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 bacteria *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 (prosthecae). 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; Roggentin & 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.

Table 1. Phenotypic characteristics of *H. indicum* and the reference strains of the genera *Photobacterium* and *Hyphomicrobium*

Strains: 1, *Photobacterium profundum*; 2, *P. phosphoreum*; 3, *Hyphomicrobium vulgare*; 4, *H. indicum*. Some data were from Johnson & Weisrock (1969) and Nogi *et al.* (1998). All of these species are motile and L-arabinose cannot be utilized. ND, Not done; NT, lack true budding and hyphal branching.

Characteristic	1	2	3	4
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	+
Catalase	+	+	—	—
Oxidase	+	+	—	—
Production of H ₂ S	—	—	—	+
Production of indole	+	—	—	+
Nitrate reduced	+	+	+	+
Lysine decarboxylase	—	—	—/+	—
Carbon utilization				
Maltose	+	+	—	+
Sucrose	—	—	—	+
G + C content (mol%)	42	39	61	40
Budding formation	—	—	+	NT

sequences were aligned using the CLUSTAL W software package (Thompson *et al.*, 1994), and the evolutionary distances and K_{nuc} value (Kimura, 1980) were generated. Alignment gaps and ambiguous bases were not taken into consideration when the 16S rRNA gene (1262 bases) and *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 (*gyrB*).

The phenotypic characteristics of *H. indicum* compared with the reference strains of the genera *Photobacterium* and *Hyphomicrobium* are shown in Table 1. The cells of *Hyphomicrobium* release buds from the ends of long and thin hyphae, but *H. indicum* lacks true budding and hyphal branching, demonstrating a pleomorphic shape (Hirsch, 1989), while *Photobacterium* cells are rod-shaped. The major quinone system of *H. indicum* is ubiquinone Q-8, which is the same as genus *Photobacterium* but different from *Hyphomicrobium* (Q-9). *H. indicum*, like the marine bacteria of the genus *Photobacterium*, can be tolerant of sea water, while other *Hyphomicrobium* species cannot. The G + C content of the DNA of *H. indicum* is 40 mol%, which is similar to the genus *Photobacterium* (39.3–42.0 mol%), but is 20 mol% lower than the genus *Hyphomicrobium* (59–67 mol%). The cellular fatty acids of *H. indicum* are 16:0, 16:1 and 18:1 as the major components, and the

Table 2. The fatty acid composition of *H. indicum* and reference strains of the genera *Photobacterium* and *Hyphomicrobium*

Values are percentages of total fatty acids. Strains: 1, *Photobacterium angustum*; 2, *P. damsela*; 3, *P. leiognathi*; 4, *P. phosphoreum*; 5, *P. profundum*; 6, *Hyphomicrobium vulgare*; 7, *H. methylovorum*; 8, *H. indicum*.

Fatty acid	1	2	3	4	5	6*	7*	8
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
19:0 cyclo	—	—	—	—	—	—	2	—
20:5 ω 3c	—	—	—	—	13	—	—	—

*The total fatty acid composition included an unknown peak (Urakami & Komagata, 1987). Some data were from Nogi *et al.* (1998).

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.

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 (5'-TTCATTACGAGCGG-3') 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 H_2S 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).

Basonym: *Hyphomicrobium indicum* Johnson and Weisrock 1969.

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, chloromycetin, neomycin, kanamycin, novobiocin and streptomycin. The cellular fatty acids are 16:0, 16:1 and 18:1 as the major components, and 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 NBRC 14233^T (=ATCC 19614^T), isolated from sea mud at a depth of 400 m.

References

- Borodina, E., Kelly, D. P., Schumann, P., Rainey, F. A., Ward-Rainey, N. L. & Wood, A. P. (2002). Enzymes of dimethylsulfone metabolism and the phylogenetic characterization of the facultative methylotrophs *Arthrobacter sulfonivorans* sp. nov., *Arthrobacter methylotrophus* sp. nov., and *Hyphomicrobium sulfonivorans* sp. nov. *Arch Microbiol* **177**, 173–187.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of the 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Gillis, M., Vandamme, P., De Vos, O., Swings, J. & Kersters, K. (2001). Polyphasic taxonomy. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, pp. 43–88. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. London: Springer.
- Hirsch, P. (1974). Budding bacteria. *Annu Rev Microbiol* **28**, 391–444.
- Hirsch, P. (1989). Genus *Hyphomicrobium* Stutzer and Hartleb 1898, 76^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 9th edn, vol. 3, pp. 1897–1904. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.
- Holm, N. C., Gliesche, C. G. & Hirsch, P. (1996). Diversity and structure of *Hyphomicrobium* populations in a sewage treatment plant and its adjacent receiving lake. *Appl Environ Microbiol* **62**, 522–528.
- Johnson, R. M. & Weisrock, W. P. (1969). *Hyphomicrobium indicum* sp. nov. *Hyphomicrobiaceae* douglas. *Int J Syst Bacteriol* **19**, 295–307.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Layton, A. C., Karanth, P. N., Lajoie, C. A., Meyers, A. J., Gregory, I. R., Stapleton, R. D., Taylor, D. E. & Sayler, G. S. (2000). Quantification of *Hyphomicrobium* populations in activated sludge from an industrial wastewater treatment system as determined by 16S rRNA analysis. *Appl Environ Microbiol* **66**, 1167–1174.
- McDonald, I. R., Doronina, N. V., Trotsenko, Y. A., McAnulla, C. & Murrell, J. C. (2001). *Hyphomicrobium chloromethanicum* sp. nov. and *Methylobacterium chloromethanicum* sp. nov. chloromethane-utilizing bacteria isolated from a polluted environment. *Int J Syst Evol Microbiol* **51**, 119–122.
- Nogi, Y., Masui, N. & Kato, C. (1998). *Photobacterium profundum* sp. nov., a new, moderately barophilic bacterial species isolated from a deep-sea sediment. *Extremophiles* **2**, 1–7.
- Rainey, F. A., Ward-Rainey, N., Gliesche, C. G. & Stackebrandt, E. (1998). Phylogenetic analysis and intragenetic structure of the genus *Hyphomicrobium* and the related genus *Filomicrobium*. *Int J Syst Bacteriol* **48**, 635–639.
- Roggentin, T. & Hirsch, P. (1989). Ribosomal RNA cistron similarities among *Hyphomicrobium* species and several other hyphal, budding bacteria. *Syst Appl Microbiol* **11**, 140–147.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Stackebrandt, E. R. G., Fischer, A., Roggentin, T., Wehmeyer, U., Bomar, D. & Smida, J. (1988). A phylogenetic survey of budding, and/or prosthecate, non-phototrophic eubacteria: membership of *Hyphomicrobium*, *Hyphomonas*, *Pedomicrobium*, *Filomicrobium*, *Caulobacter* and “*Dichotomicrobium*” to the alpha-subdivision of purple non-sulfur bacteria. *Arch Microbiol* **149**, 547–556.
- Swofford, D. L. (1998). PAUP* Phylogenetic analysis using parsimony (* and other methods), version 4. Sunderland, MA: Sinauer Associates.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Tsuji, K., Tsien, H. C., Hanson, R. S., DePalma, S. R., Scholtz, R. & LaRoche, S. (1990). 16S ribosomal RNA sequence analysis for determination of phylogenetic relationship among methylotrophs. *J Gen Microbiol* **136**, 1–10.
- Urakami, T. & Komagata, K. (1987). Characterization and identification of methanol-utilizing *Hyphomicrobium* strains and a comparison with species of *Hyphomonas* and *Rhodomicrobium*. *J Gen Appl Microbiol* **33**, 521–542.
- Xie, C. & Yokota, A. (2003). Phylogenetic analysis of *Lampropedia hyalina* based on the 16S rRNA gene sequence. *J Gen Appl Microbiol* **49**, 345–349.
- Yamamoto, S. & Harayama, S. (1995). PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. *Appl Environ Microbiol* **61**, 1104–1109.