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

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The phylogenetic position of \textit{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 \textit{Photobacterium} species and is clearly distinct from other \textit{Vibrio} species. Its nearest phylogenetic neighbour is \textit{Photobacterium phosphoreum}. From these results, it is concluded that \textit{V. iliopiscarius} should be reclassified as \textit{Photobacterium iliopiscarium} comb. nov., the type strain of which is PS1\textsuperscript{T} (= ATCC 51760\textsuperscript{T}).

Keywords: \textit{Vibrio iliopiscarius}, \textit{Photobacterium iliopiscarium}, \textit{Vibrionaceae}, \textit{Vibrio}, \textit{Photobacterium}

\section*{INTRODUCTION}

\textit{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 \textit{Vibrio} by Onarheim \textit{et al.} (1994). These authors determined the phenotypic characteristics and partial 16S rRNA sequence of \textit{V. iliopiscarius} and concluded that this species was different from all the described species of the genus \textit{Vibrio}, its nearest phylogenetic neighbours being \textit{Vibrio salmonicida} and \textit{Vibrio fischeri} (Onarheim \textit{et al.}, 1994). The type strain of \textit{V. iliopiscarius} is ATCC 51760\textsuperscript{T}.

Recently, RFLP analysis on the basis of PCR-amplified 16S rRNA genes among the members of the family \textit{Vibrionaceae} was performed (Urakawa \textit{et al.}, 1997, 1998). The RFLP profile of \textit{V. iliopiscarius} (ATCC 51760\textsuperscript{T}) by \textit{HhaI} digestion showed the same restriction fragment pattern as did species of the genus \textit{Photobacterium} (Urakawa \textit{et al.}, 1998). In addition, this species showed the same RFLP profile as did \textit{Photobacterium phosphoreum} and \textit{Photobacterium leiognathi} with the four-base-specific restriction enzymes \textit{HhaI}, \textit{DdeI}, \textit{RsaI}, \textit{Sau3AI} and \textit{MspI} (Urakawa \textit{et al.}, 1997). Considering these results, the taxonomic position of \textit{V. iliopiscarius} was reassessed on the basis of almost complete 16S rRNA gene sequence data.

\section*{METHODS}

DNA extraction and PCR amplification of 16S rRNA genes were carried out as described previously (Urakawa \textit{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 \textit{w} program (version 1.60) (Thompson \textit{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 \textit{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 \textit{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.
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 iliopiscarius 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...
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, 30–508 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, \textit{V. iliopiscarius} should be transferred to the genus \textit{Photobacterium}, as \textit{P. iliopiscarium} comb. nov.

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

The description of \textit{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 PSIT (= ATCC 51760\textsuperscript{T}).

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**REFERENCES**


