Dyadobacter beijingensis sp. nov., isolated from the rhizosphere of turf grasses in China

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Strain A54^T was isolated from rhizospheric soil of turf grasses irrigated with reclaimed water in Taoranting Park, Beijing, China. Phenotypic, chemotaxonomic and polygenetic analyses established the affiliation of the isolate to the genus *Dyadobacter*. Strain A54^T possessed 97.7, 94.4 and 94.7 % 16S rRNA gene sequence similarity with respect to the type strains of *Dyadobacter fermentans*, *D. hamtensis* and *D. crusticola*, respectively. Furthermore, DNA–DNA hybridization did not show significant relatedness (< 25 % hybridization) between strain A54^T and *D. fermentans* ATCC 700827^T. Therefore, these results indicate that strain A54^T belongs to a novel species of the genus *Dyadobacter*, for which the name *Dyadobacter beijingensis* sp. nov. is proposed. The type strain is strain A54^T (= CGMCC 1.6375^T = JCM 14200^T).

The genus *Dyadobacter* was first described by Chelius & Triplett (2000), and the genus currently comprises three species: *Dyadobacter fermentans*, isolated from surface-sterilized *Zea mays* stems (Chelius & Triplett, 2000), *Dyadobacter crusticola*, from biological soil crusts (Reddy & Garcia-Pichel, 2005), and *Dyadobacter hamtensis*, from the 'snout' of the Hamta glacier (Chaturvedi *et al.*, 2005). Recently, using culture-dependent methods, we examined rhizosphere-associated bacterial communities of the perennial grass *Poa pratensis* L. irrigated with reclaimed water, a practice which has been in place for more than 4 years. A total of 138 isolates were obtained from Luria–Bertani (LB) medium seeded with a rhizospheric soil sample. Most isolates belonged to the *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, *Firmicutes* and *Actinobacteria* by 16S rRNA gene sequencing (X. Guo and Z. Dong, unpublished results). In the present study, one of the isolates, designated A54^T, was assigned to the genus *Dyadobacter* on the basis of phenotypic, chemotaxonomic and phylogenetic analyses.

Strain A54^T and *D. fermentans* ATCC 700827^T, as a reference strain, were cultivated on LB medium or R2A medium at 28 °C and maintained in R2A medium at 4 °C or as a glycerol suspension (20 %, v/v) at –20 °C.

R2A was used for determination of growth of strain A54^T at various temperatures, at different pH values and in the presence of various concentrations of NaCl. Phenotypic characteristics such as colony morphology, cell morphology, various enzyme activities (Hugh & Leifson, 1953), growth in various media such as YM (Beringer, 1974), R2A and peptone water (Chelius & Triplett, 2000) and sensitivity to antibiotics at 28 °C were ascertained by using standard methods (Lanyi, 1987; Smibert & Krieg, 1994). API 50CH (bioMérieux) was employed to test carbon assimilation according to the manufacturer's instructions. The catalase test was done with cells scraped from R2A and treated with 3 % (w/v) hydrogen peroxide. The presence of flexirubin-like pigments was tested by measuring the absorbance spectrum of absolute ethanol and alkaline-ethanol extracts of lysed cells (Weeks, 1981). Fatty acid analysis was performed using standard methods and compared to the database of fatty acids in the MIDI Sherlock Microbial Identification System (Microbial ID). Genomic DNA was extracted and purified from cells using the procedure of

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain A54^T is DQ335125.

Marmur & Doty (1962). DNA base compositions were determined using the thermal melting protocol (De Ley, 1970) with *Escherichia coli* K-12 as a reference strain. Levels of DNA relatedness were estimated by measurement of the initial reassociation rate (De Ley *et al.*, 1970).

The 16S rRNA gene was amplified by PCR using universal primers 27f and 1492r (Lane, 1991). The amplified 16S rRNA gene fragment was purified from agarose gels by using a Tiangen kit and cloned into the pGEM-T Easy vector (Promega) according to the technical manual. Sequence

Table 1. Phenotypic characteristics that differentiate strain A54^T, *D. fermentans*, *D. crusticola* and *D. hamtensis*

Strains: 1, strain A54^T (*D. beijingensis* sp. nov.); 2, *D. fermentans* NS-114^T; 3, *D. crusticola* CP183-8^T; 4, *D. hamtensis* HHS 11^T. +, Positive; –, negative; W, weak reaction; NA, data not available. Data for A54^T and *D. fermentans* NS-114^T were obtained in the present study; data for *D. crusticola* CP183-8^T and *D. hamtensis* HHS 11^T were taken from Chaturvedi *et al.* (2005).

Characteristic	1	2	3	4
Growth at:				
5 °C	+	–	+	–
37 °C	–	+	–	+
Growth on peptone water	+	+	NA	+
Acid from:				
Sucrose	+	+	–	–
D-Glucose	+	+	–	+
D-Fructose	+	–	+	–
Carbon-source utilization				
D-Arabinose	–	+	–	+
D-Mannitol	–	+	–	–
Dulcitol	+	W	+	–
myo-Inositol	+	+	+	–
Sodium acetate	–	+	–	+
Tartrate	–	+	–	+
L-Rhamnose	–	+	–	–
Inositol	+	+	+	–
D-Mannitol	+	W	–/+	–
D-Sorbitol	–	+	+	–
Antibiotic resistance (µg per disc)*				
Ampicillin (25)	+	+	+	–
Rifampicin (25)†	–	–	+	+
Streptomycin (10)	+	+	+	–
Gentamicin (10)	–	+	–	+
Erythromycin (10)	–	+	+	+
Roxithromycin (30)	–	+	+	–
Vancomycin (30)	+	–	+	+
DNA G + C content (mol%)	49.2	48	48	49

*The amounts of antibiotic used by Chaturvedi *et al.* (2005) to generate these results are not necessarily the same as the amounts used in this study.

†Rifampicin was tested at 25 µg ml^{–1} in this study.

data were obtained by single-pass double-stranded analysis using primers T7 and SP6, which flank the cloning region in the pGEM-T Easy vector. The sequence of A54^T was aligned with those of closely related species of *Dyadobacter* and members of other related genera using CLUSTAL X version 1.8 (Thompson *et al.*, 1997) and phylogenetic affiliations were inferred using TREECON (version 1.3b) and the PHYLIP package. The Kimura two-parameter method was used for distance calculations (Kimura, 1980) and the resultant tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Morphological, physiological, biochemical and chemotaxonomic characteristics of strain A54^T are given in the species description below and in Table 1. Strain A54^T is Gram-negative, aerobic, oxidase- and catalase-positive and ferments glucose but not sucrose and cells are non-motile, rod-shaped in both exponential and stationary phases and appear in pairs. The DNA G + C content of strain A54^T was 49.2 mol%, which is in the range for known *Dyadobacter* species (Table 1).

In addition, strain A54^T produces a flexirubin-like yellow pigment, a characteristic feature of the genus *Dyadobacter*. The strain exhibited the three peaks characteristic of flexirubin at 428, 452 and 478 nm in ethanol (Fig. 1) (Chelius & Triplett, 2000; Chaturvedi *et al.*, 2005; Reddy & Garcia-Pichel, 2005). The addition of alkali (20 % KOH) changed the colour of the pigment to orange and broadened the peak, thus confirming that it is a flexirubin-type pigment (Weeks, 1981). Strain A54^T contained the following major fatty acids: iso-C_{15:0} (19.22 %), C_{16:1ω7c} (17.46 %), iso-C_{15:0} 2-OH (23.38 %), C_{16:1ω5c} (10.26 %), C_{16:0} 3-OH (3.09 %) and iso-C_{17:0} 3-OH (12.41 %). In *D. fermentans* NS114^T, these fatty acids were also present; however, the level of iso-C_{17:0} 3-OH was lower, while C_{16:1ω5c} and C_{16:1ω7c} were more abundant [although for *D. fermentans* NS114^T, Chelius & Triplett (2000) reported C_{16:1ω7c} as summed feature 3, which may also contain iso-C_{15:0} 2-OH] (Table 2).

Fig. 1. Absorbance spectrum of ethanol (solid line) and alkaline ethanol (dashed line) extracts of strain A54^T.

Table 2. Fatty acid compositions (%) of strain A54^T, *D. fermentans*, *D. crusticola* and *D. hamtensis*

Strains: 1, A54^T (*D. beijingensis* sp. nov.); 2, *D. fermentans* NS-114^T (data from Chelius & Triplett, 2000); 3, *D. crusticola* CP183-8^T (Reddy & Garcia-Pichel, 2005); 4, *D. hamtensis* HHS 11^T (Chaturvedi *et al.*, 2005). Strain A54^T was grown in R2A medium at 28 °C for determination of the fatty acid composition. —, Fatty acid not reported; NA, not applicable (see footnote).

Fatty acid	1	2	3	4
C _{14:0}	0.57	—	1.4	1.23
iso-C _{15:1}	0.50	—	1.5	3.7
iso-C _{15:0}	19.22	16.8	13.4	26.49
anteiso-C _{15:0}	1.04	—	—	—
C _{16:1} ω5c	10.26	17.5	21.4	19.75
C _{16:1} ω7c	17.46	43.5*	41.2	14.81
C _{16:0}	2.91	4.76	12.4	8.64
iso-C _{15:0} 2-OH	23.38	NA	—	—
iso-C _{15:0} 3-OH	2.63	2.74	2.4	—
Unknown ECL 13.57	2.11	1.32	0.3	—
C _{16:0} 3-OH	3.09	4.66	2.2	2.46
iso-C _{17:0} 3-OH	12.41	7.38	2.9	22.22

*Reported as summed feature 3, which contains C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

The placement of strain A54^T within the genus *Dyadobacter* is further supported by phylogenetic analysis (Fig. 2) based on the 16S rRNA gene sequence. It forms a robust clade with the type strains of species of the genus *Dyadobacter*. The nearly complete 16S rRNA gene sequence of strain A54^T (1411 bp) showed 97.7, 94.4 and 94.7 % similarity,

respectively, to those of *D. fermentans* NS114^T, *D. hamtensis* HHS 11^T and *D. crusticola* DSM 16708^T. The results of DNA–DNA hybridization did not show significant relatedness (< 25 % hybridization) between strain A54^T and *D. fermentans* ATCC 700827^T, indicating that strain A54^T is different from *D. fermentans* ATCC 700827^T at the species level when the recommendation of a threshold value of 70 % DNA–DNA relatedness for definition of bacterial species is considered (Wayne *et al.*, 1987).

Therefore, on the basis of morphological, physiological, biochemical, chemotaxonomic and phylogenetic data, isolate A54^T should be classified as representing a novel species, for which we propose the name *Dyadobacter beijingensis* sp. nov.

Description of *Dyadobacter beijingensis* sp. nov.

Dyadobacter beijingensis (bei.jing.en’sis. N.L. masc. adj. *beijingensis* pertaining to Beijing, the geographical origin of the type strain).

Colonies on R2A agar are yellow, mucoid, flaky, convex and smooth. Cells are aerobic, non-motile, Gram-negative rods. Growth occurs at 4–35 °C (optimum 28 °C) but not at 37 °C, at pH 6–8 (optimum pH 7) and in the presence of up to 1.5 % NaCl. Growth occurs in peptone water, on LB medium and R2A medium. Positive for catalase and oxidase and negative for urease, gelatinase, hydrolysis of casein, cellulose, starch and gelatin, H₂S production, the methyl red, indole and Voges–Proskauer tests and reduction of nitrate to nitrite. Acid is produced from sucrose, D-glucose and D-fructose, but not from D-arabinose, D-galactose or D-rhamnose. Utilizes erythritol, L-arabinose, D- and L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, dulcitol, inositol,

Fig. 2. Neighbour-joining tree showing the phylogenetic position of strain A54^T and representatives of related taxa, based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 resamplings) > 50 % are shown at branch points. Bar, 0.1 substitutions per nucleotide position.

D-mannitol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, arbutin, aesculin, citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, gentiobiose and D-fucose as sole carbon sources. Does not utilize glycerol, D-arabinose, D-mannitol, D-ribose, lactic acid, *myo*-inositol, sodium acetate, tartrate, L-rhamnose, L-sorbose, D-sorbitol, N-acetylglucosamine, amygdalin, xylitol, D-turanose, D-lyxose, L-fucose, D-arabitol, L-arabitol, glycogen, D-tagatose, starch, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate or methanol as sole carbon sources. Cells are sensitive to (μ g per disc) acetylspiramycin (100), amoxicillin (30), ampicillin (25), carbenicillin (100), cephalothin (30), chloramphenicol (25), erythromycin (10), gentamicin (10), kanamycin (50), lincomycin (20), norfloxacin (10), penicillin (10), roxithromycin (30), spectinomycin (10) and streptomycin (10), but resistant to levofloxacin (100), rifampicin (25), tetracycline (10) and vancomycin (30). The pigment present is of the flexirubin type, with absorption maxima at 428, 452 and 478 nm. The G + C content of the DNA is 49.2 mol%. The major cellular fatty acids are 15:0 iso, 16:1 ω 7c, 15:0 iso 2-OH, 16:1 ω 5c, 16:0 3-OH and 17:0 iso 3-OH.

The type strain, A54^T (=CGMCC 1.6375^T=JCM 14200^T), was isolated from the rhizosphere of turf grasses.

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