuri* LMG 19643^T (Iwamoto *et al.*, 1995). However, we found positive results for these features for the same strain. One might relate these discrepancies between both results to differences in the protocols used in each study. The protocol used in this study was carefully established since its reproducibility and consistency have been analysed previously.

Based on our polyphasic approach combining phylogenetic, genotypic and phenotypic data, we propose that *Vibrio trachuri* Iwamoto *et al.* 1995 should be considered a junior synonym of *Vibrio harveyi*.

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