Ochrobactrum daejeonense sp. nov., a nitrate-reducing bacterium isolated from sludge of a leachate treatment plant

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A Gram-reaction-negative, non-spore-forming, rod-shaped, aerobic bacterial strain, designated MJ11^T, was isolated from sludge of a leachate treatment plant in Daejeon, South Korea, and was characterized taxonomically by using a polyphasic approach. Comparative 16S rRNA gene sequence analysis showed that strain MJ11^T belonged to the family *Brucellaceae*, class *Alphaproteobacteria*, and was most closely related to *Ochrobactrum ciceri* Ca-34^T (97.9 % sequence similarity) and *Ochrobactrum pituitosum* CCUG 50899^T (96.4 %). Comparative sequence analyses of the additional phylogenetic marker genes *dnaK*, *groEL* and *gyrB* confirmed the affiliation of strain MJ11^T to the genus *Ochrobactrum*. The G+C content of the genomic DNA of strain MJ11^T was 59.3 mol%. The detection of a quinone system with ubiquinone Q-10 as the predominant respiratory lipoquinone, a fatty acid profile with $C_{18:1}\omega7c$ (62.6 %) and $C_{19:0}$ cyclo $\omega8c$ (14.2 %) as the major components, a polar lipid profile with phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylmonomethylethanolamine, diphosphatidylglycerol and unknown aminolipids AL1 and AL2 as major polar lipids and spermidine and putrescine as the predominant polyamines also supported the affiliation of strain MJ11^T to the genus *Ochrobactrum*. The DNA–DNA relatedness between strain MJ11^T and *Ochrobactrum ciceri* DSM 22292^T was 29 ± 7 %, clearly showing that the isolate constitutes a new genospecies. Strain MJ11^T could be clearly differentiated from its closest neighbours on the basis of its phenotypic, genotypic and chemotaxonomic features. Therefore, strain MJ11^T represents a novel species of the genus *Ochrobactrum*, for which the name *Ochrobactrum daejeonense* sp. nov. is proposed. The type strain is MJ11^T (=KCTC 22458^T =JCM 16234^T).

The genus *Ochrobactrum*, a member of the family *Brucellaceae* in the class *Alphaproteobacteria*, was established by Holmes *et al.* (1988). At the time of writing, the genus *Ochrobactrum* comprised 16 species with validly published names, including the recently described species *Ochrobactrum pecoris* (Kämpfer *et al.*, 2011). Type strains of species of the genus *Ochrobactrum* have been recovered from a diverse range of sources, including soil (Lebuhn *et al.*, 2000), plants and their rhizospheres (Trujillo *et al.*, 2005; Tripathi *et al.*, 2006; Zurdo-Piñeiro *et al.*, 2007; Kämpfer *et al.*, 2008; Imran *et al.*, 2010), industrial environments (Kämpfer *et al.*, 2008; Huber *et al.*, 2010), animals (Kämpfer *et al.*, 2003, 2011) and humans (Holmes *et al.*, 1988; Velasco *et al.*, 1998; Teyssier *et al.*, 2007; Kämpfer *et al.*, 2007a). In the present paper, we describe a Gram-reaction-negative, nitrate-reducing bacterial strain, designated MJ11^T, which was isolated from sludge of a leachate treatment plant. On the basis of 16S rRNA gene sequence data, the strain was found to be a member of the genus *Ochrobactrum*. Further study of this strain was based on a polyphasic approach that included chemotaxonomic and physiological analyses and DNA–DNA hybridization,

Abbreviations: AL, aminolipid; DPG, diphosphatidylglycerol; HE, hydroxyethyl; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid; PME, phosphatidylmonomethylethanolamine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MJ11^T is HQ171203. The GenBank/EMBL/DDBJ accession numbers for the partial *dnaK*, *gyrB* and *groEL* sequences of strains MJ11^T and *Ochrobactrum ciceri* DSM 22292^T are HQ285866, HQ285868 and HQ285870, and HQ285867, HQ285869 and HQ285871, respectively.

Two supplementary figures and one supplementary table are available with the online version of this paper.

and confirmed its position as a representative of a novel species within the genus *Ochrobactrum*.

Strain MJ11^T was isolated from sludge of the aerobic treatment tanks of a municipal leachate treatment plant located in Daejeon, South Korea. The sludge sample had a pH of 6.8 and a total solids content of 35.4 g l^{-1}. The sludge was resuspended and spread on Luria–Bertani (LB) agar (Difco) plates after being serially diluted with 50 mM phosphate buffer (pH 7.0). The plates were incubated at 30 °C for 2 weeks. Single colonies were purified by transferring them onto fresh plates and incubating under the same conditions. Strain MJ11^T was routinely cultured on LB agar at 30 °C and maintained in a 20 % (w/v) glycerol suspension at −70 °C. *Ochrobactrum anthropi* KCTC 22833^T, *Ochrobactrum ciceri* DSM 22292^T, *Ochrobactrum intermedium* KACC 11952^T, *Ochrobactrum oryzae* KACC 12297^T, *Ochrobactrum pituitosum* DSM 22207^T and *Ochrobactrum pseudintermedium* KCTC 22760^T were used as reference strains in this study.

For phylogenetic analysis of strain MJ11^T, genomic DNA was extracted by using a commercial genomic DNA-extraction kit (Solgent) followed by PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product, which were carried out according to Kim *et al.* (2005). The almost full-length sequence of the 16S rRNA gene was 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). A phylogenetic tree was reconstructed using the neighbour-joining method (Saitou & Nei, 1987) in the MEGA4 program (Tamura *et al.*, 2007), with bootstrap values based on 1000 replications (Felsenstein, 1985).

The nearly complete 16S rRNA gene sequence of strain MJ11^T was obtained (1389 bp). The isolate exhibited an elongated 16S rRNA gene sequence characterized by an insertion of 47 nt. This insertion has been described previously in the type strains of *Ochrobactrum ciceri* (Imran *et al.*, 2010) and *Ochrobactrum pituitosum* (Huber *et al.*, 2010), which are the closest phylogenetic relatives of strain MJ11^T. In addition, the 47 nt insertion has been reported for some isolates of *O. intermedium*, including strains ADV1–ADV7, ADV9, PR17/sat (Teyssier *et al.*, 2003), CCUG 1838, CCUG 44770 and CCM 7036 (Scholz *et al.*, 2008). As mentioned by Imran *et al.* (2010), BLAST searches in GenBank using the insertion sequence detected 100 % sequence similarity to the 16S rRNA gene sequences of more than 10 partially characterized *O. tritici* isolates (e.g. accession numbers AY972337, AY972330, AY972328), a few isolates of *O. anthropi* and *O. intermedium* and some uncultured bacterial clones (accession numbers EU769179, EU149209 and DQ917822).

Strain MJ11^T showed the highest 16S rRNA gene sequence similarity to the type strains of the species *Ochrobactrum ciceri* (97.9 %) and *Ochrobactrum pituitosum* (96.4 %). Sequence similarities <95 % were found with other recognized species of the family *Brucellaceae*. When the sequence was corrected for the 47 bp insertion sequence, strain MJ11^T shared highest 16S rRNA gene sequence similarity with *Ochrobactrum intermedium* LMG 3301^T (97.2 %), *Ochrobactrum oryzae* MTCC 4195^T (96.5 %) and *Ochrobactrum pseudintermedium* ADV31^T (96.3 %). The relationship between strain MJ11^T and other members of the genus *Ochrobactrum* was also evident in the neighbour-joining phylogenetic tree (Fig. 1). Recently, sequence analyses of genes encoding the DNA gyrase B subunit (*gyrB*), chaperonin GroEL (*groEL*) and the 70 kDa heat-shock protein (*dnaK*) have been applied for the accurate identification of species of the genus *Ochrobactrum* (Teyssier *et al.*, 2007; Huber *et al.*, 2010). Comparative sequence analyses of these genes were, therefore, performed in the present study. Partial *gyrB* and *groEL* sequences of strain MJ11^T and *Ochrobactrum ciceri* DSM 22292^T were analysed according to Huber *et al.* (2010) using the primer pairs GyrB_f/GyrB_r and GroEL_f/GroEL_r, respectively. Partial *dnaK* sequences of strain MJ11^T and *O. ciceri* DSM 22292^T were obtained using primers 289f and 1142r as described previously (Teyssier *et al.*, 2007). Phylogenetic trees based on *dnaK*, *groEL* and *gyrB* gene sequences were reconstructed using the neighbour-joining method (Supplementary Fig. S1, available in IJSEM Online). The *dnaK*, *groEL* and *gyrB* trees revealed only moderate consistency with the tree topology based on 16S rRNA gene sequences and showed marked differences in the branching order. In the *gyrB* gene sequence-based tree, strain MJ11^T grouped with type strain of *O. anthropi* and in the *dnaK*- and *groEL* gene sequence-based trees, strain MJ11^T occupied a distinct phylogenetic position within the genus *Ochrobactrum* (Supplementary Fig. S1). Discrepancies may be explained by different datasets (not all sequences of all species were available), different sequence lengths of genes or differences in the apparent rate of evolution of protein-encoding genes with respect to the 16S rRNA gene. Nevertheless, each of these trees supported the status of strain MJ11^T as a separate species and its affiliation to the genus *Ochrobactrum*. The maximum similarity levels were 93.9 % with *O. cicero*, based on *groEL* gene sequences; 91.5 % with *O. ciceri* and *O. pseudintermedium*, based on *dnaK* gene sequences; and 89.8 % with *O. ciceri* and *O. intermedium*, based on *gyrB* gene sequences. The presence of the *recA* gene, another alternative phylogenetic marker, was determined using the primer pairs *rec*A-BrucOchro-f/*rec*A-BrucOchro-r, *rec*A-PsOchro-f/*rec*A-PsOchro-r and *rec*A-wob-f/*rec*A-wob-r, as described by Scholz *et al.* (2006, 2008). *O. intermedium* KACC 11952^T was used as a positive control. However, all attempts to amplify a *recA* gene fragment from the DNA of strain MJ11^T using these primer sets were unsuccessful, suggesting that this strain was closely related to *O. ciceri* Ca-34^T (Imran *et al.*, 2010) but differed markedly from strains of *O.*

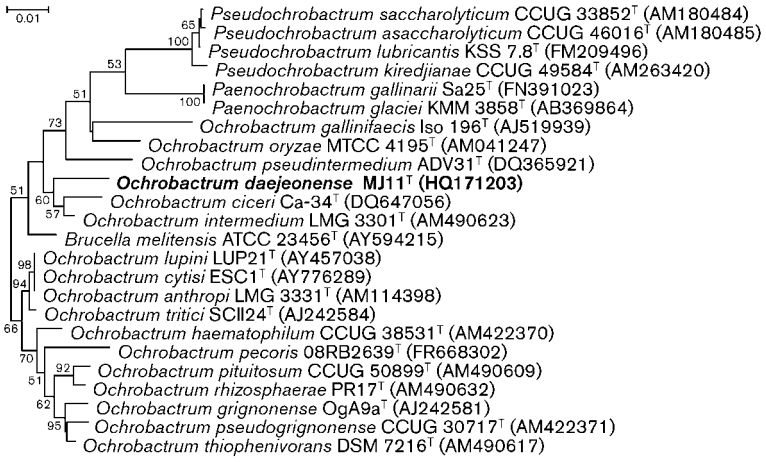

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain MJ11^T among recognized members of the genus *Ochrobactrum* and other related species of the family *Brucellaceae*. Bootstrap values >50 %, based on 1000 replications, are shown at branch points. *Bradyrhizobium japonicum* ATCC 10324^T (accession no. U69638) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

pituitosum (Huber *et al.*, 2010), *O. anthropi* and *O. intermedium* (Scholz *et al.*, 2006, 2008). These data indicated that strain MJ11^T could be clearly separated from other members of the genus *Ochrobactrum* with the exception of *O. ciceri*. To differentiate strain MJ11^T from its closest phylogenetic relative, DNA–DNA hybridization experiments were performed.

The Gram reaction was determined by using a Gram-stain kit (Difco) according to the manufacturer's instructions. Cell morphology and motility were observed under a Nikon light microscope (×1000 magnification) using exponential- and stationary-phase cultures grown at 30 °C on R2A agar and by using the hanging drop technique. Tests for anaerobic growth, catalase and oxidase activities and nitrate and nitrite reduction were performed as described previously (Ten *et al.*, 2006). Assimilation of single carbon sources, enzyme activities, acid production from substrates and other physiological characteristics were determined using API ID 32 GN (at 30 °C for 48 h), API ZYM (at 30 °C for 4 h), API 20 NE (at 30 °C for 48 h) and API 50 CHB (at 30 °C for 48 h) galleries (bioMérieux) and Biolog GN MicroPlate panels (at 30 °C for 48 h) according to the manufacturers' instructions. Nitrogen-fixing ability was determined as previously reported by Im *et al.* (2006). The primer system PolF–PolR (Poly *et al.*, 2001) was used to amplify the *nifH* gene according to the methods described by Im *et al.* (2004). Biochemical tests for hydrolysis of aesculin, casein, gelatin and starch, arginine dihydrolase and chitinase activities, Voges–Proskauer reaction and hydrogen sulfide production were performed as described by Tindall *et al.* (2007). Tests for the degradation of DNA, using DNase agar (Scharlau) with DNase activity detected by flooding plates with 1 M HCl (Collins & Lyne, 1984), and tests for the degradation of xylan and hydroxyethyl (HE)-cellulose (Ten *et al.*, 2004) were performed and evaluated after 7 days. Growth at 10, 15, 20, 25, 30, 34, 37 and 42 °C was assessed on R2A agar, nutrient agar, LB agar, trypticase soy agar (TSA; Difco) and MacConkey agar after 5 days of incubation. Growth at pH 5.0–10.0 (at intervals of 0.5 pH units) was evaluated in LB broth at 30 °C.

Cells of strain MJ11^T were Gram-reaction-negative, aerobic, non-spore-forming rods (0.7–0.8 × 0.8–0.9 μm) that exhibited motility at exponential phase but no motility at stationary phase. Colonies grown on LB agar plates for 2 days at 30 °C were 2–5 mm in diameter, slightly convex, shiny, viscous and pale yellow. On LB agar, good growth was observed between 25–30 °C and weak growth was observed at 20 and 34 °C. Strain MJ11^T was not able to grow at 15 or 37 °C. Good growth also occurred on R2A agar, nutrient agar, TSA and MacConkey agar at 25–30 °C. Strain MJ11^T reduced nitrate to nitrite but did not reduce nitrite to nitrogen gas. The isolate did not grow in a nitrogen-free liquid medium, suggesting that it may not have a nitrogen-fixing ability. Attempts to amplify the *nifH* gene (encoding the iron protein of nitrogenase) failed with the specific primers used (Poly *et al.*, 2001). These findings are consistent with results obtained with *O. ciceri* Ca-34^T (Imran *et al.*, 2010). Phenotypic and chemotaxonomic characteristics that differentiate strain MJ11^T from closely related members of the genus *Ochrobactrum* are listed in Table 1. In contrast to the reference strains, the novel isolate was positive for assimilation of itaconate, 3- and 4-hydroxybenzoate, phenylacetate, salicin, suberate and valerate as well as acid production from gluconate, 5-ketogluconate and methyl β-D-xylopyranoside. The isolate was also negative for utilization of L-rhamnose. In addition, strain MJ11^T could be differentiated from the reference strains and other recognized members of the genus *Ochrobactrum* based on the results of Biolog GN MicroPlate tests (Supplementary Table S1).

For the measurement of chromosomal DNA G+C content, the genomic DNA of the strain was extracted and purified as described by Moore & Dowhan (1995) and degraded enzymically into nucleosides. The DNA G+C content was then determined as described by Mesbah *et al.* (1989) using reversed-phase HPLC. Cell biomass for isoprenoid quinone analysis was obtained from cultures

Table 1. Differential phenotypic characteristics between strain MJ11^T and closely related type strains in the genus *Ochrobactrum*

Strains: 1, MJ11^T; 2, *O. ciceri* DSM 22292^T; 3, *O. pituitosum* DSM 22207^T; 4, *O. intermedium* KACC 11952^T; 5, *O. anthropi* KCTC 22833^T; 6, *O. oryzae* KACC 12297^T; 7, *O. pseudintermedium* KCTC 22760^T. All data are from the present study. All strains were positive for nitrate reduction and were negative for indole and hydrogen sulfide production and lysine decarboxylase and ornithine decarboxylase activities. In API ID 32 GN, API 20 NE (assimilation of carbon sources), API ZYM and API 50 CHB tests, all strains showed identical biochemical characteristics except for those substrates indicated here and in the species description. +, Positive; −, negative; w, weakly positive.

Characteristic	1	2	3	4	5	6	7
Growth at:							
20 °C	+	−	+	+	+	+	−
37 °C	−	+	−	+	+	+	+
Aesculin and gelatin hydrolysis	−	+	−	−	−	−	−
Arginine dihydrolase	+	+	−	−	−	−	−
Voges–Proskauer reaction	+	+	+	−	−	+	−
Enzyme activities (API ZYM and API 20 E):							
Alkaline phosphatase	+	+	+	−	−	w	−
Cystine arylamidase, α-glucosidase	+	+	+	−	−	−	−
Esterase lipase (C8)	−	+	+	+	−	+	+
β-Galactosidase, β-glucosidase	−	+	−	−	−	−	−
Naphthol-AS-BI-phosphohydrolase	+	+	+	+	−	−	+
Tryptophan deaminase	+	+	+	−	−	+	−
Valine arylamidase	−	+	−	−	−	−	−
Urease	−	+	−	+	+	+	−
Assimilation of (API ID 32 GN and API 20 NE):							
Acetate	+	+	−	+	+	+	+
Caprate	+	−	−	+	+	−	+
Citrate	−	+	+	+	+	−	−
Gluconate	+	+	−	w	+	+	w
Inositol	+	+	+	−	+	−	+
Itaconate, 3- or 4-hydroxybenzoate, salicin, phenylacetate, suberate, valerate	+	−	−	−	−	−	−
DL-3-Hydroxybutyrate	+	+	w	−	+	+	−
D-Mannitol	+	+	+	−	+	+	−
Propionate	+	+	−	+	+	+	−
L-Rhamnose	−	+	+	+	+	+	+
D-Ribose	+	−	w	−	+	−	−
D-Sorbitol	+	+	+	−	+	+	+
Acid production from (API 50 CHB):							
N-Acetylglucosamine, lactose	+	+	−	−	−	+	+
Adonitol, maltose	+	+	−	+	+	+	+
Aesculin, inositol, gentiobiose, sucrose and xylitol	+	+	−	+	−	+	+
L-Arabinose	+	+	+	−	−	+	+
D-Arabitol, dulcitol	+	−	−	+	−	+	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7
Arbutin, cellobiose, salicin	+	+	−	−	−	−	−
D-Galactose	+	−	+	+	+	+	+
Gluconate, 5-ketogluconate, methyl β-D-xylopyranoside	+	−	−	−	−	−	−
D-Glucose	+	+	+	−	−	+	−
Glycerol	+	+	+	−	+	−	−
Glycogen, starch	−	+	−	−	−	−	+
Inulin, raffinose	−	−	−	−	−	−	+
D-Mannitol	+	−	−	−	−	+	−
D-Mannose, trehalose, methyl α-D-glucopyranoside	+	+	−	−	−	+	−
Melibiose	−	+	−	−	−	+	+
Methyl α-D-mannopyranoside	−	+	+	−	−	−	−
L-Rhamnose	−	+	−	+	−	+	+
D-Ribose	+	+	+	+	+	+	−
Sorbitol	−	+	+	−	−	+	+
D-Xylose	+	+	+	+	−	+	+

grown in LB broth for 2 days at 30 °C. Quinones were extracted, fractionated and analysed by HPLC as described previously (Hiraishi *et al.*, 1996). Cellular fatty acids were analysed using cultures grown on nutrient agar for 48 h at 30 °C. The 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) using the Microbial Identification software package (Sasser, 1990). Polyamines were extracted and analysed according to the methods of Busse & Auling (1988) and Schenkel *et al.* (1995). Polar lipids were extracted using the procedure described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents as described previously (Lee *et al.*, 2008).

The cellular fatty acid composition of strain MJ11^T was similar to that of the type strains of six reference species of the genus *Ochrobactrum*, containing a large amount of $C_{18:1}\omega7c$, moderate amounts of $C_{19:0}$ cyclo $\omega8c$, $C_{16:0}$ and $C_{18:0}$ and a small amount of $C_{18:1}$ 2-OH (Table 2). Some quantitative differences were found between the proportions of these components in strain MJ11^T and the six reference strains. In particular, the summed proportions of the major fatty acids $C_{18:1}$ $\omega7c$ and $C_{19:0}$ cyclo $\omega8c$, which was used to compare fatty acid profiles between strains of the genus *Ochrobactrum* (Huber *et al.*, 2010), was smaller in strain MJ11^T (74.8 %) than was found in its closest neighbours, *O. ciceri* DSM 22292^T (82.9 %) and *O. pituitosum* 22207^T (77.2 %). Apart from these differences, strain MJ11^T differed from its closest neighbours mainly by the absence of $C_{17:0}$ cyclo. Two-dimensional TLC analysis of polar lipids extracted from strain MJ11^T (Supplementary Fig. S2) showed that the isolate contained the characteristic unknown aminolipid AL2, which was detected in polar lipid

Table 2. Fatty acid compositions of strain MJ11^T and closely related type strains in the genus *Ochrobactrum*

Strains: 1, MJ11^T; 2, *O. ciceri* DSM 22292^T; 3, *O. pituitosum* DSM 22207^T; 4, *O. intermedium* KACC 11952^T; 5, *O. anthropi* KCTC 22833^T; 6, *O. oryzae* KACC 12297^T; 7, *O. pseudintermedium* KCTC 22760^T. All data are from the present study. All strains were grown on nutrient agar for 48 h at 30 °C prior to fatty acid analysis. Values are percentages of total fatty acids. −, Not detected.

Fatty acid	1	2	3	4	5	6	7
$C_{10:0}$	−	−	−	1.1	−	−	−
$C_{16:0}$	11.5	5.3	6.9	6.1	9.6	10.4	12.4
$C_{17:0}$	0.8	1.2	2.4	2.1	0.9	0.2	1.4
$C_{18:0}$	8.5	6.7	9.1	6.5	10.7	9.7	4.5
$C_{17:0}$ cyclo	−	0.4	0.5	−	0.6	−	1.1
$C_{19:0}$ cyclo $\omega 8c$	14.2	15.1	42.1	28.3	27.3	11.4	33.0
$C_{12:1}$ at 11-12	−	−	−	3.8	−	−	−
$C_{18:1}\omega 7c$	62.6	67.8	35.1	50.3	50.6	66.7	40.8
$C_{18:1}$ 2-OH	1.4	3.5	1.7	1.8	0.3	−	3.9
$C_{18:1}$ 3-OH	−	−	0.3	−	−	−	−
Summed feature 3*	1.0	−	1.9	−	−	1.6	2.9

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 comprises $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH.

extracts of other members of the genus *Ochrobactrum* but was not produced by representatives of the closely related genus *Pseudochrobactrum* (Kämpfer *et al.*, 2006, 2007b). The overall polar lipid pattern of strain MJ11^T corresponded to that described for the genus *Ochrobactrum* (Kämpfer *et al.*, 2003, 2007a, 2008; Teyssier *et al.*, 2007), and comprised large amounts of phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylmonomethylethanolamine (PME) as well as moderate amounts of diphosphatidylglycerol (DPG) and unknown aminolipid AL1. Minor unknown aminolipids AL3 and AL4, phospholipids PL2 and PL4 and polar lipid L2 were also detected and have previously been found in other members of the genus *Ochrobactrum* (Kämpfer *et al.*, 2007a; Huber *et al.*, 2010). The polyamine pattern of strain MJ11^T, with putrescine and spermidine as predominant compounds and *sym*-homospermidine as a minor component, was in agreement with polyamine compositions reported for other members of the genus *Ochrobactrum* (Kämpfer *et al.*, 2007a, 2008). Analysis of the respiratory lipoquinones of strain MJ11^T revealed ubiquinone Q-10 as the predominant compound with trace amounts of Q-9. The DNA G+C content of the novel isolate was 59.3 mol%, which is slightly higher than values reported for other type strains in the genus *Ochrobactrum* (54.5–59.0 mol%) (Lebuhn *et al.*, 2000; Teyssier *et al.*, 2007). However, the value still lies within the range recommended for members of the same genus and therefore should be taken into account in the range of G+C contents of members of the genus *Ochrobactrum*.

DNA–DNA hybridizations were performed fluorometrically according to the method of Ezaki *et al.* (1989), 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 were quoted as the DNA–DNA hybridization values. SD was also calculated based on these three values. Strain MJ11^T exhibited relatively low levels of DNA–DNA relatedness with respect to *Ochrobactrum ciceri* DSM 22292^T (29±7 %), indicating that they are not related at the species level (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). These results are also in agreement with the recommendation that the 16S rRNA gene sequence similarity threshold be increased from 97 % to 98.7–99 % when determining the uniqueness of a new strain (Stackebrandt & Ebers, 2006).

The phenotypic and phylogenetic data presented here indicate that strain MJ11^T belongs to the genus *Ochrobactrum*. The phylogenetic distinctiveness, together with the DNA–DNA hybridization data, confirmed that this isolate represents a species that is distinct from recognized species of the genus *Ochrobactrum*. Strain MJ11^T can also be differentiated from its closest phylogenetic relatives based on several phenotypic characteristics (Table 1, Supplementary Table S1). Therefore, on the basis of the data presented here, strain MJ11^T represents a novel species of the genus *Ochrobactrum*, for which the name *Ochrobactrum daejeonense* sp. nov. is proposed.

Description of *Ochrobactrum daejeonense* sp. nov.

Ochrobactrum daejeonense (dae.jeon.en′se. N.L. fem. adj. *daejeonense* pertaining to Daejeon, a city in South Korea, from where the type strain was isolated).

Cells are Gram-reaction-negative, aerobic, non-spore-forming rods. The pH range for growth is pH 6.0–8.0, with an optimum of pH 7.0. Aesculin, casein, chitin, DNA, HE-cellulose, gelatin, starch and xylan are not hydrolysed. The results of tests for carbon source utilization (API ID 32 GN and API 20 NE), enzyme activities (API ZYM and API 20 E) and acid production (API 50 CHB) are reported in Table 1. *N*-Acetylglucosamine, L-alanine, L-arabinose, L-fucose, D-glucose, L-histidine, 2-ketogluconate, lactate, malate, maltose, D-mannose, L-proline, L-serine and sucrose are utilized for growth but adipate, glycogen, 5-ketogluconate, malonate and melibiose are not utilized. Positive for acid phosphatase, esterase (C4), leucine arylamidase and trypsin activities but negative for *N*-acetyl-β-glucosaminidase, α-chymotrypsin, α-fucosidase, α-galactosidase, β-glucuronidase, lipase (C14), lysine decarboxylase, α-mannosidase and ornithine decarboxylase activities. Acid is produced without gas from D-arabinose, erythritol, D-fructose, D- and L-fucose, D-lyxose, D-tagatose, turanose and L-xylose but not from amygdalin, L-arabitol, 2-ketogluconate, melezitose or L-sorbose. Ubiquinone Q-10 is the predominant respiratory lipoquinone. The major fatty acids are $C_{18:1}\omega 7c$, $C_{19:0}$ cyclo

$\omega 8c$, $C_{16:0}$ and $C_{18:0}$. The major polar lipids are PG, PE, PC, PME, DPG and two unknown AL. The major respiratory quinone is ubiquinone Q-10 with a trace amount of Q-9. The major polyamines are putrescine and spermidine and the minor polyamine is *sym*-homospermidine.

The type strain, MJ11^T (=KCTC 22458^T =JCM 16234^T), was isolated from sludge of the aerobic treatment tanks of a municipal leachate treatment plant located in Daejeon, South Korea. The DNA G+C content of the type strain is 59.3 mol%.

Acknowledgements

This work was supported by the GAIA Project (173-101-034), Ministry of Environment, Republic of Korea, and by the Korea Brain Pool Program of 2011 (grant 111S-4-5-0032).

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