Xenophilus aerolatus sp. nov., isolated from airSoo-Jin Kim,¹ Yi-Seul Kim,¹ Hang-Yeon Weon,¹ Rangasamy Anandham,² Hyung-Jun Noh³ and Soon-Wo Kwon¹

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A novel aerobic, Gram-negative, motile, rod-shaped bacterial strain designated 5516S-2^T was isolated from an air sample taken in Suwon, Republic of Korea. Colonies were yellow-pigmented and circular with entire margins. 16S rRNA gene sequence analysis showed that strain 5516S-2^T was closely related to *Xylophilus ampelinus* DSM 7250^T (97.6 % sequence similarity), *Variovorax soli* KACC 11579^T (97.5 %) and *Xenophilus azovorans* DSM 13620^T (97.1 %). However, the phylogenetic tree indicated that strain 5516S-2^T formed a separate clade from *Xenophilus azovorans*. Strain 5516S-2^T displayed 42, 31 and 30 % DNA–DNA relatedness to the type strains of *Xenophilus azovorans*, *Xylophilus ampelinus* and *V. soli*, respectively. The major fatty acids (>10 % of total fatty acids) were C_{16:0} (33.3 %), C_{17:0} cyclo (18.8 %), C_{18:1}ω7c (17.5 %) and summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 13.0 %). The DNA G + C content was 69 mol%. The major quinone was ubiquinone Q-8. The predominant polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and two unknown aminophospholipids. Genotypic and phenotypic characteristics clearly distinguished strain 5516S-2^T from closely related species and indicated that it represents a novel species within the genus *Xenophilus*, for which the name *Xenophilus aerolatus* sp. nov. is proposed. The type strain is 5516S-2^T (=KACC 12602^T=DSM 19424^T).

The genus *Xenophilus* was proposed as a member of the family Comamonadaceae by Blümel *et al.* (2001), with *Xenophilus azovorans* as the type species. Members of this genus are characterized as rod-shaped, motile, oxidase-positive bacteria containing a high DNA G + C content (70 mol%), C_{16:0}, summed feature 7 (containing C_{18:1}ω7c and/or C_{18:1}ω9t and/or C_{18:1}ω12c), C_{17:0} cyclo and summed feature 3 (containing C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) as the major fatty acids, and ubiquinone 8 as the major quinone. This genus was shown to be closely related to the genera *Xylophilus* and *Hydrogenophaga*. However, the genus *Xenophilus* could be clearly differentiated from

these two genera based on phylogenetic dendrograms, a unique fatty acid pattern and several physiological features.

During a course of study on the cultivable bacterial diversity of air samples, one yellow-coloured bacterium, designated strain 5516S-2^T, was isolated and subjected to a taxonomic investigation. Air samples were collected with an MAS-100 air sampler (Merck; single-stage multiple-hole impactor) in the downtown outdoor region of Suwon City, Republic of Korea, on May 16, 2005. The sampler contained Petri dishes with R2A agar (BBL) containing cycloheximide (200 µg ml⁻¹; Sigma). After sampling, plates were incubated at 30 °C for 5 days. *Variovorax soli* KACC 11579^T, obtained from the Korean Agricultural Culture Collection, Suwon, Korea, and *Xenophilus azovorans* DSM 13620^T and *Xylophilus ampelinus* DSM 7250^T, both obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, were used as reference strains.

Phenotypic, biochemical and physiological characterizations were carried out in R2A medium at 30 °C as described previously (Smibert & Krieg, 1994; Weon *et al.*, 2007). For transmission electron microscopy, cells were

Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5516S-2^T is EF660342.

Polar lipid profiles and cellular fatty acid compositions of strain 5516S-2^T and related strains, and a maximum-parsimony tree based on almost-complete 16S rRNA gene sequences showing the phylogenetic position of strain 5516S-2^T, are available as supplementary material with the online version of this paper.

grown for 24 h, negatively stained with 0.5 % (w/v) uranyl acetate and examined with a LEO model 912AB electron microscope. Biochemical characteristics were determined using the API 20NE, API ID 32 GN and API ZYM (bioMérieux) systems 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 10 days at 37 °C.

Cells of strain 5516S-2^T were aerobic, Gram-negative, motile, non-spore-forming, short rods, 0.6–0.8 µm in width and 1.0–1.4 µm in length. Colonies grown on R2A agar for 3 days were yellow-coloured and circular with entire margins. Strain 5516S-2^T grew well on R2A, trypticase soy agar (Difco) and nutrient agar (Difco); weak growth was observed on MacConkey agar (Difco). Phenotypic characteristics of strain 5516S-2^T and closely related species are shown in Table 1.

Whole-cell fatty acids were extracted from cell mass collected after incubation on R2A medium for 2 or 3 days and analysed according to the standard protocol of the

MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990). Polar lipid profiles were determined according to Minnikin *et al.* (1984). Isoprenoid quinones were extracted and analysed as described by Groth *et al.* (1996). The DNA G+C content was analysed by using HPLC (Mesbah *et al.*, 1989).

The major cellular fatty acid constituents of strain 5516S-2^T were C_{16:0} (33.3 %), C_{17:0} cyclo (18.8 %), C_{18:1}ω7c (17.5 %) and summed feature 3 (containing C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 13.0 %) (Supplementary Table S1, available in IJSEM Online). The overall cellular fatty acid profile of strain 5516S-2^T was generally in agreement with that of *Xenophilus azovorans* DSM 13620^T. Strain 5516S-2^T and *Xenophilus azovorans* could be clearly differentiated from *Xylophilus ampelinus* based on the presence of C_{10:0}, C_{10:0} 3-OH, C_{17:0} cyclo, C_{18:0} and C_{18:1} 2-OH. Strain 5516S-2^T, *Xenophilus azovorans* and *Xylophilus ampelinus* could be differentiated from some *Variovorax* species based on the absence of C_{12:0} and C_{17:0}, and the presence of C_{18:0} and C_{18:1} 2-OH. The predominant polar lipids of

Table 1. Differential phenotypic traits of strain 5516S-2^T and strains of closely related species

Strains: 1, 5516S-2^T (*Xenophilus aerolatus* sp. nov.); 2, *Xenophilus azovorans* DSM 13620^T; 3, *Xylophilus ampelinus* DSM 7250^T; 4, *Variovorax soli* KACC 11579^T; 5, *Variovorax paradoxus* DSM 30034^T. Except where indicated, all data were determined in this study. +, Positive; (+), weakly positive; –, negative; NA, not available.

Characteristic	1	2	3	4	5
Isolation source	Air sample	Soil*	Plant†	Soil‡	Soil§
Morphology	Rods	Rods*	Rods†	Rods‡	Rods§
Flagella	One, polar	NA	One, polar†	One, polar‡	Peritrichous§
Catalase/oxidase	+/+	+/+*	+/-†	+/+‡	NA/+§
Nitrate reduction	–	–	–	+‡	+‡
Urease	+	–	+	–‡	–‡
Utilization of:					
Sodium acetate	–	+	–	–	+
Ascorbic acid	–	+	–	–	+
Casein	–	–	–	–	+
Sodium citrate	–	–	–	–	+
Dextrin	+	+	–	–	+
Formic acid	(+)	+	(+)	+	+
D-Glucose	–	+	–	–	+
Glycine	–	–	–	–	+
α-D-Lactose	–	+	–	–	+
L-Malic acid	–	+	+	+	+
Mannitol	–	+	+	–	+
Methanol	–	–	–	+	–
Starch	+	–	–	–	–
Succinic acid	–	+	–	–	+
Sucrose	+	+	+	(+)	+
Tartaric acid	–	–	–	–	+
DNA G+C content (mol%)	69	70.4*	68†	67.1‡	67.0§

*Data from Blümel *et al.* (2001).

†Data from Willems *et al.* (1987).

‡Data from Kim *et al.* (2006).

§Data from Willems *et al.* (1991).

strain 5516S-2^T were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and two unknown aminophospholipids (Supplementary Fig. S1, available in IJSEM Online); the polar lipid profiles of strain 5516S-2^T and *Xenophilus azovorans* DSM 13620^T were similar. The major isoprenoid quinone was ubiquinone 8, which is commonly found in members of the family *Comamonadaceae*. The DNA G+C content of strain 5516S-2^T was 69 mol%, which is close to that reported for *Xenophilus azovorans* DSM 13620^T (70 mol%).

The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Kwon *et al.*, 2003). The sequence of the amplified 16S rRNA gene was analysed using an Applied Biosystems DNA sequencer (ABI 3100). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (Chun *et al.*, 2007). The 16S rRNA gene sequences were aligned using CLUSTAL W software (Thompson *et al.*, 1994). Phylogenetic trees for the datasets were inferred from the neighbour-joining (NJ; Saitou & Nei, 1987), maximum-likelihood (ML; Felsenstein, 1981) and maximum-parsimony (MP; Fitch, 1971) methods using MEGA version 3.0 (Kumar *et al.*, 2004). The stability of relationships was assessed by performing bootstrap analyses based on 1000 resamplings. DNA–DNA hybridization was carried out as described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG-High Prime system (Roche) and hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad).

For 16S rRNA gene sequence analysis, continuous stretches of 1407 bp were obtained and analysed. Strain 5516S-2^T exhibited 97.6, 97.5 and 97.1 % 16S rRNA gene sequence similarities to *Xylophilus ampelinus* DSM 7250^T, *V. soli*

KACC 11579^T and *Xenophilus azovorans* DSM 13620^T, respectively. The NJ tree indicated that strain 5516S-2^T formed a monophyletic clade with *Xenophilus azovorans* DSM 13620^T, which was supported by a moderate bootstrap value (66 %) (Fig. 1). A similar phenomenon was also observed in the ML tree (data not shown). The MP tree showed loose clustering between strain 5516S-2^T and *Xenophilus azovorans* DSM 13620^T (Supplementary Fig. S2, available in IJSEM Online). Strain 5516S-2^T displayed 42, 31 and 30 % DNA–DNA relatedness to the type strains of *Xenophilus azovorans*, *Xylophilus ampelinus* and *V. soli*, respectively.

Although strain 5516S-2^T showed highest sequence similarity to the type strains of *Xylophilus ampelinus* and *V. soli*, the phylogenetic dendrograms indicated that strain 5516S-2^T was closely related to *Xenophilus azovorans*. The fatty acid composition and polar lipid pattern clearly revealed that strain 5516S-2^T was a member of the genus *Xenophilus*. However, strain 5516S-2^T could be differentiated from *Xenophilus azovorans* on the basis of the presence of urease, differences in the assimilation patterns of several substrates embedded in API test strips, and qualitative and quantitative differences in fatty acid composition. Based on the data obtained in this study, it is concluded that strain 5516S-2^T represents a novel *Xenophilus* species, for which the name *Xenophilus aerolatus* sp. nov. is proposed.

Description of *Xenophilus aerolatus* sp. nov.

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

Cells are Gram-negative, non-spore-forming, aerobic rods, 0.6–0.8 µm in width and 1.0–1.4 µm in length. Motile with

Fig. 1. Neighbour-joining tree based on almost-complete 16S rRNA gene sequences showing the phylogenetic position of strain 5516S-2^T. Numbers at nodes indicate percentages of 1000 bootstrap resamplings; only values >40 % are given. Bar, 0.005 substitutions per nucleotide position.

a single polar flagellum. Catalase- and oxidase-positive. Colonies are yellow and circular with entire margins. Grows at 10–35 °C (optimum, 25–30 °C), at pH 5.0–9.0 (optimum, pH 6.0–8.0) and with 0–2 % NaCl (optimum, 0–1 %). Hydrolyses hypoxanthine, tyrosine, Tween 80 and xanthine, but not casein, chitin, carboxymethylcellulose, DNA, pectin or starch. In addition to the characteristics shown in Table 1, assimilates itaconic acid, suberic acid, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid and L-proline, but does not assimilate L-rhamnose, D-ribose, inositol, sucrose, sodium malonate, glycogen, L-serine, salicin, melibiose or L-fucose. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8) and leucine arylamidase; negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. The major isoprenoid quinone is ubiquinone Q-8. The major cellular fatty acids are C_{16:0}, C_{17:0} cyclo, C_{18:1 ω 7c} and summed feature 3 (containing C_{16:1 ω 7c} and/or iso-C_{15:0} 2-OH). Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and two unknown aminophospholipids are the predominant polar lipids. The DNA G + C content of strain 5516S-2^T is 69 mol%.

The type strain is 5516S-2^T (=KACC 12602^T=DSM 19424^T), isolated from air samples, Suwon, Republic of Korea.

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