Pedobacter himalayensis sp. nov., from the Hamta glacier located in the Himalayan mountain ranges of India

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Strain HHS 22^T was isolated from a glacial water sample from the snout of the Hamta glacier located in the Himalayan mountain ranges of India. Phenotypic, chemotaxonomic and phylogenetic analyses established the affiliation of the isolate to the genus *Pedobacter*. HHS 22^T exhibits high 16S rRNA gene sequence similarity with *Pedobacter cryoconitis* (98 %). However, the level of DNA–DNA relatedness between HHS 22^T and *P. cryoconitis* is only 42 %. Furthermore, HHS 22^T differs from *P. cryoconitis* and the four other recognized species of *Pedobacter* in a number of phenotypic characteristics. These data suggest that HHS 22^T represents a novel species of the genus *Pedobacter*, for which the name *Pedobacter himalayensis* sp. nov. is proposed. The type strain is HHS 22^T (=JCM 12171^T=MTCC 6384^T).

The genus *Pedobacter* was created to accommodate species characterized by Gram-negative rods that are heparinase-producing, obligately aerobic, with or without gliding motility, negative for urease, lipase, gelatinase, arginine dihydrolase, indole production and nitrate reduction, and contain iso-C_{15:0}, iso-C_{15:0} 2-OH, iso-C_{15:0} 3-OH, C_{16:0}, C_{16:1}ω5c, C_{16:1}ω7c, C_{16:0} 3-OH, iso-C_{17:0} 3-OH and iso-C_{17:1}ω9c fatty acids (Steyn *et al.*, 1998). Five species of *Pedobacter* have been described thus far, and they have been isolated from soil (*Pedobacter heparinus*, *Pedobacter africanus* and *Pedobacter saltans*), fish (*Pedobacter piscium*) and a glacier in the Tyrolean Alps (Austria) (*Pedobacter cryoconitis*) (Steyn *et al.*, 1998; Margesin *et al.*, 2003). In the present report, we have characterized a bacterium isolated from a glacial water sample collected from the snout of a glacier. Phenotypic, chemotaxonomic and phylogenetic analyses establish the affiliation of the isolate to the genus *Pedobacter*. The data also suggest that the isolate is different from the recognized species of *Pedobacter*. Therefore, it is proposed to assign the isolate to a novel species, for which the name *Pedobacter himalayensis* sp. nov. is proposed.

Three water samples were collected in sterile tubes from the Hamta glacier (4270 m above sea level) located in the Himalayan mountain ranges of India. When plated on nutrient agar plates [0.5 % (w/v) peptone, 0.3 % (w/v) beef

extract, 0.5 % (w/v) NaCl, 1.5 % (w/v) agar, pH 7.0] and incubated at 22 °C for 3 days, these samples yielded about 1.8×10^4 to 4×10^5 c.f.u. ml⁻¹. Based on their morphology these colonies could be grouped into 26 different morphotypes. Representative strains from each morphotype were initially characterized based on their 16S rRNA gene sequence. Analysis using the BLAST program indicated that all the strains were closely related to recognized species (>98 % sequence similarity). Strain HHS 1 is closely related to *Pseudomonas meridiana* (GenBank/EMBL/DDBJ accession no. AJ537602), HHS 5 and HHS 23 to *Pseudomonas antarctica* (AJ537601), HHS 12, HHS 13, HHS 24 and HHS 29 to *Pseudomonas veronii* (AY267192), HHS 3 and HHS 15 to *Pseudomonas migulae* (AY047218), HHS 2, HHS 9 and HHS 16 to *Pseudomonas fluorescens* (AY538263), HHS 8 to *Pseudomonas lini* (AY035996), HHS 17, HHS 18, HHS 19 and HHS 20 to *Pseudomonas jessinii* (AY391278), HHS 4 to *Serratia marcescens* (AF076038), HHS 6, HHS 7 and HHS 32 to *Janthinobacterium lividum* (AY247410), HHS 10 to *Bacillus subtilis* (AY672765), HHS 27 and HHS 28 to *Hafnia alvei* (AY572428), HHS 31 to *Exiguobacterium acetylicum* (AY297792) and HHS 22^T to *Pedobacter cryoconitis* (AJ585231). Because these isolates were isolated from glacial water they are likely to be psychrophilic and may thus represent either a psychrophilic variant of the previously reported strain or a novel species. Strain HHS 22^T was selected for detailed analysis to establish its identity based on a polyphasic taxonomic approach; a major consideration for choosing this strain was that only five species have been described so far for *Pedobacter*.

Strain HHS 22^T was maintained on ABM [0.5 % (w/v) peptone, 0.2 % (w/v) yeast extract, pH 7.0], LB (1 %

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of HHS 22^T is AJ583425.

Phenotypic characteristics that differentiate strain HHS 22^T and *Pedobacter cryoconitis* DSM 14825^T are given in Supplementary Table A in IJSEM Online.

tryptone, 0.5 % yeast extract, 1 % NaCl, pH 7.0) or nutrient agar medium. It also grows on MacConkey's medium [1.7 % (w/v) peptone from casein, 0.3 % (w/v) peptone, 0.5 % (w/v) NaCl, 1 % (w/v) lactose, 0.15 % bile salt mixture, 0.03 % (w/v) neutral red, 0.001 % (w/v) crystal violet, 1.35 % (w/v) agar], Ayers' agar [0.1 % (w/v) $\text{NH}_4\text{H}_2\text{PO}_4$, 0.02 % (w/v) KCl, 0.02 % (w/v) MgSO_4 , 0.015 % bromothymol blue, pH 7.0] (Ayers *et al.*, 1919), R₂A medium [0.05 % (w/v) yeast extract, 0.05 % (w/v) peptone, 0.05 % (w/v) Casamino acids, 0.05 % (w/v) glucose, 0.05 % (w/v) starch, 0.03 % (w/v) sodium pyruvate, 0.03 % (w/v) K_2HPO_4 , 0.005 % (w/v) MgSO_4 , pH 7], TSA medium [1.7 % (w/v) casein enzyme hydrolysate, 0.3 % (w/v) papaic digest of soyabean meal, 0.5 % (w/v) NaCl, 2.5 % (w/v) K_2HPO_4 , 2.5 % (w/v) glucose, pH 7.3] and minimal medium [1.05 % (w/v) K_2HPO_4 , 0.45 % (w/v) KH_2PO_4 , 0.1 % (w/v) $(\text{NH}_4)_2\text{SO}_4$, 1.5 % (w/v) agar]. Phenotypic characteristics such as colony morphology, cell morphology, motility, various enzyme activities, carbon assimilation, acid and gas production, tolerance to temperature, pH and NaCl, growth in various media and sensitivity to antibiotics at 22 °C were ascertained as described by Reddy *et al.* (2000) using standard methods (Lanyi, 1987; Smibert & Krieg, 1994). Heparinase activity was detected according to the method of Zimmermann *et al.* (1990). In addition, the ability of the culture to utilize a carbon compound as the sole carbon source was investigated by testing 0.5 % carbon compound in minimal medium. Isolation of DNA and determination of the G+C content of the DNA was determined as described by Shivaji *et al.* (1989a, b). Fatty acid methyl esters were prepared according to the method of Sato & Murata (1988) and were analysed by gas chromatography as described by Shivaji *et al.* (2004, 2005).

To establish the phylogenetic position of strain HHS 22^T further, its 16S rRNA gene was sequenced. For this purpose, the genomic DNA was prepared, the 16S rRNA gene was amplified and sequenced as described by Shivaji *et al.* (2000), and sequences were aligned with those of recognized species of *Pedobacter* from the EMBL database using CLUSTAL V (Higgins *et al.*, 1992). The PHYLIP package version 3.5c (Felsenstein, 1993) was implemented to compute pairwise evolutionary distances using DNADIST (Kimura, 1980), to resample the original sequence data 1000 times using SEQBOOT, to construct phylogenetic trees using FITCH, KITSCH and UPGMA, and for parsimony analysis using DNAPARS. In all cases, the input order of species added to the topology being constructed was randomized with the 'jumble' option with a random seed of seven and ten replications. Majority rule (50 %) consensus trees were constructed. *Pedobacter cryoconitis* DSM 14825^T was used as a reference strain in studies related to morphology, biochemical tests, identification of fatty acids and DNA–DNA hybridization (which was carried out using the membrane filter method; Shivaji *et al.*, 2004, 2005; Tourova & Antonov, 1987).

Strain HHS 22^T has morphological and biochemical

characteristics similar to those reported for the genus *Pedobacter* (Steyn *et al.*, 1998); i.e. cells are Gram-negative rods that are non-motile, it grows on heparin, is positive for oxidase, catalase, gelatinase, phosphatase and β -galactosidase, hydrolyses aesculin, assimilates lactose, glucose and mannose, is negative for H_2S production, indole production, urease, lipase and nitrate reduction, and does not produce acid from a large number of substrates (Table 1) (see species description for further details). In addition, the presence of iso-C_{15:0}, C_{16:0}, C_{16:1 ω 7c}, iso-C_{17:1 ω 9c} and iso-C_{17:0} 3-OH as the predominant fatty acids and a DNA G+C content of 41.25 mol% (Table 1 and Table 2) further confirmed the similarity of strain HHS 22^T with representative strains of members of the genus *Pedobacter* (Steyn *et al.*, 1998). Furthermore, the almost-complete 16S rRNA gene sequence of HHS 22^T (1455 nt) exhibits high similarity (>95 %) with four out of the five *Pedobacter* species with validly published names, namely *Pedobacter cryoconitis* (98 %), *Pedobacter africanus* (96 %), *Pedobacter piscium* (96 %) and *Pedobacter heparinus* (95 %). 16S rRNA gene sequence similarity with *Pedobacter saltans* is only 91 %. The phylogenetic tree constructed by using UPGMA and neighbour-joining shows that strain HHS 22^T forms a coherent clade with those species showing greater than 95 % sequence similarity and with a bootstrap resampling value of greater than 95 %, and is clearly separated from *Pedobacter saltans* (Fig. 1).

It is clear that strain HHS 22^T represents a species of *Pedobacter* and that phylogenetically it is closely related to *Pedobacter cryoconitis*, which is a psychrophile. The level of DNA–DNA relatedness between HHS 22^T and *Pedobacter cryoconitis* was only 42 %. In addition, HHS 22^T differs from *Pedobacter cryoconitis* (see Supplementary Table A in IJSEM Online) and the other four *Pedobacter* species with validly published names (Table 1 and Table 2) in a number of phenotypic characteristics. Thus, we assign strain HHS 22^T to a novel species, for which the name *Pedobacter himalayensis* sp. nov. is proposed. To our knowledge, this is the first species described from a Himalayan glacier.

Description of *Pedobacter himalayensis* sp. nov.

Pedobacter himalayensis (him.a.lay.en'sis. N.L. masc. adj. *himalayensis* pertaining to Himalaya).

Colonies on nutrient agar medium are round (1.6–2 mm in diameter), convex with complete margins, mucoid and pale white. Cells are aerobic, psychrotolerant, Gram-negative and non-motile. Grows between 4 and 25 °C and pH 6–10. Optimum temperature and pH for growth are 22 °C and pH 7, respectively. Growth occurs in the presence of 5.8 % NaCl. Grows on MacConkey's medium, Ayers' agar, R₂A medium, TSA medium, LB and minimal medium. Positive for catalase, oxidase, phosphatase, gelatinase, arginine decarboxylase, arginine dihydrolase, β -glucosidase, β -galactosidase, heparinase, tryptophan deamination and aesculin hydrolysis; negative for urease, lipase, lysine decarboxylase, ornithine decarboxylase, H_2S production,

Table 1. Phenotypic characteristics that differentiate *Pedobacter himalayensis* sp. nov. from related *Pedobacter* species

All strains are positive for the following: aerobic growth, catalase, oxidase, phosphatase, β -galactosidase, aesculin hydrolysis; utilization of mannose, glucose and *N*-acetylglucosamine as the sole carbon source. All strains are negative for the following: Gram stain; indole production; urease; reduction of nitrate to nitrite; utilization of erythritol, L-sorbose, xylitol, melezitose, dulcitol, citric acid, lysine and inositol as the sole carbon source; acid production from D-rhamnose and adonitol. +, Positive; –, negative; $\pm$, variable; NA, data not available; S, susceptible; R, resistant.

Biochemical tests	<i>P. himalayensis</i> *	<i>P. cryoconitis</i> *	<i>P. heparinus</i> *	<i>P. africanus</i> *	<i>P. piscium</i> *	<i>P. saltans</i> *
Habitat	Glacier water	Glacier cryoconite	Soil	Soil, sludge	Fish	Soil
Colony size (mm)	1.6–2	1.6–2	1–4	2–4	2–4	2–4
Colony shape	Round, convex, mucoid with entire margins	Round, convex, mucoid with entire margins	Round, convex, or slightly umbonate with entire margins	Round, convex, smooth with entire scalloped margins	Round, convex, opaque, smooth, entire margin	Round, convex with entire margin
Colony colour	Pale white	Creamish white	Translucent yellow	Translucent yellow	Yellow or creamish white	Light yellow
Growth range (°C)	4–25	1–25	5–30	NA	5–30	NA
Maximum growth temperature (°C)	25	25	37	37	25–30	25–30
Growth on MacConkey's medium	+	–	–	–	–	–
Motility	Non-motile	Motile/gliding	Gliding	Non-motile	Non-motile	Gliding
Cell shape	Long rods	Long rods	Short rods	NA	Long rods	NA
Lipase	–	–	–	+	+	+
Gelatinase	+	+	–	$\pm$	–	–
H ₂ S production	–	–	–	–	NA	–
Lysine decarboxylase	–	–	+	+	+	+
Tryptophan deaminase	+	–	–	–	NA	–
Heparinase	+	–	+	+	–	+
Arginine dihydrolase	+	–	–	–	NA	–
Lipase	–	–	–	+	$\pm$	+
β -Glucosidase	+	+	–	$\pm$	+	+
Acid production from:						
D-Arabinose	–	–	$\pm$	$\pm$	$\pm$	$\pm$
D-Glucose	–	–	+	+	+	$\pm$
D-Mannose	+	–	–	–	NA	NA
Sucrose	–	–	–	–	+	–
D-Lactose	–	–	–	–	+	–
D-Melibiose	–	–	–	–	$\pm$	–
Mannitol	+	+	–	–	$\pm$	–
Inositol	+	–	–	–	–	–
Sorbitol	+	–	–	–	–	–
Utilization of carbon sources:						
D-Arabinose	+	–	–	$\pm$	–	$\pm$
D-Xylose	+	+	+	$\pm$	+	+
Ribose	+	–	–	$\pm$	–	–
D-Glucose	+	+	–	–	–	+
D-Galactose	+	+	+	+	$\pm$	+
D-Fructose	+	+	+	$\pm$	+	$\pm$
Methyl α -D-mannoside	+	–	+	+	–	–
Methyl α -D-glucoside	+	+	+	+	+	$\pm$
L-Fucose	+	+	+	$\pm$	–	–
L-Rhamnose	+	–	+	+	$\pm$	+
L-Melibiose	+	+	+	+	+	$\pm$
D-Cellobiose	+	+	+	+	+	–
Sucrose	+	+	+	+	+	$\pm$
Trehalose	+	+	+	+	+	$\pm$
D-Raffinose	+	+	–	–	+	$\pm$
D-Maltose	+	+	+	$\pm$	+	+

Table 1. cont.

Biochemical tests	<i>P. himalayensis</i> *	<i>P. cryoconitis</i> *	<i>P. heparinus</i> *	<i>P. africanus</i> *	<i>P. piscium</i> *	<i>P. saltans</i> *
Starch	+	—	—	±	+	—
Inulin	+	+	—	—	—	—
Glycogen	—	+	—	—	—	—
Arbutin	+	+	±	±	+	+
Salicin	+	+	+	±	+	+
Mannitol	+	—	+	—	—	—
Glycerol	+	—	—	—	NA	NA
Adonitol	+	—	+	—	—	+
D-Sorbitol	+	—	+	—	—	—
Sodium fumarate	+	—	—	—	—	—
Sodium succinate	+	—	—	±	—	—
Pyruvate	+	—	+	±	—	—
L-Alanine	+	—	—	±	—	—
L-Serine	+	—	±	+	±	—
L-Threonine	+	—	±	+	±	—
L-Arginine	+	—	—	±	—	—
L-Glutamic acid	+	—	—	+	+	—
L-Aspartic acid	+	+	—	±	—	—
L-Tyrosine	+	—	—	+	—	—
L-Proline	+	—	—	±	+	—
L-Histidine	—	—	—	±	—	—
L-Creatinine	+	+	—	±	—	—
Antibiotics used (µg per disc):						
Tobramycin (15)	S	R	R	NA	NA	NA
Amikacin (30)	S	R	R	NA	NA	NA
Penicillin (10)	R	R	S	±	NA	±
Kanamycin (30)	R	R	R	NA	NA	NA
Chloramphenicol (30)	R	R	S	±	NA	S
Ampicillin (10)	R	R	R	R	NA	±
Tetracycline (30)	R	R	S	R	NA	S
Sulfamethoxazole (50)	S	S	S	S	NA	S
G + C content of DNA (mol%)	41.0	43.0	42–43	43–45	40–43	36–38

*Data for *Pedobacter himalayensis* HHS 22^T and *Pedobacter cryoconitis* DSM 14825^T are from the present study, whereas data for *Pedobacter heparinus* LMG 10339^T, *Pedobacter africanus* LMG 10353^T, *Pedobacter piscium* LMG 14024^T and *Pedobacter saltans* LMG 10337^T are from Steyn *et al.* (1998).

methyl red test, indole test, Voges–Proskauer test, hydrolysis of starch and reduction of nitrate to nitrite. Acid is produced from D-xylose, D-mannose, sorbitol, mannitol and inositol, but not from D-arabinose, D-ribose, D-glucose, D-fructose, D-galactose, D-rhamnose, sucrose, D-lactose, D-maltose, D-cellobiose, D-melibiose, trehalose, D-raffinose or adonitol. Utilizes D-arabinose, L-arabinose, L-xylose, D-xylose, D-ribose, D-glucose, D-galactose, D-fructose, D-mannose, N-acetylglucosamine, methyl α-D-mannoside, methyl α-D-glucoside, L-rhamnose, L-fucose, L-melibiose, D-cellobiose, sucrose, trehalose, lactose, D-maltose, D-raffinose, cellulose, starch, inulin, arbutin, salicin, D-mannitol, glycerol, adonitol, D-sorbitol, citrate, malonate, lactic acid, hydroxybutyric acid, hydroxybenzoic acid, sodium gluconate, sodium fumarate, sodium acetate, sodium succinate, succinic acid, pyruvate, polyethylene

glycol, sodium thioglycolate, L-alanine, L-valine, L-leucine, L-isoleucine, L-serine, L-threonine, L-arginine, L-glutamic acid, L-aspartic acid, L-glutamine, L-asparagine, L-methionine, L-cysteine, L-tyrosine, L-phenylalanine, L-tryptophan, L-proline and L-creatinine; but not L-sorbose, dextran, glycogen, dulcitol, erythritol, xylitol, inositol, L-malate, sodium formate, DL-malic acid, citric acid, L-glycine, L-lysine or L-histidine as sole carbon source. Cells are sensitive to (µg per disc): norfloxacin (10), lomefloxacin (30), tobramycin (15), amikacin (30), vancomycin (30), cotrimoxazole (25), sulfamethoxazole (50), doxycycline (100), clindamycin (50) and trimethoprim (50), but resistant to roxithromycin (30), ciprofloxacin (30), nitrofurantoin (300), penicillin (10), cefoperazone (75), cefuroxime (20), lincomycin (2), cephotaxime (30), colistin (10), cefazolin (30), kanamycin (30), novobiocin (30), chloramphenicol

Table 2. Fatty acid composition (%) of *Pedobacter himalayensis* sp. nov. and related *Pedobacter* species

Pedobacter himalayensis HHS 22^T and *Pedobacter cryoconitis* DSM 14825^T were grown in TSA medium at 22 °C for the determination of fatty acid composition in the present study. Data for *Pedobacter heparinus* DSM 2366^T, *Pedobacter africanus* DSM 12126^T, *Pedobacter piscium* DSM 11725^T and *Pedobacter saltans* DSM 12145^T are from Steyn *et al.* (1998). Trace, <1%; —, not detected; ECL, equivalent chain length.

Fatty acid	<i>P. himalayensis</i>	<i>P. cryoconitis</i>	<i>P. heparinus</i>	<i>P. africanus</i>	<i>P. piscium</i>	<i>P. saltans</i>
ECL 13:566	—	—	5.0	2.6	Trace	1.1
C _{14:0}	1.0	1.0	1.1	1.5	1.3	Trace
C _{15:0}	—	—	1.1	Trace	—	1.4
C _{15:1ω6c}	—	—	—	Trace	—	1.4
Iso-C _{15:0}	33.0	15.0	28.2	26.6	26.2	31.4
Anteiso-C _{15:0}	—	—	Trace	Trace	2.6	2.9
Iso-C _{15:0} 2-OH	2.0	2.0	10.4	10.4	10.7	8.9
Iso-C _{15:0} 3-OH	1.5	1.9	2.5	2.1	2.5	2.8
C _{16:0}	7.3	9.1	3.0	3.8	3.3	4.0
C _{16:1ω5c}	—	—	1.4	2.1	3.5	Trace
C _{16:1ω7c}	20.0	27.0	20.2	23.7	31.4	19.6
C _{16:0} 3-OH	—	—	1.5	3.1	4.5	Trace
ECL 16:580	—	—	1.0	1.0	Trace	1.2
Iso-C _{17:1ω9c}	4.0	8.6	6.3	4.4	1.6	6.6
Anteiso-C _{17:1ω9c}	—	—	—	—	1.2	—
Iso-C _{17:0} 3-OH	6.0	4.2	15.2	14.7	9.2	12.7

(30), ampicillin (10), tetracycline (30), amoxicillin (100), erythromycin (15), streptomycin (10) and nalidixic acid (30). The G + C content of the DNA is 41 mol%. The major menaquinones present are MK-7 (68%), MK-7(H₂) (5%), MK-8 (6%), MK-8(H₂) (13%) and MK-9(H₂) (8%). The cellular fatty acids are C_{14:0} (1%), iso-C_{15:0} (33%), iso-C_{15:0} 2-OH (2%), iso-C_{15:0} 3-OH (1.5%), C_{16:0} (7.3%), C_{16:1 ω 7c} (20%), iso-C_{17:1 ω 9c} (4%) and iso-C_{17:0} 3-OH (6%).

The type strain, HHS 22^T (=JCM 12171^T=MTCC 6384^T), was isolated from a glacial water sample.

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Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences (1455 bases) showing the phylogenetic relationship between *Pedobacter himalayensis* sp. nov. and species of the genus *Pedobacter* and *Sphingobacterium spiritivorum*. Bootstrap values (expressed as percentage of 1000 replications) greater than 50% are given at the nodes.

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