Hymenobacter daecheongensis sp. nov., isolated from stream sediment

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A Gram-negative, strictly aerobic, non-spore-forming, rod-shaped, red–pink bacterium, designated strain Dae14^T, was isolated from stream sediment collected near Daecheong Dam, South Korea, and its taxonomic position was investigated by using a polyphasic approach. Phylogenetic analysis of 16S rRNA gene sequences showed that strain Dae14^T belonged to the genus *Hymenobacter*. Sequence similarities between strain Dae14^T and the type strains of *Hymenobacter* species with validly published names ranged from 91.3 to 94.3%. The predominant cellular fatty acids of strain Dae14^T were iso-C_{15:0}, C_{16:1}ω5c, summed feature 5 (iso-C_{17:1} I and/or anteiso-C_{17:1} B) and iso-C_{16:0}. The DNA G+C content was 62.2 mol%. Results of phylogenetic, chemotaxonomic and phenotypic characterization indicated that strain Dae14^T can be distinguished from all known *Hymenobacter* species and represents a novel species, for which the name *Hymenobacter daecheongensis* sp. nov. is proposed, with Dae14^T (=KCTC 22258^T=LMG 24498^T) as the type strain.

The genus *Hymenobacter* was first described by Hirsch *et al.* (1998) and was known to be unique because of its relatively high DNA G+C content (55–65 mol%); other members of the family 'Flexibacteraceae' in the class 'Sphingobacteria' (Garrity & Holt, 2001) have low G+C contents (27–48 mol%) (Buczolits *et al.*, 2002). At the time of writing, the genus *Hymenobacter* comprised the following 10 species: *Hymenobacter roseosalivarius* (Hirsch *et al.*, 1998); *H. actinosclerus* (Collins *et al.*, 2000); *H. aerophilus* (Buczolits *et al.*, 2002); *H. norwichensis*, *H. ocellatus*, *H. gelipurpurascens* and *H. chitinivorans* (Buczolits *et al.*, 2006); *H. rigui* (Baik *et al.*, 2006); *H. xinjiangensis* (Zhang *et al.*, 2007); and *H. soli* (Kim *et al.*, 2008).

During the course of a study on the culturable aerobic bacteria of soils and streams near Daejeon, South Korea, a large number of novel bacterial strains was isolated (Im *et al.*, 2003). One of these isolates, strain Dae14^T, was found to be a member of the genus *Hymenobacter* and was subjected to further taxonomic investigation. In this study, its genotypic, chemotaxonomic and phenotypic characteristics were analysed in order to establish its phylogenetic affiliation.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Dae14^T is EU370958.

A table showing cellular fatty acid data for strain Dae14^T and related strains is available as supplementary material with the online version of this paper.

Strain Dae14^T was isolated from stream sediment collected near Daecheong Dam, South Korea, in May 2003. The sediment sample was thoroughly suspended in 50 mM phosphate buffer (pH 7.0) and the suspension was spread onto R2A agar (Scharlau). After incubation at 25 °C for 2 weeks, colonies that formed were picked and subcultured several times to confirm that they were pure cultures. Isolates were cultured routinely on R2A agar at 30 °C and maintained as glycerol suspensions (20%, w/v) at –70 °C.

PCR-mediated amplification of the 16S rRNA gene and sequencing of purified PCR products were carried out according to Ten *et al.* (2008). The near-complete 16S rRNA gene sequences were compiled using SEQMAN software (DNASTAR). Related sequences were obtained from Ribosomal Database Project II (Cole *et al.*, 2005) and GenBank (Benson *et al.*, 2005) and multiple alignment was done using the program CLUSTAL_X (Thompson *et al.*, 1997). Some ambiguous sequences, gaps, and the 5' and 3' ends of the alignment were edited manually using the program BIOEDIT (Hall, 1999). Phylogenetic trees were reconstructed by using the neighbour-joining method (Saitou & Nei, 1987) in the program MEGA3 (Kumar *et al.*, 2004) with bootstrap analysis based on 1000 resamplings (Felsenstein, 1985). Distance matrices were calculated by using the Kimura two-parameter model (Kimura, 1983).

The Gram reaction was performed by using the non-staining method as described by Buck (1982). Cell

morphology was examined by light microscopy (E600; Nikon). Catalase activity was determined by bubble production in 3% (v/v) H₂O₂ and oxidase activity was determined using 1% (w/v) tetramethyl *p*-phenylenediamine. The presence of flexirubin-type pigments was determined by suspending cells in 20% KOH (Fautz & Reichenbach, 1980). Enzyme activities, substrates that could be utilized as sole carbon sources and some physiological characteristics were determined with API ZYM, API 20E and API 20NE galleries according to the instructions of the manufacturer (bioMérieux). Because of the failure of carbon assimilation tests using API test strips, agar plates for single-carbon-source assimilation studies were made manually using a defined agar medium containing basal salt media composed of (l⁻¹): 1.8 g K₂HPO₄, 1.08 g KH₂PO₄, 0.5 g NaNO₃, 0.5 g NH₄Cl, 0.1 g KCl, 0.1 g MgSO₄, 0.05 g CaCl₂ and 15 g agar. After autoclaving, a vitamin solution (Widdel & Bak, 1992), trace element solution SL-10 (Widdel *et al.*, 1983) and selenite/tungstate solution (Tschech & Pfennig, 1984) were added to this medium and filter-sterilized carbon sources were also added (individually at 0.1%, w/v). Growth was examined visually on plates incubated at 30 °C for up to 7 days. A negative control well containing no carbon source was included. A positive control culture was grown in a well containing R2A agar.

Anaerobic growth was assessed in a serum bottle containing R2A broth supplemented with sodium thioglycollate (1 g l⁻¹) and nitrogen gas as the gas phase. Tests for degradation of DNA [in which DNase agar (Scharlau) plates were flooded with 1 M HCl], casein, chitin, starch (Atlas, 1993), lipid (Kouker & Jaeger, 1987), xylan and cellulose (Ten *et al.*, 2004) were performed and evaluated after 10 days. Growth at different temperatures (4, 15, 20, 25, 30, 37 and 42 °C) and pH (pH 5.0–10.0 at intervals of 0.5 pH units) was assessed after 7 days incubation. Salt tolerance was tested after incubation for 7 days on R2A medium supplemented with 1–10% (w/v) NaCl. Growth on nutrient agar, trypticase soy agar and MacConkey agar was also evaluated at 30 °C.

For the measurement of chromosomal DNA G+C content, the genomic DNA of the isolate was extracted and purified as described by Moore & Dowhan (1995) and determined as described by Mesbah & Whitman (1989) using reversed-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under a vacuum and then re-extracted in *n*-hexane/water (1:1, v/v). The crude *n*-hexane/quinone solution was purified using Sep-Pak Vac Silica Cartridges (Waters) and subsequently analysed by HPLC, as described by Hiraishi *et al.* (1996). Cellular fatty acid profiles were determined for cells grown on R2A agar for 3 days at 30 °C. Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids analysed by GC (Hewlett Packard 6890) were identified by using the Microbial Identification software package (Sasser, 1990).

An almost-complete 16S rRNA gene sequence (1468 bp in length) was obtained for strain Dae14^T. Comparisons with corresponding sequences showed that strain Dae14^T had highest levels of similarity to *H. chitinivorans* Txc1^T (94.3%), *H. actinosclerus* CCUG 39621^T (93.4%), *H. roseosalivarius* DSM 11622^T (93.0%), *H. gelipurpurascens* Txg1^T (93.0%), *H. aerophilus* I/26-Cor1^T (92.3%), *H. rigui* WPCB131^T (92.5%), *H. xinjiangensis* X2-1g^T (92.0%), *H. ocellatus* Myx 2105^T (91.6%), *H. soli* PB17^T (91.3%) and *H. norwichensis* DSM 15439^T (91.3%). In the neighbour-joining tree constructed using 16S rRNA gene sequences of the novel strain and strains of the genus *Hymenobacter*, strain Dae14^T clustered within the radiation of the genus *Hymenobacter*, but was separate from recognized *Hymenobacter* species. Its distinct phylogenetic position was supported by high bootstrap values (96–100%) (Fig. 1). Low levels of similarity with respect to recognized *Hymenobacter* species (91.3–94.3%) indicate that strain Dae14^T represents a novel species.

Cells of strain Dae14^T were Gram-negative, non-motile, non-spore-forming and rod-shaped. Colonies grown on R2A agar for 3 days were circular, entire, smooth, red-pink, transparent and 0.5–2.0 mm in diameter. Cells became larger (approx. 10 µm in length) after 7 days incubation. Physiological characteristics of strain Dae14^T are summarized in the species description. Selected characteristics that differentiate strain Dae14^T from related species of the genus *Hymenobacter* are shown in Table 1.

MK-7 was the predominant isoprenoid quinone. The DNA G+C content of strain Dae14^T was 62.2 mol%; such relatively high values are unique to the genus *Hymenobacter*, distinguishing it from other genera of the family 'Flexibacteraceae'. The major cellular fatty acids of strain Dae14^T were iso-C_{15:0}, C_{16:1ω5c}, summed feature 5 (iso-C_{17:1} I and/or anteiso-C_{17:1} B) and iso-C_{16:0}. Strain Dae14^T had a similar profile to other members of the genus qualitatively, but it showed quantitative differences such as high proportions of iso-C_{16:0} and iso-C_{16:1} H and a low proportion of summed feature 4 (iso-C_{15:0} 2-OH and/or C_{16:1ω7c/t}) (see Supplementary Table S1, available in IJSEM Online).

Phenotypic and phylogenetic analysis, plus the relatively high G+C content, indicated that strain Dae14^T belongs to the genus *Hymenobacter*. However, low levels of 16S rRNA gene sequence similarity (91.3–94.3%) between strain Dae14^T and members of the genus, its quantitative fatty acid profiles and its differential phenotypic characteristics support the proposal that strain Dae14^T represents a novel species. Therefore, on the basis of the data and observations described above, strain Dae14^T represents a novel species of the genus *Hymenobacter*, for which the name *Hymenobacter daecheongensis* sp. nov. is proposed.

Description of *Hymenobacter daecheongensis* sp. nov.

Hymenobacter daecheongensis (dae.che.ong.en'sis. N.L. masc. adj. *daecheongensis* referring to the Daecheong Dam, from where the type strain was isolated).

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain Dae14^T and related strains. Bootstrap values (percentages based on 1000 replications) >70 % are shown at branch points. Bar, 2 substitutions per 100 nucleotide positions.

Cells are Gram-negative, rod-shaped, non-spore-forming and non-motile. Obligately aerobic and chemo-organotrophic. Cells are 0.6–1.0 µm wide and 2.0–5.0 µm long. Cells become larger, around 10 µm in length, after 7 days incubation. Cells grow on R2A agar, but not on trypticase soy agar or MacConkey agar. Colonies on R2A agar are circular, entire, smooth and red–pink. No flexirubin-type pigments are formed. Oxidase- and catalase-positive. Grows at 4–30 °C and pH 5.0–10.0, but not at 37 °C. The presence of more than 1 % NaCl inhibits growth on R2A agar. No growth occurs under anaerobic and denitrifying conditions. The major respiratory quinone is MK-7. The major cellular fatty acids are iso-C_{15:0}, C_{16:1}ω5c, summed feature 5 (iso-C_{17:1} I and/or anteiso-C_{17:1} B) and iso-C_{16:0}. Hydrolyses skim milk, but not xylan, starch, cellulose, chitin or olive oil. Positive for assimilation of the following substrates: D-galactose, L-sorbose, D-arabinose, L-xylose, N-acetyl-D-glucosamine, formate, acetate, propionate, 3-hydroxybutyrate, valerate, caprate, maleic acid, phenylacetate, benzoic acid, 3-hydroxybenzoate, salicin, citrate, lactate, malate, malonate, succinic acid, glutaric acid, tartaric acid, itaconate, adipate, suberate, oxalic acid, lactose, raffinose, D-adonitol, D-mannitol, D-sorbitol, xylitol, amygdalin, glycerol, glycogen, inulin,

histidine, proline and tyrosine. Negative for assimilation of the following substrates: D-glucose, D-mannose, L-rhamnose, D-fructose, L-arabinose, D-ribose, D-xylose, pyruvic acid, fumaric acid, 4-hydroxybenzoate, cellobiose, maltose, melibiose, sucrose, trehalose, gluconate, dulcitol, inositol and dextran. Negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and tryptophan deaminase activities and production of hydrogen sulfide, acetoin and indole. Gelatinase activity is positive (API 20E). API 20NE tests for nitrate reduction, arginine dihydrolase, urease and aesculin hydrolysis are negative. According to the API ZYM gallery (bioMérieux), positive for acid phosphatase, alkaline phosphatase, esterase lipase (C8), esterase (C4) and leucine arylamidase and negative for cystine arylamidase, α-chymotrypsin, α-fucosidase, β-glucuronidase, lipase (C14), α-mannosidase, trypsin, β-galactosidase, α-glucosidase, naphthol-AS-BI-phosphohydrolase, valine arylamidase, N-acetyl-β-glucosaminidase, α-galactosidase and β-glucosidase.

The type strain, Dae14^T (=KCTC 22258^T=LMG 24498^T), was isolated from sediment of a stream near Daecheong Dam, South Korea. The DNA G+C content of the type strain is 62.2 mol%.

Table 1. Differential characteristics of strain Dae14^T and members of the genus *Hymenobacter*

Strains: 1, Dae14^T (*Hymenobacter daecheongensis* sp. nov.); 2, *H. soli* PB17^T; 3, *H. ocellatus* Myx 2105^T; 4, *H. roseosalivarius* DSM 11622^T; 5, *H. aerophilus* I/26-Cor1^T; 6, *H. actinosclerus* CCUG 39621^T; 7, *H. chitinivorans* Txc1^T; 8, *H. gelipurpurascens* Txg1^T; 9, *H. norwichensis* DSM 15439^T; 10, *H. rigui* WPCB131^T; 11, *H. xinjiangensis* X2-1g^T. Data for reference strains are from Hirsch *et al.* (1998), Collins *et al.* (2000), Buczolits *et al.* (2002, 2006), Baik *et al.* (2006), Zhang *et al.* (2007) and Kim *et al.* (2008). +, Positive; –, negative; (+), weakly positive; ND, not determined. All strains are negative for assimilation of L-arabinose and indole production.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Growth in/at:											
1 % NaCl	–	–	+	+	+	ND	(+)	(+)	–	+	–
3 % NaCl	–	–	+	ND	(+)	ND	(+)	(+)	–	–	–
4 °C	(+)	+	–*	+	+	–	+	(+)	(+)	+	+
37 °C	–	–	+	–	–	+	–	–	–	+	(+)
Assimilation of:											
Adonitol	+	–	–	–	–	–	–	–	–	–	–
Sorbitol	+	–	–	–	–	–	–	–	–	–	+
Phenylacetate	+	–	–	–	–	–	–	–	–	ND	ND
D-Galactose	+	+	–	–	–	–	–	–	(+)	+	(+)
D-Mannose	–	+	–	–	(+)	(+)	–	–	(+)	–	(+)
D-Ribose	–	(+)	–	–	–	(+)	–	–	(+)	+	ND
D-Glucose	–	–	–	–	(+)	(+)	–	–	(+)	+	–
D-Fructose	–	–	–	–	(+)	–	–	–	(+)	+	(+)
Cellobiose	–	–	–	–	–	(+)	–	–	(+)	+	+
N-Acetyl-D-glucos-amine	+	–	–	–	–	(+)	–	–	–	–	(+)
Sucrose	–	–	–	–	(+)	–	–	–	–	+	+
Adipate	+	–	–	–	–	(+)	–	–	–	ND	ND
Citrate	+	–	–	–	–	(+)	–	–	–	–	–
Arginine dihydrolase	–	+	–	ND	–	ND	–	–	–	–	ND
Urease	–	+	–	ND	–	–	–	–	–	–	–
API ZYM (enzyme activities)											
Alkaline phosphatase	+	+	+	ND	+	+	+	+	+	+	+
Leucine arylamidase	+	+	(+)	ND	+	+	+	+	+	+	+
Cystine arylamidase	–	+	–	ND	–	+	–	–	–	(+)	(+)
Valine arylamidase	–	+	–	ND	+	+	–	(+)	(+)	–	+
Naphthol-AS-BI-phosphohydrolase	–	+	(+)	ND	+	ND	(+)	(+)	+	+	+
Esterase (C4)	+	+	–	ND	+	ND	(+)	(+)	(+)	+	+
Esterase lipase (C8)	+	+	(+)	ND	+	+	(+)	(+)	(+)	+	+
α-Galactosidase	–	(+)	–	+	–	–	–	–	–	–	–
β-Galactosidase	–	+	–	ND	–	–	–	–	–	–	–
α-Glucosidase	–	+	–	+	–	ND	–	–	–	–	–
β-Glucosidase	–	(+)	–	+	–	–	–	–	(+)	(+)	+
N-Acetyl-β-glucos-aminidase	–	(+)	–	ND	–	+	–	–	–	+	+
Acid phosphatase	+	+	–	ND	+	+	–	–	(+)	+	+
Growth on†	R2A	R2A	PYES, R2A, C-D, TSA	PYGV, R2A	PYES, R2A, TSA	YP, TSA	PYES, R2A, C-D, TSA	PYES, R2A, C-D, TSA	PYES, R2A	TSA, Nu, R2A	TSA, PYES
No growth on†	TSA, MC	TSA, MC	MC	ND	C-D, MC	ND	MC	MC	TSA‡, C-D, MC	ND	ND
Source of isolation	Sediment	Soil	Soil	Soil	Air	Pork	Soil	Soil	Air	Freshwater	Desert
DNA G + C content (mol%)	62.2	58.8	~65	56	63.1	62	61	57–58	ND	65	54

*No growth at 5 °C (Collins *et al.*, 2000).

†C-D, Czapek-Dox agar; MC, MacConkey agar; Nu, nutrient agar/broth; PYES, PYES agar (Buczolits *et al.*, 2002); PYGV, peptone-yeast extract-glucose-vitamins; TSA, trypticase soy agar; YP, yeast extract-peptone broth.

‡Poor growth on this medium.

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