Transfer of Hyphomicrobium indicum to the genus Photobacterium as Photobacterium indicum comb. nov.

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Hyphomicrobium indicum Johnson and Weisrock 1969 lacks true budding and hyphal branching, and some phenotypic characteristics are in contrast to other true hyphomicrobia. The major quinone system (ubiquinone Q-8), the G+C content of the DNA (40 mol%) and the cellular fatty acid composition (16:0, 16:1 and 18:1 as the major components, and 12:0 3-OH and 14:0 3-OH as the hydroxy fatty acids) of H. indicum are different from the genus Hyphomicrobium, but similar to the genus Photobacterium. Like the marine bacterium Photobacterium, H. indicum can be tolerant of sea water, while Hyphomicrobium cannot. Phylogenetic analyses of 16S rRNA and gyrB gene sequences revealed that H. indicum is most closely related to the genus Photobacterium of the γ-Proteobacteria. Based on the phylogenetic, phenotypic and chemotaxonomic evidence, the results indicate that H. indicum should be transferred to the genus Photobacterium, and the name Photobacterium indicum comb. nov. (type strain, NBRC 14233 T = ATCC 19614 T) is proposed.

The genus Hyphomicrobium Stutzer and Hartleb 1898 contains budding or appendage bacteria that can form various kinds of cytoplasmic extrusions: hyphae or appendage (prosthecate). A comprehensive review of the taxonomy, physiology and ecology of budding bacteria was given by Hirsch (1974). Based on 16S rRNA gene sequence analysis, Hyphomicrobium species were classified as members of the α-subclass of the Proteobacteria and showed a heterogeneous phylogenetic relationship (Stackebrandt et al., 1988; Ruggertin & Hirsch, 1989; Tsuji et al., 1990, Rainey et al., 1998). These facultatively methylotrophic bacteria can grow well on methanol, monomethylamine, chloromethane and some other C1 compounds. Many investigations have revealed evidence indicating that these bacteria play an important role in the bacterial community structure of activated sludge of wastewater treatment, and novel species have recently been isolated from soil, swamp sludge and freshwater by using the special medium for Hyphomicrobium species (Borodina et al., 2002; Layton et al., 2000; McDonald et al., 2001; Holm et al., 1996). To our knowledge, however, Hyphomicrobium indicum has not been isolated from these ecological environments and cannot grow on the special medium for hyphomicrobia, therefore indicating that it is not a methylotrophic bacterium. H. indicum has not been studied by phylogenetic analysis since it was proposed by Johnson & Weisrock (1969). In Bergey's Manual of Systematic Bacteriology, the placement of H. indicum within the genus Hyphomicrobium is questioned by Hirsch (1974) because some phenotypic characteristics are in contrast to those of other true hyphomicrobia. The intent of the present study was to elucidate the phylogenetic relationship of H. indicum based on 16S rRNA gene, gyrB gene and chemotaxonomic characteristics.

H. indicum NBRC 14233 T was obtained from the culture collection of NBRC (NITE Biological Resource Center, Chiba, Japan) and cultured on medium (10 g peptone, 2 g yeast extract, 0.5 g MgSO4.7H2O, 750 ml sea water, 250 ml distilled water) at 24 °C. Cellular fatty acid methyl esters were prepared, separated and identified using the Microbial Identification system as described by Xie & Yokota (2003). The respiratory quinone system was extracted and determined by HPLC (Shimadzu), and the genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of the PCR products were carried out as described previously (Xie & Yokota, 2003), and a 1412 bp 16S rRNA gene sequence of H. indicum NBRC 14233 T was determined. PCR-mediated amplification of gyrB and sequencing of the PCR products were carried out as described by Yamamoto & Harayama (1995), and a 1137 bp gyrB sequence of H. indicum was determined. The DNA sequences of H. indicum NBRC 14233 T were compared with the sequences obtained from the DNA database. The

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence is AB159513 and that for the gyrB sequence is AB159514.
sequences were aligned using the CLUSTAL W software package (Thompson et al., 1994), and the evolutionary distances and K\textsubscript{nuc} value (Kimura, 1980) were generated. Alignment gaps and ambiguous bases were not taken into consideration when the 16S rRNA gene (1262 bases) and \textit{gyrB} (1134 bases) nucleotides were compared. The phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The similarity values were calculated using PAUP 4.068 4.0b1 (Swofford, 1998). The 16S rRNA gene sequences determined in this study were submitted to GenBank/EMBL/DDBJ with the following accession numbers: AB159513 (16S rRNA gene) and AB159514 (\textit{gyrB}).

The phenotypic characteristics of \textit{H. indicum} compared with the reference strains of the genera \textit{Photobacterium} and \textit{Hyphomicrobium} are shown in Table 1. The cells of \textit{Hyphomicrobium} release buds from the ends of long and thin hyphae, but \textit{H. indicum} lacks true budding and hyphal branching, demonstrating a pleomorphic shape (Hirsch, 1989), while \textit{Photobacterium} cells are rod-shaped. The major quinone system of \textit{H. indicum} is ubiquinone Q-8, which is the same as genus \textit{Photobacterium} but different from \textit{Hyphomicrobium} (Q-9). \textit{H. indicum}, like the marine bacteria of the genus \textit{Photobacterium}, can be tolerant of sea water, while other \textit{Hyphomicrobium} species cannot. The G+C content of the DNA of \textit{H. indicum} is 40 mol\%, which is similar to the genus \textit{Photobacterium} (39–42 mol\%), but is 20 mol\% lower than the genus \textit{Hyphomicrobium} (59–67 mol\%). The cellular fatty acids of \textit{H. indicum} are 16:0, 16:1 and 18:1 as the major components, and the

\begin{table}[h]
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\begin{tabular}{|l|l|l|l|l|l|l|}
\hline
\textbf{Characteristic} & \textbf{1} & \textbf{2} & \textbf{3} & \textbf{4} \\
\hline
Quinone & Q-8 (95\%), Q-7 & Q-8 & Q-9 & Q-8 \\
Optimum growth temperature (°C) & 18 & 10 & 30 & 25 \\
Sea-water tolerance & + & + & - & + \\
Gas produced with growth on the glucose & - & + & ND & + \\
Catalse & + & + & - & - \\
Oxidase & + & + & - & - \\
Production of H\textsubscript{2}S & - & - & - & + \\
Production of indole & + & - & - & + \\
Nitrate reduced & + & + & + & + \\
Lysine decarboxylase & - & - & /+ & - \\
Carbon utilization & - & - & + & NT \\
Maltose & + & + & - & + \\
Sucrose & - & - & - & + \\
G+C content (mol\%) & 42 & 39 & 61 & 40 \\
Budding formation & - & - & + & NT \\
\hline
\end{tabular}
\caption{Phenotypic characteristics of \textit{H. indicum} and the reference strains of the genera \textit{Photobacterium} and \textit{Hyphomicrobium}}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|l|cccccccc|}
\hline
\textbf{Fatty acid} & \textbf{1} & \textbf{2} & \textbf{3} & \textbf{4} & \textbf{5} & \textbf{6} & \textbf{7} & \textbf{8} \\
\hline
12:0 & 6 & 4 & 5 & 6 & 2 & - & - & 4 \\
12:0 3-OH & 8 & 6 & 6 & 9 & 5 & - & - & 2 \\
14:0 & 3 & 5 & 7 & 11 & 3 & - & - & 7 \\
14:1 & 1 & 2 & 1 & 1 & 3 & - & - & 1 \\
14:0 3-OH & 3 & 2 & 2 & 1 & - & 3 & 2 & - \\
15:0 iso & - & - & - & 2 & - & - & 1 \\
15:0 & 2 & 3 & 2 & - & 1 & - & - & 2 \\
16:0 iso & - & - & - & 15 & - & - & 1 \\
16:0 & 19 & 20 & 24 & 25 & 9 & 3 & 2 & 26 \\
16:0 3-OH & - & - & - & - & 2 & 3 & - \\
16:1 & 36 & 31 & 34 & 40 & 32 & - & - & 41 \\
17:0 iso & - & - & - & - & - & - & - & 1 \\
17:0 & 3 & 2 & 2 & - & - & - & 1 \\
17:1 & 1 & 1 & 1 & - & - & - & - & - \\
18:0 & 2 & 2 & 2 & 1 & 1 & 3 & 8 & 1 \\
18:1 & 17 & 23 & 15 & 3 & 9 & 85 & 74 & 3 \\
20:5o3c & - & - & - & - & 13 & - & - & - \\
\hline
\end{tabular}
\caption{The fatty acid composition of \textit{H. indicum} and reference strains of the genera \textit{Photobacterium} and \textit{Hyphomicrobium}}
\end{table}

*The total fatty acid composition included an unknown peak (Urukami & Komagata, 1987). Some data were from Nogi \textit{et al.} (1998).
Hydroxy fatty acids contain 12:0 3-OH and 14:0 3-OH. This composition is similar to that of members of the genus *Photobacterium*, but can be differentiated from members of the genus *Hyphomicrobium*, which include large amounts of 18:1, and the hydroxy fatty acids contain 14:0 3-OH and 16:0 3-OH (Table 2).

Based on phylogenetic analyses of the 16S rRNA gene sequence, *H. indicum* is most closely related to the deep-sea barophilic bacterium (growth at pressures up to 70 MPa) *P. profundum* (Nogi *et al.*, 1998), and formed a distinct monophyletic clade with 100% bootstrap support (Fig. 1). Molecular phylogeny deduced from a single locus may be unreliable due to the stochastic nature of base substitutions or rare horizontal gene-transfer events. Consequently, to identify further a reliable evolutionary position of *H. indicum*, the DNA gyrase gene *gyrB*, which has a higher molecular evolution rate than the 16S rRNA gene, was selected as another phylogenetic marker. The phylogenetic analyses based on the *gyrB* gene sequence also revealed that *H. indicum* could be placed within the genus *Photobacterium* of the *γ*-Proteobacteria, with a similarity of 82.6%. These reliable phylogenetic studies reflect that *H. indicum* has a close evolutionary relationship to the genus *Photobacterium* (Fig. 2). When comparing the 16S rRNA gene sequences of *H. indicum* and species of *Photobacterium*, we found that only *P. profundum* had a unique signature nucleotide sequence (′′-TTCATTACGAGCGG-′′) at positions 345–359 according to the *Escherichia coli* numbering system (Brosius *et al.*, 1978). The 16S rRNA gene sequence similarity level (96.9%) between *H. indicum* and *P. profundum* was found to be within the common index of 16S rRNA gene sequences for species-level differentiation (Gillis *et al.*, 2001). In contrast, *H. indicum* and *P. profundum* can easily be differentiated by their phenotypic characteristics: morphology, optimum growth temperature, catalase, oxidase, production of H2S and sucrose utilization. As such, they do not belong to the same species.

Based on the phylogenetic, phenotypic and chemotaxonomic distinctions from other species of the genus *Hyphomicrobium* and the similarity to the genus *Photobacterium*, we propose that *H. indicum* should be transferred to the genus *Photobacterium as Photobacterium indicum* comb. nov.

**Description of Photobacterium indicum** comb. nov.

*Photobacterium indicum* (in′di.cum. M.L. neut. adj. indicum named after the bacterium isolated from the Indian Ocean).


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**Fig. 1.** Neighbour-joining 16S rRNA gene phylogenetic tree showing close relationships of *H. indicum* and the genus *Photobacterium* of the γ-Proteobacteria. *Aeromonas salmonicida* was used as the outgroup. Numbers at the nodes indicate the percentages of occurrence in 100 bootstrapped trees; only values greater than 60% are shown.

**Fig. 2.** Neighbour-joining *gyrB* phylogenetic tree showing the position of *H. indicum* within the genus *Photobacterium* of the γ-Proteobacteria. *Alteromonas macleodii* was used as the outgroup. Numbers at the nodes indicate the percentages of occurrence in 100 bootstrapped trees; only values greater than 60% are shown.
The pleomorphic cells appear rod- or coccus-shaped, being 0.7–1.0 μm in width and 2.0–6.0 μm in length. Colonies are yellow. There is motility with polar monotrichous flagella. Growth occurs at 4–25 °C, pH 4.5–9.5. Acid is produced from glucose, and the cells can use glucose, sucrose and maltose for fermentation, but cannot use lactose, arabinose, gelatin, casein or starch. Positive tests regarding biochemical characteristics obtained with indole, nitrate and H2S. Negative reactions for enzyme activity and antibiotic susceptibility obtained with urease, catalase, oxidase, lysine decarboxylase, erythromycin, tetracycline, penicillin and pteridine; positive reactions obtained with phenylalanine deaminase, chloramycin, neomycin, kanamycin, novobiocin and streptomycin. The cellular fatty acids are 16:0, 16:1 and 18:1 as the major components, while the hydroxy fatty acids contain 12:0 3-OH and 14:0 3-OH. The major quinone system is ubiquinone Q-8. The G+C content of the DNA is 40 mol%. Based on the phylogenetic analyses of the 16S rRNA and gyrB gene sequences, the bacterium is closely related to the genus Photobacterium of the γ-Proteobacteria.

The type strain is NBRCC 14233T (= ATCC 19614T), isolated from sea mud at a depth of 400 m.

References


