Rheinheimera soli sp. nov., a gammaproteobacterium isolated from soil in Korea

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A Gram-negative, non-spore-forming bacterium, designated strain BD-d46^T, was isolated from a playground soil sample in Jinju, South Korea. Cells were straight or curved rods and showed catalase- and oxidase-positive reactions. Growth of strain BD-d46^T was observed between 15 and 35 °C (optimum 25–30 °C) and between pH 6.5 and 8.0 (optimum pH 7.0–7.5). The predominant fatty acids were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{12:0} 3-OH and C_{16:0}. Strain BD-d46^T contained phosphatidylethanolamine and phosphatidylglycerol as major polar lipids. Isoprenoid quinones were Q-8 (75 %) and MK-7 (25 %). The G + C content of the genomic DNA was 49.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain BD-d46^T formed a distinct lineage with *Rheinheimera chironomi* K19414^T within the genus *Rheinheimera*. Levels of 16S rRNA gene sequence similarity between strain BD-d46^T and the type strains of recognized *Rheinheimera* species ranged from 94.4 to 96.9 %. On the basis of chemotaxonomic data and molecular properties, strain BD-d46^T is considered to represent a novel species of the genus *Rheinheimera*, for which the name *Rheinheimera soli* sp. nov. is proposed. The type strain is BD-d46^T (=KCTC 22077^T =DSM 19413^T).

The genus *Rheinheimera* (family *Chromatiaceae*, order *Chromatiales*, class *Gammaproteobacteria*) was first described by Brettar *et al.* (2002) following screening for blue-coloured bacterial isolates from different depth stations in the central Baltic Sea. At the time of writing, the genus *Rheinheimera* comprises six recognized species, namely *Rheinheimera baltica* (Brettar *et al.*, 2002), *R. pacifica* (Romanenko *et al.*, 2003), *R. perlucida* (Brettar *et al.*, 2006), *R. aquimaris* (Yoon *et al.*, 2007), *R. chironomi* (Halpern *et al.*, 2007) and *R. texasensis* (Merchant *et al.*, 2007). All six species were isolated from aquatic environments and related habitats: *R. baltica*, *R. pacifica*, *R. perlucida* and *R. aquimaris* were isolated from marine environments, and *R. chironomi* and *R. texasensis* were isolated from freshwater habitats. The present study describes a novel species of the genus *Rheinheimera* isolated from a soil sample, indicating that members of this genus may be widely distributed in various environments. Here

we describe the taxonomic position of this novel organism based on a polyphasic analysis.

Strain BD-d46^T was isolated from a playground soil sample of Gyeongsang National University in Jinju, South Korea. The soil sample was serially diluted with 1 % (w/v) saline solution and spread on R2A agar (Difco) at 25 °C for 5 days. Subcultivation was performed on R2A agar at 30 °C for 3 days.

The morphological, physiological and biochemical characteristics of strain BD-d46^T were examined by using routine cultivation on R2A medium at 30 °C for 3 days except where indicated otherwise. Growth of strain BD-d46^T at various temperatures and pH was examined by growing the isolate on R2A agar at 5–50 °C (at 5 °C intervals) and in R2A broth adjusted to pH 5.0–10.0 (at 0.5 pH unit intervals) (Gomori, 1955). Gram staining was performed by using a bioMérieux Gram stain kit according to the manufacturer's instructions. Oxidase activity was tested based on oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck), and catalase activity was evaluated by the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution. Cell morphology and motility were studied by using phase-contrast and transmission electron microscopy

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BD-d46^T is EF575565.

A transmission electron micrograph of a negatively stained cell and a table detailing the cellular fatty acid composition of strain BD-d46^T and TLC of polar lipids of strain BD-d46^T and *R. chironomi* K19414^T are available as supplementary material with the online version of this paper.

(JEM-1010; JEOL) at different growth stages as described by Jeon *et al.* (2004). Hydrolysis of casein, gelatin, Tweens 80 and 20, aesculin, urea, tyrosine and starch was investigated on R2A agar after 7 days incubation in accordance with the methods described by Lányi (1987) and Smibert & Krieg (1994). Requirement for and tolerance to NaCl were determined in R2A broth supplemented with NaCl (0–10% at 1% intervals) at 30 °C. Nitrate reduction was performed according to the method of Lányi (1987), and acid production from carbohydrates was tested as described by Leifson (1963). Additional enzyme activities and biochemical features were determined by using API kits (API ZYM and API 20NE) at 30 °C as recommended by the manufacturer (bioMérieux).

Growth of strain BD-d46^T was observed at 15–35 °C and at pH 6.5–8.0. Cells were non-spore-forming rods (0.9–1.5 µm wide and 2.0–3.5 µm long) that were motile by means of a polar flagellum (see Supplementary Fig. S1 in IJSEM Online). The strain grew optimally in R2A broth supplemented with 0–1% (w/v) NaCl; no growth was observed at 5% NaCl or above. Anaerobic growth was not observed after 7 days at 30 °C on R2A agar but weak growth was observed after 16 days. Other phenotypic features of strain BD-d46^T are given in the species description below.

Analysis of polar lipids was carried out by using the method described by Batrakov *et al.* (1997), and isoprenoid quinones were analysed by using an HPLC (model LC-20A; Shimadzu) equipped with a photodiode array detector (SPD-M20A; Shimadzu) and reversed-phase column (250 × 4.6 mm, Kromasil; Akzo Nobel) as described by Komagata & Suzuki (1987). For analysis of fatty acid methyl esters, cells of strain BD-d46^T were harvested from agar plates after incubation at 30 °C for 3 days on marine agar 2216 (MA; Difco) and trypticase soy agar (Difco). Analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI, Inc.). The DNA G+C content of strain BD-d46^T was determined by using an HPLC fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM) according to the method of Tamaoka & Komagata (1984). The polar lipid profile of strain BD-d46^T contained large amounts of phosphatidylethanolamine and phosphatidylglycerol and small amounts of an unknown polar lipid and three unknown polar lipids containing amino groups, a profile similar to that of *R. chironomi* K19414^T (Supplementary Fig. S2). Ubiquinone-8 (Q-8) and menaquinone-7 (MK-7) at a peak area ratio of 75:25 at 270 nm were detected as respiratory lipoquinones. The major cellular fatty acids were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 26.26%), C_{12:0} 3-OH (18.20%) and C_{16:0} (17.28%) (when grown on MA; Supplementary Table S1). The isolate also contained C_{18:1}ω7c as a minor component (4.96%), which is listed as a major cellular fatty acid in the original description of the genus *Rheinheimera* (Brettar *et al.*, 2002). The DNA G+C content of strain BD-d46^T was 49.2 mol%. The

major lipoquinone, major fatty acids and G+C content were in accordance with those of members of the genus *Rheinheimera* (Brettar *et al.*, 2002, 2006; Romanenko *et al.*, 2003; Yoon *et al.*, 2007; Merchant *et al.*, 2007; Halpern *et al.*, 2007).

Sequencing and assembling of the 16S rRNA gene were carried out as described by Lane (1991). The resultant 16S rRNA gene sequence (1489 nt) of strain BD-d46^T was compared with available 16S rRNA gene sequences from GenBank by using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>) to determine an approximate phylogenetic affiliation, and gene sequences were aligned with those of closely related species by using the CLUSTAL W software program (Thompson *et al.*, 1994). Phylogenetic trees were constructed based on the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms, available within the PHYLIP program, version 3.6 (Felsenstein, 2002). Sequence similarity values between the novel strain and related taxa were computed by using the FASTA3 program (<http://www.ebi.ac.uk/fasta33/nucleotide.html>). Bootstrap analysis of the neighbour-joining data was performed according to Kimura's two-parameter model (Kimura, 1980). Neighbour-joining phylogenetic analysis based on 16S rRNA gene sequences indicated that strain BD-d46^T formed a distinct lineage with *R. chironomi* K19414^T within the genus *Rheinheimera* with 70% bootstrap support (Fig. 1). The topologies of phylogenetic trees built with the maximum-likelihood and maximum-parsimony algorithms also supported the proposal that strain BD-d46^T was a member of the genus *Rheinheimera* and could be differentiated from all recognized species of the genus (data not shown). Comparative 16S rRNA gene sequence analysis showed that levels of similarity between strain BD-d46^T and *R. chironomi* K19414^T, *R. texasensis* A62-14B^T, *R. pacifica* KMM 1406^T,

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain BD-d46^T and related taxa. Bootstrap values are shown as percentages of 1000 replicates (only values >50% are shown). *Pseudomonas aeruginosa* LMG 1242^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

Table 1. Differential phenotypic characteristics between strain BD-d46^T and related *Rheinheimera* species

Strains: 1, BD-d46^T (data from the present study); 2, *R. baltica* OSBAC1^T (Brettar *et al.*, 2002); 3, *R. pacifica* KMM 1406^T (Romanenko *et al.*, 2003); 4, *R. perlucida* BA131^T (Brettar *et al.*, 2006); 5, *R. aquimaris* SW-353^T (Yoon *et al.*, 2007); 6, *R. texasensis* A62-14B^T (Merchant *et al.*, 2007); 7, *R. chironomi* K19414^T (Halpern *et al.*, 2007). +, Positive; –, negative; NA, data not available.

Characteristic	1	2	3	4	5	6	7
Source of isolation	Soil	Baltic Sea	NW Pacific deep sea	Baltic Sea	Seawater	Spring-fed lake	Chironomid egg mass
Cell morphology	Straight or curved rods	Rods or cocci	Rods	Rods	Rods or cocci	Rods	Rods
Cell size (µm)							
Width	0.9–1.5	0.5–1.5	0.6–0.8	0.6–1.2	0.3–0.7	0.7–0.8	0.3–0.7
Length	2.0–3.5	0.9–2.5	1.8–2.0	0.9–2.4	0.3–5.0	1.25–2.5	1.0–2.4
Flagella	One polar	One polar	Multiple polar, and lateral	One polar	One polar	Single polar or multiple polar, and lateral	One polar
Oxygen requirement	Facultatively aerobic	Aerobic	Aerobic	Facultatively aerobic	Aerobic	Facultatively aerobic	Facultatively aerobic
Pigmentation	None	Blue	None	None	Yellowish white	None	None
Catalase	+	+	+	+	+	+	–
Nitrate reduction	+	–	–	+	+	+	+
NaCl concentration for growth (% w/v)							
Range	0–4	0–6	0–8	0–8	0–8	0–1	0–2
Optimum	0–1	1–3	NA	1–3	1–3	0	0.5–1
Temperature for growth (°C)							
Range	15–35	4–30	4–37	4–37	4–43	25–37	4–40
Optimum	25–30	20–25	NA	20–30	30–37	30–37	30–37
pH for growth							
Range	6.5–8.0	5.7–10	NA	5.7–10	5.5–10.5	6.5–9.6	6.5–10.0*
Optimum	7.0–7.5	7.0	NA	7.0	7.0–8.0	7.5–8.0	7.5–8.0*
Hydrolysis of:							
Aesculin	+	–	+	NA	+	NA	+
Starch	+	+	+	+	+	+	+
Assimilation of:							
D-Glucose	+	+	–	–	+	+	+
L-Arabinose	+	–	+	–	–	+	–
N-Acetyl-glucosamine	+	+	+	+	+	+	+
Maltose	+	+	+	–	+	–	+
Citrate	–	–	+	–	–	–	–
DNA G + C content (mol%)	49.2	48.9	49.6	48.9	50.5	48.2	49.9

*Results from the present study.

R. aquimaris SW-353^T, *R. baltica* OSBAC1^T and *R. perlucida* BA131^T were 96.9, 96.2, 95.5, 95.4, 94.9 and 94.4 %, respectively. Table 1 shows the phenotypic properties of strain BD-d46^T in comparison with those of its phylogenetically related relatives. The physiological, biochemical and phylogenetic properties of strain BD-d46^T suggest that it represents a novel species of the genus *Rheinheimera*, for which the name *Rheinheimera soli* sp. nov. is proposed.

Description of *Rheinheimera soli* sp. nov.

Rheinheimera soli (so'li. L. fem. gen. n. *soli* of soil, the source of the type strain).

Colonies are white, raised and circular with entire margins after 3 days on R2A agar. Cells are facultatively aerobic, Gram-negative, straight or curved rods that are motile by means of a polar flagellum. Optimal growth occurs at 25–30 °C and pH 7.0–7.5. Catalase- and oxidase-positive.

Nitrate is reduced to nitrite, but nitrogen gas is not produced. Casein, urea, tyrosine, Tweens 80 and 20, aesculin, starch and gelatin are hydrolysed. Produces acid from raffinose, *myo*-inositol, lactose, L-arabinose, melibiose, D-fructose, salicin and arbutin, but not from D-mannose, D-galactose or D-mannitol. Negative for indole production, glucose fermentation and arginine dihydrolase activity. Positive for assimilation of D-glucose, L-arabinose, N-acetylglucosamine and maltose, but negative for assimilation of D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid (API 20NE). Produces alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase and N-acetyl- β -glucosaminidase, but not lipase (C14), valine arylamidase, cystine arylamidase, β -galactosidase, β -glucuronidase, β -glucosidase, α -mannosidase or α -fucosidase. Weak enzyme activities are observed for acid phosphatase, α -galactosidase and α -glucosidase. Contains large amounts of phosphatidylethanolamine and phosphatidylglycerol, and small amounts of an unknown polar lipid and three unknown polar lipids containing amino groups. The isoprenoid quinones are Q-8 (75%) and MK-7 (25%). The major cellular fatty acids are summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH), C_{12:0} 3-OH and C_{16:0}. The DNA G+C content is 49.2 mol% (HPLC).

The type strain, BD-d46^T (=KCTC 22077^T =DSM 19413^T), was isolated from playground soil in Jinju, South Korea.

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