Dyadobacter hamtensis sp. nov., from Hamta glacier, located in the Himalayas, India

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Strain HHS 11^T was isolated from a water sample collected from the snout of Hamta glacier located in the Himalayan mountain ranges of India. Phenotypic, chemotaxonomic and phylogenetic analyses established the affiliation of the isolate to the genus *Dyadobacter*. HHS 11^T possessed 96 and 95 % 16S rRNA gene sequence similarity with respect to *Dyadobacter crusticola* and *Dyadobacter fermentans*, respectively. Furthermore, strain HHS 11^T differs from *D. crusticola* and *D. fermentans* in a number of phenotypic characteristics. These data suggest that strain HHS 11^T represents a novel species of the genus *Dyadobacter*, for which the name *Dyadobacter hamtensis* sp. nov. is proposed. The type strain is HHS 11^T (=JCM 12919^T=MTCC 7023^T).

The genus *Dyadobacter* Chelius and Triplett 2000 was created within the *Flexibacter* group to include Gram-negative rods that occur in pairs in young cultures but form chains of coccoid cells in older cultures. The cells are non-motile, oxidase- and catalase-positive, aerobic, capable of fermenting glucose and sucrose, do not hydrolyse cellulose or starch and produce a flexirubin-like pigment. These micro-organisms have a DNA G+C content of 48 mol% and are phylogenetically closely related to the genera *Runella* and *Microscilla* (Chelius & Triplett, 2000). To date, only two species of the genus, namely *Dyadobacter fermentans*, isolated from surface-sterilized *Zea mays* stems (Chelius & Triplett, 2000), and *Dyadobacter crusticola*, from biological soil crusts (Reddy and Garcia-Pichel, 2005), have been described. In this report, strain HHS 11^T, an isolate from the Hamta glacier in the Himalayan mountain ranges in India, has been identified, on the basis of phenotypic, chemotaxonomic and phylogenetic analyses, as representing a novel species of the genus *Dyadobacter*.

Strain HHS 11^T was isolated from a water sample collected from the 'snout' of the Hamta glacier located at a height of 4270 m above sea level. The sample was processed as described previously (Shivaji *et al.*, 2005b) and pure colonies of bacteria were isolated by repeated streaking on plates containing nutrient agar [0.5 %, w/v, peptone (HiMedia); 0.3 %, w/v, beef extract (HiMedia); 0.5 %, w/v, NaCl; 1.5 %, w/v, agar; pH 7.0]. The plates were incubated at 22 °C for 3 days.

Nutrient agar medium was used for maintaining strain HHS 11^T and for the determination of growth at various

temperatures, at different pH values and in the presence of various concentrations of NaCl (Shivaji *et al.*, 1989). Phenotypic characteristics such as colony morphology, cell morphology, motility, various enzyme activities and gas production (Hugh & Leifson, 1953), growth in various media, such as Ayers' and R2A (Ayers *et al.*, 1919; Chelius & Triplett, 2000), and sensitivity to antibiotics at 22 °C were ascertained as described previously (Shivaji *et al.*, 2004, 2005a) by using standard methods (Lanyi, 1987; Smibert & Krieg, 1994). Minimal medium [K₂HPO₄, 1.05 %, w/v; KH₂PO₄, 0.45 %, w/v; (NH₄)₂SO₄, 0.1 %, w/v; agar, 1.5 %, w/v] was used to evaluate the ability of the culture to assimilate various carbon compounds (0.5 %, w/v) when provided as the only carbon source. The presence of a flexirubin-like pigment was tested according to the method of Weeks (1981). Fatty acid methyl esters were prepared according to the method of Sato & Murata (1988) and analysed by GC (Shivaji *et al.*, 2004; 2005a). The G+C content of the DNA was determined by the spectrophotometric method (Shivaji *et al.*, 1989). *D. fermentans* NS 114^T was used as a reference strain in studies relating to morphology, biochemical tests and the identification of fatty acids.

To establish the phylogenetic position of strain HHS 11^T, DNA was purified and the 16S rRNA gene was amplified and sequenced as described previously (Shivaji *et al.*, 2000). The almost-complete sequence of 1466 bases was manually aligned against the sequences of closely related species of *Dyadobacter* and other related genera using CLUSTAL W (Thompson *et al.*, 1994) (Fig. 1) and phylogenetic affiliations were inferred using SEQBOOT, DNADIST, FITCH, DNAPARS and neighbour-joining, according to PHYLIP (Felsenstein, 1993). The Kimura two-parameter method was used for DNA distance calculation (Kimura, 1980), and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HHS 11^T is AJ619979.

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences (1455 bases), showing the phylogenetic relationship between *D. hamtensis* sp. nov. and species of the genera *Dyadobacter*, *Runella*, *Spirosoma*, *Flectobacillus* and *Cytophaga* and other closely related genera.

bootstrap values were generated for 1000 replications of the data. 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 7 and 10 replications. Majority rule (50%) consensus trees were constructed (Page, 1996).

Strain HHS 11^T conforms to the characteristics of the genus *Dyadobacter* in that it is Gram-negative, rod-shaped in both exponential and stationary phases, appears in pairs, is non-motile, aerobic, oxidase- and catalase-positive, ferments glucose but not sucrose, does not hydrolyse starch, has a DNA G + C content of 49 mol% and is phylogenetically related to *Runella* and *Microscilla* (see Fig. 1, Table 1 and the species description for more details). In addition, HHS 11^T produces a flexirubin-like yellow-coloured pigment, a characteristic feature of the genus *Dyadobacter*. The pigment was extracted according to the method of Weeks (1981) and the spectrum was obtained using a UV-visible spectrophotometer (UV-1601; Shimadzu): the pigment exhibited peaks at 422, 458 and 476 nm when extracted in ethanol. The addition of alkali (20% KOH) changed the colour of the pigment to orange and also broadened the peak, thus confirming that it is a flexirubin-type pigment (Weeks, 1981).

The affiliation of strain HHS 11^T to the genus *Dyadobacter* is further supported by the phylogenetic analysis based on the 16S rRNA gene sequence, which indicates that HHS 11^T is related to the type strains of *Dyadobacter* species with validly published names, namely *D. fermentans* NS114^T and *D. crusticola* C183-8^T. At the 16S rRNA gene level, strain HHS 11^T exhibits only 96% similarity to *D. crusticola* C183-8^T and 95% similarity to *D. fermentans* NS114^T. This difference of >4% at the 16S rRNA gene sequence level between HHS 11^T and the nearest phylogenetic neighbour suggests that strain HHS 11^T represents a novel species, according to the accepted criteria (Stackebrandt & Goebel, 1994). DNA–DNA hybridization between strain HHS 11^T and the nearest phylogenetic neighbours was not attempted since strains

differing by >2.5% at the 16S rRNA gene level are unlikely to exhibit >70% similarity at whole-genome level (Stackebrandt & Goebel, 1994). The assignment of novel species status to HHS 11^T is further strengthened on the basis of the differences the strain exhibits with respect to both of the *Dyadobacter* species with validly published names (Table 1). Distinct differences are also observed in the fatty acid composition of strain HHS 11^T, *D. crusticola* CP183-8^T and *D. fermentans* NS114^T. In strain HHS 11^T, the predominant fatty acids are iso-C_{15:1}, iso-C_{15:0}, C_{16:1}ω5c, C_{16:1}ω7c, C_{16:0} and iso-C_{17:0} 3-OH. In *D. crusticola* CP183-8^T, these fatty acids were also present; however, the level of iso-C_{15:0} was lower and the level of C_{16:1}ω7c was higher. The fatty acid composition of *D. fermentans* NS114^T was very similar to that of strain HHS 11^T except that it contains iso-C_{15:0} 3-OH and lacks C_{18:1} (Table 2). Thus, on the basis of the above phenotypic, chemotaxonomic and phylogenetic differences, it is proposed that strain HHS 11^T be assigned as the type strain of a novel species of the genus *Dyadobacter*, for which the name *Dyadobacter hamtensis* sp. nov. is proposed.

Description of *Dyadobacter hamtensis* sp. nov.

Dyadobacter hamtensis (ham.ten'sis. N.L. masc. adj. *hamtensis* pertaining to the Hamta glacier).

Colonies of strain HHS 11^T on nutrient agar medium are round (2–3 mm in diameter) and light yellow. Cells are aerobic, Gram-negative rods and are non-motile. Growth occurs between 10 and 37 °C and at pH 6–8, but not at pH 10. The optimum temperature and pH for growth are 22 °C and pH 7. Growth occurs in the presence of 11.6% NaCl. Growth occurs on nutrient agar medium, Ayers' agar and R2A medium. The strain is positive for catalase, oxidase, phosphatase, arginine decarboxylase and β-galactosidase, but negative for gelatinase, urease, lipase, lysine decarboxylase, arginine dihydrolase, utilization of citrate, H₂S production, the methyl red test, the indole test, the Voges–Proskauer test, hydrolysis of aesculin, hydrolysis of starch

Table 1. Phenotypic characteristics that differentiate *D. hamtensis* sp. nov., *D. crusticola* and *D. fermentans*

All three species have the same colony shape and all stain Gram-negative, are non-motile, rod-shaped, positive for catalase, oxidase and β -galactosidase, tolerate 1% NaCl, grow at pH 6–8, grow at 25 and 30 °C, grow in R2A medium, utilize D-glucose, D-cellobiose, sucrose, D-trehalose, D-raffinose, D-lactose and inulin and are resistant to bacitracin (10 μ g), nitrofurantoin (300 μ g), penicillin (10 μ g), vancomycin (10 μ g) and erythromycin (15 μ g). All are negative for urease, gelatinase, starch hydrolysis, citrate utilization, H₂S production, nitrate reduction, the methyl red test, indole production, the Voges–Proskauer test, arginine dihydrolase, acid production from D-lactose, D-galactose, D-ribose, growth at pH 4 and pH 10, and the utilization of sodium succinate, starch and pyruvate. All are sensitive to doxycycline (30 μ g) and tetracycline (30 μ g). Symbols: +, positive; –, negative; –/+, variable; W, weak reaction; NA, data not available. Data for HHS 11^T and *D. fermentans* NS-114^T were obtained in the present study; data for *D. crusticola* C183-8^T were taken from Reddy & Garcia-Pichel (2005).

Characteristic	<i>D. hamtensis</i> HHS 11 ^T	<i>D. crusticola</i> C183-8 ^T	<i>D. fermentans</i> NS-114 ^T
Colony size (mm)	2–3	NA	2–3
Colony colour	Light yellow	Yellow	Orange
Cell shape	Long rods	Rods	Rods
Growth at 5 °C	–	+	–
Growth at 37 °C	+	–	+
Maximum growth temp. (°C)	37	25	37
Fermentative metabolism	+	–	+
Growth on:			
Peptone	+	NA	+
Ayers' agar	+	NA	+
Lipase activity	–	+	–
Phosphatase activity	+	+	–
Aesculin hydrolysis	–	+	+
Lysine decarboxylase activity	–	–	+
Arginine decarboxylase activity	+	–	–
Oxidation/fermentation*			
Glucose	+	–	F
Sucrose	–	–	F
Acid production from:			
D-Glucose	+	–	+
Sucrose	–	–	+
D-Fructose	–	+	–
D-Maltose	–	–	+
D-Arabinose	+	–	+
D-Xylose	+	–	+
Carbon-source utilization			
D-Galactose	+	–	+

Table 1. cont.

Characteristic	<i>D. hamtensis</i> HHS 11 ^T	<i>D. crusticola</i> C183-8 ^T	<i>D. fermentans</i> NS-114 ^T
L-Sorbose	+	–	+
D-Rhamnose	–	–	+
D-Arabinose	–	–	+
L-Arabinose	+	–	+
L-Melibiose	–	+	+
D-Fructose	–	–	+
D-Mannose	–	–	+
D-Xylose	–	+	+
D-Mannitol	–	–/+	+
Glycerol	+	–	W
D-Ribose	–	+	–
Lactic acid	+	+	–
D-Adonitol	+	–	–
D-Sorbitol	–	+	W
Dulcitol	–	+	W
Dextran	+	–	–
Citric acid	+	–	–
Sodium acetate	+	–	+
Cellulose	+	–	–
myo-Inositol	–	+	+
Fumarate	+	–	+
Malonate	+	–	+
Tartrate	+	–	+
Amino acids			
L-Glycine	+	–	+
L-Alanine	+	–	–
L-Valine	+	–	–
L-Leucine	+	–	–
L-Isoleucine	+	–	–
L-Serine	+	–	–
L-Threonine	+	–	–
L-Lysine	+	–	+
L-Arginine	+	–	+
L-Glutamic acid	+	–	–
L-Aspartic acid	+	–	–
L-Glutamine	+	–	+
L-Asparagine	+	–	–
L-Methionine	+	–	+
L-Cysteine	+	–	–
L-Tyrosine	+	–	–
L-Phenylalanine	+	–	–
L-Tryptophan	+	–	+
L-Proline	+	–	–
L-Histidine	+	–	–
Effect of antibiotics (µg per disc)†			
Ciprofloxacin (30)	S	R	S
Colistin (10)	S	R	R
Novobiocin (30)	R	S	R
Chloramphenicol (30)	S	R	R
Streptomycin (25)	S	R	R

Table 1. cont.

Characteristic	<i>D. hamtensis</i> HHS 11 ^T	<i>D. crusticola</i> C183-8 ^T	<i>D. fermentans</i> NS-114 ^T
Rifampicin (25 µg ml ⁻¹)	S	S	R
Trimethoprim (25 µg ml ⁻¹)	S	R	R
DNA G + C content (mol%)	49	48	48

*F, Fermentation positive.

†R, Resistant; S, sensitive.

and reduction of nitrate to nitrite. Acid is produced from D-xylose, D-arabinose and D-glucose, but not from D-ribose, D-fructose, D-galactose, L-rhamnose, sucrose, D-lactose, D-maltose or D-mannose. Utilizes L-arabinose, D-glucose, D-galactose, L-sorbose, sucrose, *N*-acetylglucosamine, methyl α -D-mannoside, methyl α -D-glucoside, L-fucose, melezitose, D-cellobiose, D-trehalose, D-lactose, D-raffinose, cellulose, amygdalin, dextran, glycogen, arbutin, salicin, glycerol, D-erythritol, D-adonitol, citrate, malonate, lactic acid, citric acid, 2-ketogluconate, inulin, sodium formate, sodium fumarate, sodium malate, sodium tartrate, sodium acetate, PEG, L-glycine, L-alanine, L-valine, L-leucine, L-isoleucine, L-serine, L-threonine, L-lysine, L-arginine, L-glutamic acid, L-aspartic acid, L-glutamine, L-asparagine, L-methionine, L-cysteine, L-tyrosine, L-phenylalanine, L-tryptophan, L-proline, L-histidine and L-creatinine as sole carbon sources.

Table 2. Fatty acid composition (%) of *D. hamtensis* sp. nov. HHS 11^T, *D. crusticola* CP183-8^T and *D. fermentans* NS-114^T

D. hamtensis sp. nov. HHS 11^T and *D. fermentans* NS-114^T were grown in R2A medium at 25 °C for the determination of the fatty acid composition. Data for *D. crusticola* CP183-8^T are from Reddy & Garcia-Pichel (2005). —, Fatty acid not present.

Fatty acid	<i>D. hamtensis</i> HHS 11 ^T	<i>D. crusticola</i> CP183-8 ^T	<i>D. fermentans</i> NS 114 ^T
C _{14:0}	1.23	1.4	—
iso-C _{15:1}	3.7	1.5	—
iso-C _{15:0}	24.69	13.4	22.85
C _{16:1} ω5c	19.75	21.4	14.28
C _{16:1} ω7c	14.81	41.2	28.57
C _{16:0}	8.64	12.4	5.71
iso-C _{15:0} 3-OH	—	2.4	2.85
Unknown	—	0.3	—
C _{16:0} 3-OH	2.46	2.2	2.85
C _{18:0}	—	0.16	—
C _{18:1}	2.46	0.83	—
iso-C _{17:0} 3-OH	22.22	2.9	22.85

Does not utilize D-rhamnose, D-arabinose, L-melibiose, D-fructose, D-mannose, D-mannitol, D-xylose, L-xylose, D-ribose, D-maltose, D-sorbitol, dulcitol, *myo*-inositol, gluconate, thioglycolate, starch, methanol or agar as sole carbon sources. Cells are sensitive to (µg per disc) ciprofloxacin (30), cefoperazone (75), ceftaxime (10), doxycycline (30), colistin (10), sulphamethazole (50), kanamycin (30), tetracycline (30), nalidixic acid (30), chloramphenicol (25), rifampicin (25), streptomycin (10), trimethoprim (25), norfloxacin (10), lomefloxacin (30), tobramycin (15) and amikacin (30), but resistant to cotrimoxazole (25), roxithromycin (30), nitrofurantoin (300), penicillin (10), cefuroxime (20), lincomycin (20), cefazolin (30), novobiocin (30), ampicillin (25 µg µl⁻¹), amoxicillin (30), bacitracin (10), vancomycin (10) and erythromycin (15). The pigment is flexirubin-like. The G + C content of the DNA is 49 mol%. The major cellular fatty acids are C_{14:0}, iso-C_{15:0}, iso-C_{15:1}, C_{16:1}ω5c, C_{16:0}, C_{16:1}ω7c, C_{16:0} 3-OH, C_{18:1} and iso-C_{17:0} 3-OH.

The type strain, HHS 11^T (=JCM 12919^T = MTCC 7023^T), was isolated from a glacial water sample.

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