Deinococcus aerolatus sp. nov. and *Deinococcus aerophilus* sp. nov., isolated from air samples

Seung-Hee Yoo, Hang-Yeon Weon, Soo-Jin Kim, Yi-Seul Kim, Byung-Yong Kim and Soon-Wo Kwon

Correspondence
Soon-Wo Kwon
swkwon@rda.go.kr

Korean Agricultural Culture Collection (KACC), National Agrobiodiversity Center, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, Republic of Korea

Two strains of pink-coloured bacteria, 5516T-9^T and 5516T-11^T, were isolated from an air sample collected in Korea. The taxonomic status of these novel strains was investigated by means of a polyphasic approach. The novel strains were Gram-positive, aerobic, non-spore-forming and coccus-shaped bacteria. The DNA G + C contents of strains 5516T-9^T and 5516T-11^T were 61.0 and 59.3 mol%, respectively. The major isoprenoid quinone for both strains was MK-8. Strain 5516T-9^T contained summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c), C_{16:0} and iso-C_{17:1}ω9c, and strain 5516T-11^T contained summed feature 3, iso-C_{17:1}ω9c, C_{17:1}ω8c and C_{15:1}ω6c as the major fatty acids (>10%). The polar lipid patterns of both strains were similar, comprising one phospholipid and one aminophospholipid as the major components. Phylogenetic analyses using 16S rRNA gene sequences showed that both novel strains were affiliated to the genus *Deinococcus*. Strain 5516T-9^T exhibited the highest sequence similarity with *Deinococcus marmoris* DSM 12784^T (96.8%) and strain 5516T-11^T showed the highest sequence similarity with *Deinococcus saxicola* DSM 15974^T (94.5%). The sequence similarity between strains 5516T-9^T and 5516T-11^T was 94.7%. On the basis of the data presented, it is evident that both strains represent separate novel species of the genus *Deinococcus* for which the names *Deinococcus aerolatus* sp. nov. (type strain 5516T-9^T=KACC 12745^T=JCM 15442^T) and *Deinococcus aerophilus* sp. nov. (type strain 5516T-11^T=KACC 12746^T=JCM 15443^T) are proposed.

At the time of writing, the genus *Deinococcus* comprised 36 species with validly published names, isolated from various environments such as soils, hot springs, food, faeces, airborne dust, alpine environments, activated sludge, freshwater and the rhizosphere. Members of the genus are non-spore-forming, non-motile, coccoid or rod-shaped cells and are bright red, orange or pink in colour. With a few exceptions (Callegan *et al.*, 2008; Peng *et al.*, 2009), the majority of the members of the genus *Deinococcus* are characterized by extreme resistance to UV and gamma radiation and to desiccation.

During a study of bacterial populations in air samples, two pink-coloured bacterial strains were isolated. The air samples were collected outdoors in the Taean region, Korea, using an MAS-100 air sampler (Merck; single-stage multiple-hole impactor). The sampler contained Petri

dishes containing R2A agar (BBL) amended with 0.02% cycloheximide (Sigma). The air samples were collected directly onto the Petri dishes. After sampling, the plates were incubated at 30 °C for 5 days and strains 5516T-9^T and 5516T-11^T were recovered. Routine cultivation was conducted at 28 °C with R2A media. For comparisons *Deinococcus frigens* DSM 12807^T, *Deinococcus marmoris* DSM 12784^T, *Deinococcus radiopugnans* DSM 12027^T and *Deinococcus saxicola* DSM 15974^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany).

Phenotypic characteristics such as Gram staining, catalase and oxidase activities and the hydrolysis of carboxymethyl-cellulose, casein, chitin from crab shells, DNA, pectin, tyrosine, Tween 80 and starch were performed using the methods of Smibert & Krieg (1994). The pH range for growth (pH 4.0–10.0 at intervals of 1.0 pH unit) was determined in R2A broth which was adjusted with citrate-phosphate buffer or Tris/HCl buffer (Breznak & Costilow, 1994). Growth at 0, 1, 2, 3 and 5% NaCl (w/v) was investigated in R2A broth. Growth at various temperatures (5–50 °C) was measured on R2A agar medium. Growth under anaerobic conditions was tested in GasPak jars

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 5516T-9^T and 5516T-11^T are EU622978 and EU622979, respectively.

Photographs of the TLC plates used for polar lipid analysis and an additional phylogenetic tree constructed using the maximum-parsimony algorithm are available as supplementary figures with the online version of this paper.

(BBL) at 30 °C for 15 days. UV irradiation of the cultures was carried out according to the methods of Hirsch *et al.* (2004) and Weon *et al.* (2007). Cell morphology and motility were observed under a phase-contrast microscope (Axio; Zeiss) and a transmission electron microscope (912AB; Leo) after cultivation of strains on R2A at 30 °C. Physiological and biochemical properties were further determined with API ZYM, API 20NE and API ID32 GN kits (bioMérieux). Tests in the commercial systems were generally performed according to the manufacturer's instructions. The API ZYM test strip was read after 4 h incubation at 37 °C and other API test strips were examined after 5 days at 30 °C.

Fatty acid methyl esters were extracted and prepared by the standard protocol of the Microbial Identification System (MIDI; Microbial ID) after cells had been grown on R2A medium. Isoprenoid quinones were extracted from lyophilized cells and analysed by HPLC as described previously (Groth *et al.*, 1996). The determination of DNA G+C content was performed as described by Mesbah *et al.* (1989) using a reverse-phase column (Supelcosil LC-18 S; Supelco).

The 16S rRNA gene sequence was determined as described previously (Kwon *et al.*, 2003). The nearly complete 16S rRNA gene sequences of strains 5516T-9^T (1394 nucleotides) and 5516T-11^T (1430 nucleotides) were obtained. The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were performed using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). The MEGA (Molecular Evolutionary Genetics Analysis) version 3.1 software package (Kumar *et al.*, 2004) was used for phylogenetic tree construction. Phylogenetic trees were inferred from the neighbour-joining (Saitou & Nei, 1987), and maximum-parsimony (Fitch, 1971) methods. The stability of relationships was assessed by performing bootstrap analyses based on 1000 resamplings.

Strains 5516T-9^T and 5516T-11^T could be differentiated from each other on the basis of enzymic activities such as valine arylamidase, cystine arylamidase and *N*-acetyl- β -glucosaminidase. Both strains were clearly separable from *D. frigens*, *D. marmoris*, *D. radiopugnans* and *D. saxicola* on the basis of a variety of characteristics such as growth

Table 1. Differential phenotypic characteristics of strains 5516T-9^T and 5516T-11^T and closely related species of the genus *Deinococcus*

Strains: 1, 5516T-9^T; 2, 5516T-11^T; 3, *D. frigens* DSM 12807^T; 4, *D. marmoris* DSM 12784^T; 5, *D. radiopugnans* DSM 12027^T; 6, *Deinococcus saxicola* DSM 15974^T. Data for the reference strains are from Hirsch *et al.* (2004) and this study. All strains are coccus-shaped and are positive in tests for β -galactosidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase and α -glucosidase. All strains are negative in tests for indole production, glucose fermentation, arginine dihydrolase, urease, lipase (C14), trypsin, α -galactosidase, β -glucuronidase, α -mannosidase and α -fucosidase. +, Positive; (+), weakly positive; –, negative; A, aerobic; FA, facultative anaerobic.

Characteristic	1	2	3	4	5	6
Isolation source	Air sample	Air sample	Antarctic soil	Antarctic marble	Irradiated haddock	Antarctic sandstone
Colony colour	Pink	Pink	Pink, salmon to orange	Pink, salmon to orange	Orange to red	Pink, salmon to orange
Oxygen requirement	A	A	A, FA	A	A, FA	A
Oxidase	–	–	(+)	+	ND	+
Temperature range for growth (°C)	5–40	5–45	1–21	<4–25	<30–41	4–<37
Optimum temperature (°C)	30	30	16–18	9–16	30	9
Size (μ m)	1.2–1.7	1.4–2.0	0.9–2.0	1.0–4.5	1.0–2.0	2.0–4.0
NaCl	0–1	0–1	0–10	0–<2	0–1	0–1
Nitrate reduction*	–	–	–	–	+	–
Aesculin hydrolysis*	+	+	–	+	+	+
Gelatin hydrolysis*	+	+	–	+	+	+
Enzymic activities*						
Leucine arylamidase	+	(+)	+	(+)	+	–
Valine arylamidase	+	–	–	–	–	–
Cystine arylamidase	+	–	–	–	–	–
α -Chymotrypsin	(+)	+	–	+	+	+
β -Glucosidase	(+)	(+)	–	–	+	+
<i>N</i> -Acetyl- β -glucosaminidase	+	–	–	–	+	–
DNA G+C content (mol%)	61.0	59.3	61.8–65.5	62.8	70	59.4

*Nitrate reduction, aesculin hydrolysis, gelatin hydrolysis and enzymic activities were obtained from the present study using API 20NE and API ZYM test strips.

temperature, oxidase and other enzymic activities. Strains 5516T-9^T and 5516T-11^T were resistant to UV radiation (254 nm, 10 cm, 10 min). *Escherichia coli* ATCC 35607 and *Bacillus subtilis* ATCC 465 were used as controls and were found to be sensitive. Table 1 shows the main phenotypic differences between strains 5516T-9^T, 5516T-11^T and closely related species of the genus *Deinococcus*.

Strain 5516T-9^T contained summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c; 32.9%), C_{16:0} (19.2%) and iso-C_{17:1}ω9c (12.7%). Strain 5516T-11^T contained summed feature 3 (25.1%), iso-C_{17:1}ω9c (14.4%), C_{17:1}ω8c (10.7%) and C_{15:1}ω6c (10.5%) as the major fatty acids (>10%) (Table 2). Strain 5516T-11^T could be differentiated from strain 5516T-9^T by the presence of iso-C_{16:0} (2.6%), iso-C_{16:1} H (3.5%) and C_{17:1}ω6c (2.2%). Both strains could be separated from the other closely related species on the basis of qualitative and quantitative differences in the compositions of the fatty acids (Table 2). The major respiratory menaquinone of both novel strains was menaquinone 8. The polar lipid patterns of both strains were similar, comprising one phospholipid and one aminophospholipid as the major components (see Supplementary Fig. S1 in IJSEM Online). These were consistent with those of *Deinococcus cellulosilyticus* KACC 11606^T and *Deinococcus deserti* DSM 17065^T (Weon *et al.*,

2007). The DNA G+C contents of strains 5516T-9^T and 5516T-11^T were 61.0 and 59.3 mol%, respectively.

Strain 5516T-9^T exhibited 16S rRNA gene sequence similarity levels of 85.6–96.8% to the type strains of recognized species of the genus *Deinococcus*. The highest sequence similarities were found with *D. marmoris* DSM 12784^T (96.8%), *D. saxicola* DSM 15974^T (96.4%), *D. radiopugnans* ATCC 19172^T (96.3%) and *D. frigens* DSM 12807^T (95.0%). Strain 5516T-11^T showed 86.1–94.5% gene sequence similarity with the type strains of the genus *Deinococcus*, with the highest sequence similarities to *D. saxicola* DSM 15974^T (94.5%), *D. marmoris* DSM 12784^T (94.2%) and *D. frigens* DSM 12807^T (94.1%). The sequence similarity between strains 5516T-9^T and 5516T-11^T was 94.7%. A Neighbour-joining phylogenetic tree (Fig. 1) showed that strains 5516T-9^T and 5516T-11^T formed one clade with *D. frigens* DSM 12807^T, *D. marmoris* DSM 12784^T, *D. radiopugnans* ATCC 19172^T and *D. saxicola* DSM 15974^T with a bootstrap value of 92%. This was also supported by the topology of the maximum-parsimony phylogenetic tree with a bootstrap value of 61% (see Supplementary Fig. S2 in IJSEM Online).

On the basis of the data obtained from this polyphasic taxonomic approach, strains 5516T-9^T and 5516T-11^T represent two novel species of the genus *Deinococcus* for which the names *Deinococcus aerolatus* sp. nov. and *Deinococcus aerophilus* sp. nov. are proposed, respectively.

Table 2. Cellular fatty acid compositions of strains 5516T-9^T and 5516T-11^T and related strains

Strains: 1, 5516T-9^T; 2, 5516T-11^T; 3, *D. frigens* DSM 12807^T; 4, *D. marmoris* DSM 12784^T; 5, *D. radiopugnans* DSM 12027^T; 6, *D. saxicola* DSM 15974^T. Cells for strains 5516T-9^T and 5516T-11^T and DSM 12027^T were harvested after growth on R2A at 30 °C for 2 days. Cells for DSM 12807^T, DSM 12784^T and DSM 15974^T were collected after growth on R2A at 15 °C for 5 days. Fatty acids representing <1.0% have been omitted.

Fatty acid	1	2	3	4	5	6
iso-C _{13:0}	—	—	—	—	1.0	—
C _{14:0}	1.0	—	—	2.8	1.2	—
iso-C _{15:0}	6.6	4.6	2.3	1.7	15.1	1.4
iso-C _{15:1} F	1.1	1.2	—	—	1.8	—
C _{15:1} ω6c	5.5	10.5	9.9	23.9	6.2	18.8
C _{15:1} ω8c	3.2	5.8	2.0	5.4	4.2	5.9
C _{16:0}	19.2	8.5	8.7	20.4	10.6	6.4
iso-C _{16:0}	—	2.6	2.7	—	—	—
iso-C _{16:1} H	—	3.5	1.7	—	—	—
C _{16:1} ω9c	1.8	2.4	—	—	—	—
C _{17:0}	3.8	4.2	6.1	4.5	4.9	7.3
iso-C _{17:0}	5.0	4.0	2.6	—	7.3	2.0
C _{17:1} ω6c	—	2.2	5.2	—	1.3	6.4
C _{17:1} ω8c	5.6	10.7	13.7	2.9	7.2	18.7
iso-C _{17:1} ω9c	12.7	14.4	6.7	—	21.7	8.4
Summed feature 3*	32.9	25.1	38.5	38.6	15.8	23.7

*Summed feature 3 comprises iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

Description of *Deinococcus aerolatus* sp. nov.

Deinococcus aerolatus (ae.ro.la'tus. Gr. n. *aer* air; L. masc. part. adj. *latus* carried; N.L. masc. part. adj. *aerolatus* airborne).

Cells are Gram-positive, non-motile, aerobic cocci (1.2–1.7 µm). Colonies are pink, convex and circular with entire margins. Growth occurs in the presence of 0–1% (w/v) NaCl (optimum, 0%), at pH 7–9 (optimum, pH 7) and at 5–40 °C (optimum, 30 °C). Catalase-positive and oxidase-negative. Hydrolyses casein, hypoxanthine, Tween 80 and xanthine, but does not hydrolyse chitin, CM-cellulose, DNA, pectin, starch or tyrosine. Positive reactions for aesculin hydrolysis, gelatin hydrolysis and β-galactosidase. Negative reactions for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease (API 20NE). Assimilates D-glucose, D-mannose, D-mannitol, maltose, potassium gluconate, adipic acid, sucrose, salicin, melibiose, D-sorbitol, valeric acid, potassium 2-ketogluconate and L-proline. Weakly assimilates L-serine and L-histidine; does not assimilate L-arabinose, N-acetylglucosamine, capric acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-fucose, propionic acid, 3-hydroxybutyric acid or 4-hydroxybenzoic acid (API 20NE and API ID32 GN). Enzymic activities are shown in Table 1. MK-8 is the predominant isoprenoid quinone.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strains 5516T-9^T and 5516T-11^T and species of the genus *Deinococcus*. Numbers at nodes indicate the percentages of bootstrap support. Bootstrap values <50% are not shown. Bar, 0.02 substitutions per nucleotide position.

The polar lipids consist of one phospholipid, several polar lipids and two aminophospholipids. The major fatty acids are summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c), C_{16:0} and iso-C_{17:1}ω9c.

The type strain, 5516T-9^T (=KACC 12745^T=JCM 15442^T), was isolated from an air sample from Taean region, Korea. The G + C content of the genomic DNA of the type strain is 61.0 mol%.

Description of *Deinococcus aerophilus* sp. nov.

Deinococcus aerophilus [ae.ro.phi'lus. Gr. n. *aer* air; N.L. masc. adj. *philus* (from Gr. masc. adj. *philos*) loving; N.L. masc. adj. *aerophilus* air-loving].

Cells are Gram-positive, non-motile, aerobic cocci (1.4–2.0 μm). Colonies are pink, convex and circular with entire

margins. Growth occurs in the presence of 0–1% (w/v) NaCl (optimum, 0%), at pH 6–8 (optimum, pH 7) and at 5–45 °C (optimum, 30 °C). Catalase-positive and oxidase-negative. Hydrolyses casein, hypoxanthine and starch, but does not hydrolyse chitin, CM-cellulose, DNA, pectin, Tween 80, tyrosine or xanthine. Positive reactions for aesculin hydrolysis, gelatin hydrolysis and β-galactosidase. Negative reactions for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease (API 20NE). Assimilates D-glucose, D-mannitol, glycogen, melibiose and D-sorbitol. Weakly assimilates D-mannose, maltose, adipic acid, sucrose, L-serine, valeric acid and L-histidine; does not assimilate L-arabinose, N-acetylglucosamine, potassium gluconate, capric acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-

ketogluconate, 3-hydroxybenzoic acid, salicin, L-fucose, propionic acid, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid or L-proline (API 20NE and API ID32 GN). Enzymic activities are shown in Table 1. MK-8 is the predominant lipoquinone. The polar lipids consist of one phospholipid, several polar lipids and one aminophospholipid. The major fatty acids are summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c), iso-C_{17:1}ω9c, C_{17:1}ω8c and C_{15:1}ω6c.

The type strain, 5516T-11^T (=KACC 12746^T=JCM 15443^T), was isolated from an air sample from Taean region, Korea. The G+C content of the genomic DNA of the type strain is 59.3 mol%.

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