Skermanella aerolata sp. nov., isolated from air, and emended description of the genus *Skermanella*

Hang-Yeon Weon,¹ Byung-Yong Kim,² Seung-Beom Hong,² Jae-Ho Joa,³ Sang-Sik Nam,⁴ Ki Hwan Lee⁵ and Soon-Wo Kwon²

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

¹Applied Microbiology Division, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Republic of Korea

²Korean Agricultural Culture Collection (KACC), Microbial Genetics Division, National Institute of Agricultural Biotechnology, Rural Development Administration, Suwon 441-707, Republic of Korea

³National Institute of Subtropical Agriculture, Rural Development Administration, Jeju 690-150, Republic of Korea

⁴Mokpo Experiment Station, National Institute of Crop Science, Rural Development Administration, Muan 534-833, Republic of Korea

⁵Taeon Lily Experimental Station, Chungnam Provincial Agricultural Research and Extension Services, Taeon 357-952, Republic of Korea

A light-pink-coloured bacterium, designated strain 5416T-32^T, was isolated from an air sample in Korea. Cells of the strain were strictly aerobic, Gram-negative, motile (single polar or subpolar flagellum) and rod-shaped. Optimal growth occurred at 25–30 °C and at pH 6.0–7.0. The major quinones were Q-10 and Q-8. The major fatty acids were C_{18:1}ω7c (53.8 %) and C_{16:0} (15.9 %). The G + C content of the genomic DNA was 65.0 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain 5416T-32^T was most closely related to *Skermanella parooensis*, with a similarity of 96.2 %, but relatively low sequence similarities (<92 %) were found with respect to other species with validly published names held in GenBank. Phenotypic and genotypic analyses indicated that strain 5416T-32^T could not be assigned to any recognized species. Therefore strain 5416T-32^T represents a novel species of the genus *Skermanella*, for which the name *Skermanella aerolata* sp. nov. is proposed. The type strain is 5416T-32^T (=KACC 11604^T=DSM 18479^T).

Conglomeromonas largomobilis subsp. *parooensis* was first proposed by Skerman *et al.* (1983). Later, this taxon was reclassified as *Skermanella parooensis* gen. nov., sp. nov. on the basis of polyphasic taxonomic evidence (Sly & Stackebrandt, 1999). The genus was phylogenetically related to the genus *Azospirillum* of the *Alphaproteobacteria*, and was characterized as a Gram-negative, motile, high G + C-containing facultative anaerobe. The only species (and therefore the type species) is *S. parooensis*.

When violent sandstorms happen in northern China or Mongolia, they produce lots of fine particles that float at a

high altitude. These fine particles drift to the Korean peninsula in the spring. This is referred to as the Asian dust ('Hwangs'a' in Korean) phenomenon, and usually happens between March and May.

To compare the airborne bacteria in Asian dust with those present normally, air samples were collected with a MAS-100 air sampler (a single-stage, multiple-hole impactor; Merck) on the roof of Taeon Lily Experimental Station (Chungnam Provincial Agricultural Research and Extension Services in the Taeon district of Korea) on 16 April 2005. The sample volume (20–1000 cc) was chosen on the basis of the bacterial concentration expected at each site. The sampler contained Petri dishes with R2A agar (BBL) supplemented with 200 µg cycloheximide ml⁻¹ (Sigma). After sampling, the plates were incubated in the dark at 28 °C for 5 days. During this process, we isolated a light-pink-coloured bacterium, designated strain 5416T-32^T.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 5416T-32^T is DQ672568.

The cellular fatty acid profiles of strain 5416T-32^T and *Skermanella parooensis* CIP 106994^T are available in a supplementary table with the online version of this paper.

Unless indicated otherwise, phenotypic characteristics were studied by using standard procedures (Smibert & Krieg, 1994). Growth under anaerobic conditions was tested in a GasPak (BBL) jar at 28 °C for 15 days on R2A and nutrient agar (Difco). Strain 5416T-32^T was grown in R2A medium for 48 h at 28 °C to check the morphology using phase-contrast microscopy and transmission electron microscopy. Several other physiological characteristics, plus the utilization of various sole carbon sources, were determined with the API ID 32 GN, API 20NE, API 50 CH and API ZYM galleries, according to the instructions of the manufacturer (bioMérieux). The API ZYM tests were read after 4 h incubation at 37 °C and the other API tests were read after 4 days at 28 °C.

Strain 5416T-32^T is a strictly aerobic, Gram-negative, motile (single polar or subpolar flagellum), rod-shaped bacterium (0.6–1.2 × 1.5–3.5 µm). The colonies are light pink in colour, convex and round with clear margins. It grows on R2A, nutrient agar, trypticase soy agar (Difco) and MacConkey agar (Difco). Strain 5416T-32^T can be distinguished from *S. parooensis* in that the former can grow at higher NaCl concentrations (up to 5 %) and lower temperatures (down to 5 °C) and can hydrolyse CM-cellulose, but lacks the ability to hydrolyse DNA. According to the assimilation tests with the API 20NE and API ID 32 GN strips, two strains, 5416T-32^T and *S. parooensis* CIP 106994^T, showed some differences in the pattern of substrate utilization. However, in fermentation tests performed using API 50 CH strips, the two strains produced almost identical fermentation patterns, except that strain 5416T-32^T was able to ferment D-galactose and D-glucose. A phenotypic comparison between strain 5416T-32^T and *S. parooensis* is presented in Table 1.

Isoprenoid quinones were extracted from lyophilized cells and analysed by HPLC as described previously (Groth *et al.*, 1996). Whole-cell fatty acids were analysed according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990) after cell cultivation on R2A for 2 days at 28 °C. The DNA G + C content of strain 5416T-32^T was determined according to Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18-S; Supelco). The major isoprenoid quinones were Q-10 and Q-8. A small amount of Q-6 was also detected. Strain 5416T-32^T contained C_{18:1}ω7c (53.8 %) and C_{16:0} (15.9 %) as the predominant fatty acids, and no significant differences in the fatty acid profiles were found between strain 5416T-32^T and *S. parooensis* CIP 106994^T (see Supplementary Table S1, available in IJSEM Online). The DNA G + C content of strain 5416T-32^T was 65.0 mol%.

Amplification and sequencing of the 16S rRNA gene were performed as described previously (Kwon *et al.*, 2003). The sequence obtained was compared with those in GenBank by using DNASTAR software. The sequences were aligned by using CLUSTAL W software (Thompson *et al.*, 1994). Distances were calculated according to the methods of Kimura (1980). Phylogenetic trees were inferred by using the neighbour-joining method (Saitou & Nei, 1987). A

Table 1. Phenotypic comparison of strain 5416T-32^T and *S. parooensis* CIP 106994^T

Data for *S. parooensis* CIP 106994^T are from Sly & Stackebrandt (1999) and this study. Both strains are positive for nitrate reduction, urea hydrolysis, gelatin hydrolysis and β-galactosidase (API 20NE). Both are negative for anaerobic growth and for glucose fermentation (though these two results are not in line with the results from the original description by Sly & Stackebrandt, 1999) and for indole production, arginine dihydrolase and aesculin hydrolysis. Both strains assimilate D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, malic acid, D-ribose, sodium malonate, lactic acid, L-alanine, L-fucose, D-sorbitol, propionic acid, potassium 2-ketogluconate, 3-hydroxybutyric acid and L-proline. Neither strain assimilates N-acetylglucosamine, D-maltose, capric acid, adipic acid, trisodium citrate, phenylacetic acid, inositol, D-sucrose, itaconic acid, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, salicin, D-melibiose or L-histidine (API 20NE and API ID 32 GN). Both strains show alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities. Neither strain shows lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase or α-fucosidase activity (API ZYM).

Characteristic	Strain 5416T-32 ^T	<i>S. parooensis</i> CIP 106994 ^T
Source	Air	Fresh water*
Growth at:		
5 °C	+	—*
5 % NaCl	+	—*
Hydrolysis of:		
Cellulose	+	—*
DNA	—	+*
Assimilation of (API 20NE and API ID 32 GN):		
L-Rhamnose	—	+
Suberic acid	+	—
Sodium acetate	—	+
Valeric acid	—	+
4-Hydroxybenzoic acid	—	+
Enzymic activities (API ZYM):		
α-Glucosidase	—	+
β-Glucosidase	—	+
DNA G + C content (mol%)	65	67*

*Data from Sly & Stackebrandt (1999).

bootstrap analysis was performed on the basis of 1000 resamplings. The MEGA version 2.1 package (Kumar *et al.*, 2001) was used for the analysis.

An almost-complete 16S rRNA gene sequence (1365 nt) for isolate 5416T-32^T was obtained. Sequence comparisons with species with validly published names (obtained from GenBank) indicated that strain 5416T-32^T is related phylogenetically to members of the genera *Skermanella*, *Azospirillum* and *Rhodocista* (within the *Alphaproteo-*

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain 5416T-32^T within the family *Rhodospirillaceae*. Bar, 1 inferred substitution per 100 nt. Numbers at branch points are bootstrap percentages (of 1000 resamplings).

bacteria). The phylogenetic tree (Fig. 1) showed that strain 5416T-32^T forms a separate cluster with *S. parooensis* DSM 9527^T (with 100 % bootstrap support). The sequence comparisons also showed that the 16S rRNA gene sequence of strain 5416T-32^T exhibits the highest level of similarity (96.2 %) with *S. parooensis* DSM 9527^T but has relatively low levels of sequence similarity (<92 %) with respect to other species with validly published names held in GenBank.

The genetic and phenotypic data presented above show that strain 5416T-32^T represents a novel species of the genus *Skermanella*, for which the name *Skermanella aerolata* sp. nov. is proposed.

Emended description of the genus *Skermanella* Sly and Stackebrandt 1999

The description is as given by Sly & Stackebrandt (1999), except that the genus is negative for glucose fermentation and is strictly aerobic.

Description of *Skermanella aerolata* sp. nov.

Skermanella aerolata (ae.ro.la'ta. Gr. n. *aer* air; L. part. adj. *lata* carried; N.L. part. adj. *aerolata* airborne).

Cells are strictly aerobic, Gram-negative rods, and motile by means of a single polar or subpolar flagellum. Temperature range for growth is 5–35 °C. Growth occurs with NaCl concentrations in the range 0–5 % and at pH 4–9. Poly-β-hydroxybutyrate is produced. Positive for catalase, oxidase and hydrolysis of CM-cellulose, starch and Tween 80. Negative for Voges–Proskauer test, for the deamination of phenylalanine and for hydrolysis of casein, chitin, DNA,

pectin and tyrosine. Ferments D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-galactose, D-glucose, D-melibiose and D-lyxose, but does not ferment glycerol, erythritol, D-adonitol, methyl β-D-xylopyranoside, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, D-cellobiose, D-maltose, D-lactose, D-sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate (API 50 CH strips). Major respiratory lipoquinones are Q-10 and Q-8. Major fatty acids are C_{18:1}ω7c and C_{16:0}. The G + C content of the genomic DNA of the type strain is 65.0 mol%.

The type strain, 5416T-32^T (= KACC 11604^T = DSM 18479^T), was isolated from air in Taean, Republic of Korea.

Acknowledgements

This study was supported by the National Institute of Agricultural Biotechnology (NIAB grant no. 06-4-11-19-1), Rural Development Administration, Republic of Korea.

References

- Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diamminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001).** MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.
- Kwon, S. W., Kim, J. S., Park, I. C., Yoon, S. H., Park, D. H., Lim, C. K. & Go, S. J. (2003).** *Pseudomonas koreensis* sp. nov., *Pseudomonas umsongensis* sp. nov. and *Pseudomonas jinjuensis* sp. nov., novel species from farm soils in Korea. *Int J Syst Evol Microbiol* **53**, 21–27.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990).** *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note No. 101. Newark, DE: MIDI Inc.
- Skerman, V. B. D., Sly, L. I. & Williamson, M.-L. (1983).** *Conglomeromonas largomobilis* gen. nov., sp. nov., a sodium-sensitive, mixed-flagellated organism from fresh waters. *Int J Syst Bacteriol* **33**, 300–308.
- Sly, L. I. & Stackebrandt, E. (1999).** Description of *Skermanella parooensis* gen. nov., sp. nov. to accommodate *Conglomeromonas largomobilis* subsp. *parooensis* following the transfer of *Conglomeromonas largomobilis* subsp. *largomobilis* to the genus *Azospirillum*. *Int J Syst Bacteriol* **49**, 541–544.
- Smibert, R. M. & Krieg, N. R. (1994).** Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994).** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.