Reassessment of the taxonomic position of *Vibrio iliopiscarius* (Onarheim *et al.* 1994) and proposal for *Photobacterium iliopiscarium* comb. nov.

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The phylogenetic position of *Vibrio iliopiscarius* was inferred by the maximum-likelihood, maximum-parsimony and neighbour-joining methods on the basis of almost complete 16S rRNA gene sequences. The results showed that this species falls into the same cluster as *Photobacterium* species and is clearly distinct from other *Vibrio* species. Its nearest phylogenetic neighbour is *Photobacterium phosphoreum*. From these results, it is concluded that *V. iliopiscarius* should be reclassified as *Photobacterium iliopiscarium* comb. nov., the type strain of which is PS1^T (= ATCC 51760^T).

Keywords: *Vibrio iliopiscarius*, *Photobacterium iliopiscarium*, *Vibrionaceae*, *Vibrio*, *Photobacterium*

INTRODUCTION

Vibrio iliopiscarius was isolated from the intestines of fish (herring, coal fish, cod and salmon) living in cold seawater and reported as a new species of the genus *Vibrio* by Onarheim *et al.* (1994). These authors determined the phenotypic characteristics and partial 16S rRNA sequence of *V. iliopiscarius* and concluded that this species was different from all the described species of the genus *Vibrio*, its nearest phylogenetic neighbours being *Vibrio salmonicida* and *Vibrio fischeri* (Onarheim *et al.*, 1994). The type strain of *V. iliopiscarius* is ATCC 51760^T.

Recently, RFLP analysis on the basis of PCR-amplified 16S rRNA genes among the members of the family *Vibrionaceae* was performed (Urakawa *et al.*, 1997, 1998). The RFLP profile of *V. iliopiscarius* (ATCC 51760^T) by *Hha*I digestion showed the same restriction fragment pattern as did species of the genus *Photobacterium* (Urakawa *et al.*, 1998). In addition, this species showed the same RFLP profile as did *Photobacterium phosphoreum* and *Photobacterium leiognathi* with the four-base-specific restriction enzymes *Hha*I, *Dde*I, *Rsa*I, *Sau*3AI and *Msp*I (Urakawa *et al.*,

1997). Considering these results, the taxonomic position of *V. iliopiscarius* was reassessed on the basis of almost complete 16S rRNA gene sequence data.

METHODS

DNA extraction and PCR amplification of 16S rRNA genes were carried out as described previously (Urakawa *et al.*, 1997). The PCR product was purified and concentrated with a Microcon-100 micro-concentrator (Amicon) according to the manufacturer's instructions. Sequencing was carried out with a SequiTherm long-read cycle sequencing kit (Epicentre Technologies) and an automated sequencer (ALF DNA Sequencer; Amersham-Pharmacia Biotech). Sequences were aligned by using the CLUSTAL W program (version 1.60) (Thompson *et al.*, 1994) and then realigned manually with the MACCLADE 3.06 software (Maddison & Maddison, 1992). Nucleotide positions of ambiguous alignment and gaps were omitted from subsequent phylogenetic analysis. Maximum-likelihood (ML) analysis was performed by using the NUCML program in the MOLPHY package (version 2.3) (Adachi & Hasegawa, 1996). First, the neighbour-joining (NJ) tree (Saitou & Nei, 1987) was inferred by using a distance matrix estimated by the ML method. The optimal transition/transversion ratio was calculated by the HKY85 model (Hasegawa *et al.*, 1985). The tree thus obtained was analysed further by the local rearrangement method to search the ML tree. Bootstrap probability was estimated by the resampling of estimated log-likelihood method (Kishino *et al.*, 1990). Maximum-parsimony (MP) analysis was performed by using the PAUP program (version 3.1.1) (Swofford, 1993) with a heuristic search. Bootstrap confidence values were obtained with 100 resamplings with an option of stepwise addition.

Abbreviations: ML, maximum likelihood; MP, maximum parsimony; NJ, neighbour-joining.

The DDBJ accession number for the 16S rRNA gene sequence of *P. iliopiscarium* strain PS1^T is AB000278.

NJ analysis was also performed by using the CLUSTAL W program. The NJ tree was constructed from the distance matrix calculated by the algorithm of Kimura's two-parameter model (Kimura, 1980). Bootstrap confidence values were obtained with 1000 resamplings.

RESULTS AND DISCUSSION

Three different phylogenetic analyses were carried out with sequence data from almost all *Vibrio* species available in the databases (DDBJ, EMBL and GenBank). A total of 1244 aligned sites were used for the analysis. The ML tree is shown in Fig. 1. *V. iliopiscarius* clustered with the genus *Photobacterium* with high bootstrap confidence values and was clearly

distinguished from other *Vibrio* species (Fig. 1). The closest relative to *V. iliopiscarius* was *P. phosphoreum* (99.7% identity). This relationship was also supported by both the MP and NJ methods with 100% bootstrap confidence values (data not shown). The data show clearly that *V. iliopiscarius* should be transferred from the genus *Vibrio* to the genus *Photobacterium* as *Photobacterium iliopiscarium* comb. nov. In addition to the phylogenetic information, several phenotypic traits of *V. iliopiscarius* that are common to most or all *Photobacterium* species and rarely seen in *Vibrio* species support the transfer to the genus *Photobacterium*, such as gas production from glucose, positive Voges-Proskauer, arginine dihydrolase and lysine decarboxylase tests and the inability to use

Fig. 1. Maximum-likelihood tree deduced from complete 16S rRNA sequences of *V. iliopiscarius* and members of the genera *Vibrio*, *Photobacterium*, *Salinivibrio* and *Moritella* (HKY85 model; $\alpha/\beta = 2.168$; $\ln L = -6188.20$). Bar, one nucleotide substitution per 100 nucleotides. Bootstrap confidence values (percentage) obtained with 1000 resamplings are given at the branch-points. Accession numbers of reference organisms used in the phylogenetic analysis are: *Moritella marina* (= *Vibrio marinus*) NCIMB 1144^T, X82142; *Photobacterium angustum* ATCC 25915^T, D25307; *Photobacterium damsela* ATCC 33539^T, X74700; *Photobacterium histaminum* C-8^T, D25308; *P. leiognathi* ATCC 25521^T, D25309; *P. phosphoreum* ATCC 11040^T, D25310; *Salinivibrio costicola* NCIMB 701^T, X95527; *Vibrio aestuarianus* ATCC 35048^T, X74689; *Vibrio alginolyticus* ATCC 17749^T, X74690; *Vibrio anguillarum* ATCC 19264^T, X16895; *Vibrio campbellii* ATCC 25920^T, X74692; *Vibrio carchariae* ATCC 35084^T, X74693; *Vibrio cholerae* ATCC 14033, X74694; *Vibrio cincinnatiensis* ATCC 35912^T, X74698; *Vibrio diabolus* HE800^T, X99762; *Vibrio diazotrophicus* ATCC 33466^T, X74701; *V. fischeri* ATCC 7744^T, X74702; *Vibrio fluvialis* ATCC 33809^T, X74703; *Vibrio furnissii* ATCC 35016^T, X74704; *Vibrio gazogenes* ATCC 29988^T, X74705; *Vibrio harveyi* ATCC 14126^T, X74706; *Vibrio hollisae* ATCC 33564^T, X56583; *V. logei* ATCC 15382, X74708; *Vibrio mediterranei* CIP 103203^T, X74710; *Vibrio metschnikovii* CIP 69.14^T, X74711; *Vibrio mimicus* ATCC 33653^T, X74713; *Vibrio mytili* CECT 632^T, X99761; *Vibrio natriegens* ATCC 14048^T, X74714; *Vibrio navarrensis* CIP 103381^T, X74715; *Vibrio nereis* ATCC 25917^T, X74716; *Vibrio nigrapulchritudo* ATCC 27043^T, X74717; *Vibrio ordalii* ATCC 33509^T, X74718; *Vibrio orientalis* ATCC 33934^T, X74719; *Vibrio parahaemolyticus* ATCC 17802^T, X74720; *Vibrio pelagius* ATCC 25916^T, X74722; *Vibrio proteolyticus* ATCC 15338^T, X74723; *V. salmonicida* NCMB 2262^T, X70643; *Vibrio scopthalmi* CECT 4638^T, U46579; '*Vibrio shiloi*' AK-1, AF007115; *Vibrio splendidus* ATCC 33125^T, X74724; *Vibrio tapetis* CECT 4600^T, Y08430; *Vibrio tubiashii* ATCC 19109^T, X74725; and *Vibrio vulnificus* ATCC 27562^T, X74726.

Fig. 2. Neighbour-joining trees reproduced from sequence data of Onarheim *et al.* (1994) (a) and rearranged data (b). The 550 aligned sites described by Onarheim *et al.* (1994) were used for phylogenetic analysis. Bar, one nucleotide substitution per 100 nucleotides. Bootstrap confidence values obtained with 1000 resamplings are given at the branch-points. Accession numbers of reference organisms used in the phylogenetic analysis are as listed in the legend to Fig. 1 with the addition of: *V. fischeri* NCMB 1281, X70640; *V. iliopiscarius* PS1^T, X70636; *V. pelagius* NCMB 1900, X70642; *V. salmonicida* HI 11366, X70638; *V. salmonicida* HI 11391, X70639; and *V. salmonicida* PT2, X70637.

mannitol and to hydrolyse gelatin (Onarheim *et al.*, 1994). This phenotypic concordance reinforces our phylogenetic conclusion.

Stackebrandt & Rainey (1995) have insisted that phylogenetic positions of taxa should be based on analysis of complete sequences. However, the phylogenetic position of *V. iliopiscarius* was determined on the basis of partial sequence data (Onarheim *et al.*, 1994). These authors stated that the important factors are that the number of nucleotides compared is statistically meaningful, that for closely related organisms variable regions are included in the analysis and that the sequences compared are appropriately aligned. Onarheim *et al.* (1994) compared *V. iliopiscarius* only with *Vibrio* species and did not include *Photobacterium* species. Whether this misclassification was due to the use of partial sequence data or an inappropriate combination of species in the phylogenetic analysis is not clear. A phylogenetic tree has been reconstructed by using the same combination of species and sequence data used in this original analysis; the same topology among *V. salmonicida*, *V. fischeri* and *V. iliopiscarius* that was shown by Onarheim *et al.* (1994) was reproduced (Fig. 2a). In this tree, *V. iliopiscarius* clustered with *V. salmonicida* as shown by

Onarheim *et al.* (1994). 16S rRNA sequence data from *Photobacterium* species and another strain of *V. fischeri* were then added to the data used by Onarheim *et al.* (1994) to examine the validity of the combination of species used in their analysis (Fig. 2b). In this tree, *V. salmonicida*, *V. fischeri*, *V. iliopiscarius* and other *Photobacterium* species fell into the same cluster. Onarheim *et al.* (1994) used 550 nucleotides in their analysis, consisting of nucleotides 1–185, 300–508 and 932–1089. From these results, it is assumed that the partial sequences used by Onarheim *et al.* (1994) were not sufficient to separate *Photobacterium* species from *V. salmonicida*, *V. fischeri* and other *Vibrio* species. As shown in Fig. 1, phylogenetic analysis with complete sequences showed that *V. iliopiscarius* did not fall within the *Vibrio* cluster and that it was clearly separated from *V. salmonicida*, *Vibrio logei* and *V. fischeri*. Thus, it is clear that the misclassification was caused by the effect of partial sequence analysis.

In summary, the taxonomic position of *V. iliopiscarius* has been re-examined. The phylogenetic position of this species, on the basis of almost complete 16S rRNA sequences, was determined by ML, MP and NJ analyses. *V. iliopiscarius* was most closely related to *P. phosphoreum* in all phylogenetic analyses carried out in

this study, with high bootstrap probabilities. From these results, *V. iliopiscarius* should be transferred to the genus *Photobacterium*, as *P. iliopiscarium* comb. nov.

Emended description of *Photobacterium iliopiscarium* (Onarheim et al. 1994) Urakawa, Kita-Tsukamoto and Ohwada comb. nov.

The description of *Photobacterium iliopiscarium* comb. nov. is identical to that given by Onarheim et al. (1994) except the description of 16S rRNA gene sequence analysis. The type strain is PS1^T (= ATCC 51760^T).

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