Dyadobacter soli sp. nov., a starch-degrading bacterium isolated from farm soil

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A Gram-negative, non-motile, aerobic bacterial strain, designated MJ20^T, was isolated from farm soil near Daejeon (South Korea) and was characterized taxonomically by using a polyphasic approach. Comparative 16S rRNA gene sequence analysis showed that strain MJ20^T belongs to the family *Cytophagaceae*, class *Sphingobacteria*, and was related most closely to *Dyadobacter fermentans* DSM 18053^T (98.9 % sequence similarity), *Dyadobacter beijingensis* JCM 14200^T (98.0 %) and *Dyadobacter ginsengisoli* KCTC 12589^T (96.4 %). The G+C content of the genomic DNA of strain MJ20^T was 48.5 mol%. The detection of MK-7 as the predominant menaquinone and a fatty acid profile with summed feature 4 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), iso-$C_{15:0}$, $C_{16:0}$ and $C_{16:1}\omega5c$ as major components supported the affiliation of strain MJ20^T to the genus *Dyadobacter*. The new isolate exhibited relatively low levels of DNA–DNA relatedness with respect to *D. fermentans* DSM 18053^T (mean ± SD of three determinations, 47 ± 7 %) and *D. beijingensis* JCM 14200^T (38 ± 8 %). On the basis of its phenotypic and genotypic properties together with phylogenetic distinctiveness, strain MJ20^T (=KCTC 22481^T =JCM 16232^T) should be classified in the genus *Dyadobacter* as the type strain of a novel species, for which the name *Dyadobacter soli* sp. nov. is proposed.

The genus *Dyadobacter* was erected by Chelius & Triplett (2000) within the phylum *Bacteroidetes*, class *Sphingobacteria*, to accommodate Gram-negative rods that occur in pairs in young cultures but form chains of coccoid cells in old cultures. Cells are non-motile, oxidase- and catalase-positive, aerobic and capable of fermenting glucose and sucrose, do not hydrolyse cellulose or starch and produce a flexirubin-like pigment. At the time of writing, the genus comprises eight recognized species: *Dyadobacter fermentans* (Chelius & Triplett, 2000) (the type species), *D. alkalitolerans* (Tang *et al.*, 2009), *D. beijingensis* (Dong *et al.*, 2007), *D. crusticola* (Reddy & Garcia-Pichel, 2005), *D. ginsengisoli* (Liu *et al.*, 2006), *D. hamtensis* (Chaturvedi *et al.*, 2005), *D. koreensis* (Baik *et al.*, 2007) and *D. psychrophilus* (Zhang *et al.*, 2010).

During the course of screening polysaccharide-degrading micro-organisms, via an agar plate screening technique (Ten *et al.*, 2004), a number of bacteria were isolated from farm soil near Daejeon (Republic of Korea). Among these isolates was strain MJ20^T, which was able to break down starch. On the basis of preliminary 16S rRNA gene sequence data, this strain was found to be a member of the genus *Dyadobacter*. Further study of this strain, based on a polyphasic approach that included chemotaxonomic, physiological and DNA–DNA hybridization analyses, indicated that it represents a novel species of the genus *Dyadobacter*.

Strain MJ20^T was isolated by using nutrient agar plates supplemented with insoluble coloured substrates, as described by Ten *et al.* (2004). Single polysaccharide-degrading colonies on the plates were purified by transferring them onto fresh plates of R2A agar and incubating again. Strain MJ20^T was one of the isolates that showed starch-degrading ability under aerobic conditions. The strain was cultured routinely on R2A agar at 25 °C and was maintained as a glycerol suspension (20 %, w/v) at −70 °C. *D. fermentans* DSM 18053^T and *D. beijingensis* JCM 14200^T were used as reference strains for DNA–DNA hybridization and other experiments.

For phylogenetic analysis of strain MJ20^T, genomic DNA was extracted by using a commercial genomic DNA

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MJ20^T is GQ241324.

A table detailing differential phenotypic characteristics between strain MJ20^T and its phylogenetically closest relatives is available as supplementary material with the online version of this paper.

extraction kit (Solgent) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled by using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Multiple alignments were performed by using the program CLUSTAL X (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated by using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with the MEGA4 program (Tamura *et al.*, 2007), with bootstrap values based on 1000 replications (Felsenstein, 1985).

The 16S rRNA gene sequence of strain MJ20^T obtained was a continuous stretch of 1398 bp. Sequence similarity calculations after a neighbour-joining analysis indicated that the closest relatives of strain MJ20^T were *D. fermentans* DSM 18053^T (98.9 %), *D. beijingensis* JCM 14200^T (98.0 %) and *D. ginsengisoli* KCTC 12589^T (96.4 %). Lower levels of 16S rRNA gene sequence similarity (<96.0 %) were found with the type strains of other recognized species in the genus *Dyadobacter*. This relationship between strain MJ20^T and other members of the family *Cytophagaceae* was also evident in the phylogenetic tree (Fig. 1). Strain MJ20^T and the type strains of all recognized *Dyadobacter* species formed a monophyletic clade with a high bootstrap value (100 %), which was supported by the two tree-making methods employed in this study. These data indicate that strain MJ20^T can be clearly separated from other members of the family *Cytophagaceae* with the exception of *D. fermentans* and *D. beijingensis* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). To differentiate strain MJ20^T from *D. fermentans* and *D. beijingensis*, DNA–DNA hybridization experiments were performed.

The Gram reaction of strain MJ20^T was determined by using the non-staining method, as described by Buck (1982). Cell morphology was observed under a Nikon light microscope at ×1000 magnification with cells grown for 2 days at 25 °C on R2A agar. Catalase and oxidase tests were performed as outlined by Cappuccino & Sherman (2002). Anaerobic growth was determined at 25 °C as described by Liu *et al.* (2006). Assimilation of single carbon sources, enzyme activities and other physiological characteristics were determined with the API ID 32 GN, API ZYM, API 20NE and API 50CH galleries according to the manufacturer's instructions (bioMérieux). Tests for degradation of DNA [DNase agar (Scharlau), with DNase activity detected by flooding plates with 1 M HCl], casein and chitin (Atlas, 1993), cellulose, starch and xylan (Ten *et al.*, 2004) were performed and evaluated after 7 days. Susceptibility to antibiotics was studied by placing antibiotic discs (KB Disk; Eiken) on R2A agar plates inoculated with suspensions of strain MJ20^T. Growth at 10, 15, 20, 25, 30, 37 and 42 °C was assessed on R2A agar, nutrient agar, Luria–Bertani agar (LB; Difco) and trypticase soy agar (TSA; Difco) after 5 days' incubation. Growth at pH 5.0–11.0 (at intervals of 0.5 pH units) was evaluated in R2A broth at 25 °C.

Strain MJ20^T grew well on all complex media such as LB, R2A agar, TSA and nutrient agar at 25 °C, but growth was not observed at 10 or 37 °C. Colonies grown on R2A agar plates for 2 days at 25 °C were 2–4 mm in diameter, circular, convex, shiny and yellow. Strain MJ20^T was aerobic, Gram-negative, non-motile, rod-shaped and oxidase- and catalase-positive, fermented glucose and sucrose and was unable to hydrolyse cellulose, and cells appeared in pairs, characteristics consistent with its placement in the genus *Dyadobacter*. The isolate produced a flexirubin-like yellow pigment, a characteristic feature of the genus *Dyadobacter* (Chelius & Triplett, 2000). The pigment was extracted according to the method of Weeks (1981) and the spectrum was obtained by using a Beckman DU 650 UV/visible spectrophotometer. This pigment exhibited peaks at 431, 452 and 476 nm when extracted in ethanol. The addition of an alkali (20 % KOH) changed the colour of the pigment to orange and also broadened the peaks, thus confirming that it is a flexirubin-type pigment (Weeks, 1981). Phenotypic and chemotaxonomic characteristics that differentiate strain MJ20^T from recognized *Dyadobacter* species are listed in Table 1. In particular, strain MJ20^T could be differentiated readily from all

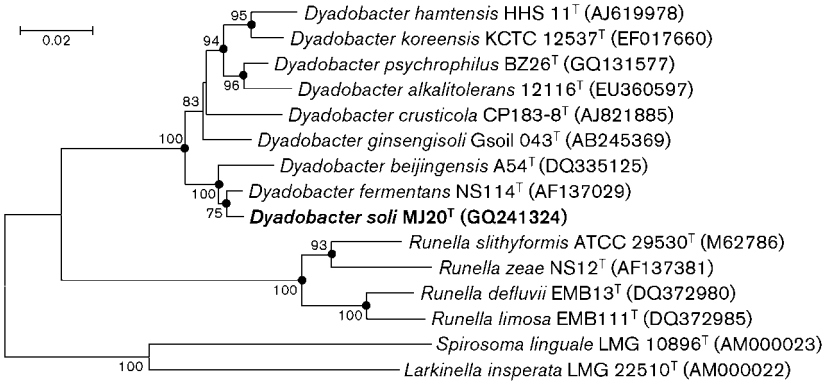

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain MJ20^T among related taxa. Bootstrap values (expressed as percentages of 1000 replications) of >70 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.02 substitutions per nucleotide position.

Table 1. Differential phenotypic characteristics between strain MJ20^T and the type strains of recognized *Dyadobacter* species

Strains: 1, MJ20^T; 2, *D. fermentans* DSM 18053^T (data from Chelius & Triplett, 2000); 3, *D. beijingensis* JCM 14200^T (except where indicated, data from Dong *et al.*, 2007); 4, *D. ginsengisoli* KCTC 12589^T (Liu *et al.*, 2006); 5, *D. crusticola* CP183-8^T (Reddy & Garcia-Pichel, 2005); 6, *D. koreensis* KCTC 12537^T (Baik *et al.*, 2007); 7, *D. alkalitolerans* 12116^T (Tang *et al.*, 2009); 8, *D. hamtensis* HHS 11^T (Chaturvedi *et al.*, 2005); 9, *D. psychrophilus* DSM 22270^T (Zhang *et al.*, 2010). All strains are positive for aerobic growth, catalase and oxidase reactions and assimilation of D-glucose and sucrose. All strains are negative for the Gram reaction, motility, urease, H_2S and indole production. +, Positive; −, negative; W, weakly positive; NA, no data available.

Characteristic	1	2	3	4	5	6	7	8	9
Growth at 5 °C	−	−	+	+	+	+	+	−	+
Growth at 37 °C	−	+	−	−	−	−	−	+	−
pH range for growth	5–8	6–8	6–8	5.5–8.5	6–8	5–11	5–12	6–8	6–8
Nitrate reduction	−	−	−	+	−	−	+	−	−
Hydrolysis of:									
Aesculin	+	+	NA	−	+	+	+	−	+
Gelatin	+	−	−	−	+	−	−	−	+
Starch	+	−	−	−	−	−	−	−	−
Assimilation of:									
L-Arabinose	+	+	+	+	−	+	−	+	−
D-Mannose	+	+	+	+	−	+	+	−	−
L-Rhamnose	+	+	−	+	−	W	−	−	−
D-Ribose	−	−	−	−	+	−	−	−	NA
Inositol	+	+	+	−	+	−	−	−	−
D-Mannitol	−	+	−*	−	+	−	−	−	−
D-Sorbitol	−	W	−	−	+	−	−	−	−
Acetate	−	+	−	−	−	−	−	+	−
Citrate	−	−	+	−	−	NA	−	+	−
Acid production from:									
D-Glucose	+	+	+	−	−	−	+	+	−
D-Fructose	+	−	+	−	+	NA	+	−	−
Sucrose	+	+	+	−	−	−	+	−	−
Antibiotic resistance (μg per disc)									
Ampicillin (25)	+	+	+	+	+	−	+	−	+
Erythromycin (15)	+	+	−	NA	+	NA	+	+	NA
Rifampicin (25)	−	−	−	+	+	−	−	+	−
Tetracycline (30)	−	−	−	+	−	−	−	−	−
Vancomycin (30)	−	−	+	NA	+	−	+	+	NA
Streptomycin (10)	+	+	+	+	+	+	NA	−	+
Gentamicin (10)	+	+	−	NA	−	−	NA	+	NA
DNA G+C content (mol%)	48.5	48.0	49.2	48.0	48.0	44.0	46.3	49.0	48.9

*Data from the present study.

recognized *Dyadobacter* species based on its ability to hydrolyse starch. Furthermore, strain MJ20^T differed from its closest phylogenetic relatives, *D. fermentans* and *D. beijingensis*, in utilization of nine of the 32 carbon sources tested, in the production of six of 19 enzyme activities examined and in acid production from 11 of 48 compounds tested (Supplementary Table S1 available in IJSEM Online).

For measurement of the chromosomal DNA G+C content, genomic DNA of strain MJ20^T was extracted and purified as described by Moore & Dowhan (1995) and was degraded enzymically into nucleosides; the DNA G+C content was determined as described by Mesbah *et al.* (1989) by using reversed-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), evaporated under vacuum conditions and re-extracted in n-hexane/water (1 : 1, v/v). The crude quinone in n-hexane was purified by using Sep-Pak Vac silica cartridges (Waters) and was then analysed by HPLC, as described by Hiraishi *et al.* (1996). Cellular fatty acid profiles were determined for strains grown on TSA for 2 days at 28 °C. Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). Fatty acid methyl esters were then analysed by GC (model 6890; Hewlett Packard) by using the Microbial Identification software package (Sasser, 1990).

The cellular fatty acid profile of strain MJ20^T (shown in Table 2) was compared with those of the type strains of recognized *Dyadobacter* species. The major components of strain MJ20^T were summed feature 4 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), iso-$C_{15:0}$, $C_{16:0}$, $C_{16:1}\omega5c$ and substantial quantities of 3-OH fatty acids, which is a profile typical of members of the genus *Dyadobacter* (Chelius & Triplett, 2000; Tang *et al.*, 2009). Strain MJ20^T contained MK-7 as the predominant menaquinone. The DNA G+C content of strain MJ20^T was 48.5 mol%. These data are in good agreement with the properties of other members of the genus *Dyadobacter* (Chelius & Triplett, 2000; Chaturvedi *et al.*, 2005; Reddy & Garcia-Pichel, 2005; Liu *et al.*, 2006; Baik *et al.*, 2007; Dong *et al.*, 2007; Tang *et al.*, 2009).

DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989), by using photobiotin-labelled DNA probes (Sigma) and microdilution wells (Greiner), with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values are quoted as the level of DNA–DNA relatedness. Standard deviations were also calculated based on these three values. Strain MJ20^T exhibited relatively low levels of DNA–DNA relatedness to *D. fermentans* DSM 18053^T (47±7 %) and *D. beijingensis* JCM 14200^T (38±8 %), indicating that it was not related to them at the species level (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994).

Phenotypic and phylogenetic data indicated that strain MJ20^T is a member of the genus *Dyadobacter*. Phylogenetic distinctiveness, together with DNA–DNA hybridization data, confirmed that strain MJ20^T represents a species that is distinct from recognized *Dyadobacter* species. Strain MJ20^T can be differentiated from its phylogenetically closest relatives based on differences in several phenotypic characteristics (Table 1, Supplementary Table S1). Therefore, on the basis of the data presented, strain MJ20^T should be classified within the genus *Dyadobacter* as the type strain of a novel species, for which the name *Dyadobacter soli* sp. nov. is proposed.

Description of *Dyadobacter soli* sp. nov.

Dyadobacter soli (so′li. L. neut. gen. n. *soli* of soil, the source of the type strain).

Cells are Gram-negative, aerobic, non-motile and rod-shaped, 0.6–0.7 μm wide and 1.9–2.4 μm long. Growth occurs at 15–30 °C (optimum, 25 °C) and pH 5.0–8.0 (optimum, pH 6.5–7.0). Positive for oxidase and catalase.

Table 2. Cellular fatty acid compositions of strain MJ20^T and the type strains of recognized *Dyadobacter* species

Strains: 1, MJ20^T (data from the present study); 2, *D. fermentans* DSM 18053^T (Chelius & Triplett, 2000); 3, *D. beijingensis* JCM 14200^T (Dong *et al.*, 2007); 4, *D. ginsengisoli* KCTC 12589^T (Liu *et al.*, 2006); 5, *D. crusticola* CP183-8^T (Reddy & Garcia-Pichel, 2005); 6, *D. koreensis* KCTC 12537^T (Baik *et al.*, 2007); 7, *D. alkalitolerans* 12116^T (Tang *et al.*, 2009); 8, *D. hamtensis* HHS 11^T (Chaturvedi *et al.*, 2005); 9, *D. psychrophilus* DSM 22270^T (Zhang *et al.*, 2010). *D. beijingensis* JCM 14200^T contained a large amount of iso-$C_{15:0}$ 2-OH (23.38 %) and *D. alkalitolerans* 12116^T contained a small amount of iso-$C_{15:1}$ G (4.65 %). −, Not detected/not reported.

Fatty acid	1	2	3	4	5	6	7	8	9
Saturated									
$C_{14:0}$	0.86	−	0.57	−	1.4	0.66	1.53	1.23	0.8
$C_{15:0}$	0.87	1.37	−	−	−	−	−	−	−
$C_{16:0}$	15.27	4.76	2.91	11.0	12.4	9.40	4.17	8.64	1.0
$C_{18:0}$	−	−	−	−	0.16	−	3.97	−	−
iso-$C_{15:0}$	19.00	16.80	19.22	20.3	13.4	24.17	16.59	24.69	17.0
anteiso-$C_{15:0}$	−	−	1.04	−	−	−	1.48	−	−
Unsaturated									
iso-$C_{15:1}$	−	−	0.50	−	1.50	−	−	3.7	1.5
$C_{16:1}\omega5c$	7.26	17.50	10.26	7.90	21.40	10.96	8.31	19.75	19.0
$C_{16:1}\omega7c$	39.71*	43.50*	17.46	44.6	41.20	34.84	37.18†	14.81	52.7
$C_{18:1}$	−	−	−	−	0.83	−	−	2.46	−
Hydroxy									
iso-$C_{15:0}$ 3-OH	3.10	2.74	2.63	3.9	2.4	2.84	2.55	−	2.1
$C_{16:0}$ 3-OH	4.86	4.66	3.09	2.3	2.2	2.47	1.98	2.46	0.6
iso-$C_{17:0}$ 3-OH	6.79	7.38	12.41	7.6	2.9	9.53	9.70	22.22	2.1
Unknown‡									
ECL 13.57	1.36	1.32	2.11	0.8	0.3	−	−	−	−

*Identified as summed feature 4 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH). Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system.

†Identified as summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$).

‡Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified.

Nitrate is not reduced to nitrite. Able to hydrolyse DNA, aesculin, gelatin, casein and starch, but not cellulose, chitin or xylan. Utilizes *N*-acetylglucosamine, L-arabinose, D-glucose, maltose, D-mannose, melibiose, L-rhamnose, sucrose, inositol, salicin, 2-ketogluconate, malate and phenylacetate for growth, but not D-mannitol, L-fucose, D-ribose, D-sorbitol, acetate, adipate, caprate, citrate, gluconate, 3-hydroxybenzoate, 4-hydroxybenzoate, DL-3-hydroxybutyrate, itaconate, 5-ketogluconate, lactate, malonate, propionate, suberate, valerate, glycogen, L-alanine, L-serine, L-histidine or L-proline. In API ZYM tests, positive for *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), α-galactosidase, α-glucosidase, β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase, but negative for α-chymotrypsin, esterase lipase (C8), α-fucosidase, β-galactosidase, β-glucuronidase, lipase (C14), α-mannosidase and trypsin. In API 50 CHB tests, acid is produced without gas from D- and L-arabinose, cellobiose, D-galactose, gentiobiose, D-glucose, D-fructose, D-fucose, lactose, D-lyxose, maltose, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, D-ribose, L-sorbose, sucrose, D-tagatose, trehalose, turanose, D-xylose, L-xylose, adonitol, inositol, sorbitol, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylopyranoside, *N*-acetylglucosamine, arbutin, aesculin, inulin, salicin, starch and 5-ketogluconate, but not from amygdalin, D- or L-arabitol, dulcitol, glycerol, glycogen, gluconate, erythritol, D-mannitol, xylitol or 2-ketogluconate. Sensitive to (μg per disc) tetracycline (30), rifampicin (25) and vancomycin (30), but resistant to ampicillin (25), erythromycin (15), streptomycin (10) and gentamicin (10). The pigment present is a flexirubin type with absorption maxima at 431, 452 and 476 nm. The predominant menaquinone is MK-7. The major fatty acids are summed feature 4 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), iso-$C_{15:0}$, $C_{16:0}$ and $C_{16:1}\omega5c$. The DNA G+C content of the type strain is 48.5 mol%.

The type strain, MJ20^T (=KCTC 22481^T =JCM 16232^T), was isolated from farm soil near Daejeon, South Korea.

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