Aeromicrobium alkaliterrae sp. nov., isolated from an alkaline soil, and emended description of the genus *Aeromicrobium*

Jung-Hoon Yoon, Choong-Hwan Lee and Tae-Kwang Oh

Correspondence

Jung-Hoon Yoon
jhyoon@kribb.re.kr

Korea Research Institute of Bioscience and Biotechnology (KRIIBB), PO Box 115, Yusong, Taejeon, South Korea

A Gram-positive, aerobic, non-motile bacterial strain, KSL-107^T, was isolated from an alkaline soil from Korea and subjected to a polyphasic taxonomic study. Strain KSL-107^T grew optimally at 25 °C and pH 7.0–7.5. Strain KSL-107^T had a cell-wall peptidoglycan based on LL-2,6-diaminopimelic acid. It contained MK-9(H₄) as the predominant menaquinone and C_{16:0}, C_{16:0} 2-OH and 10-methyl C_{18:0} as the major fatty acids. The DNA G + C content was 71.5 mol%. Comparative 16S rRNA gene sequence analyses showed that strain KSL-107^T was phylogenetically affiliated to the genus *Aeromicrobium* of the family *Nocardioidaceae*. Similarity values between the 16S rRNA gene sequence of strain KSL-107^T and those of type strains of *Aeromicrobium* species were in the range 97.9–98.2 %. The isolate could be distinguished from other *Aeromicrobium* species by levels of DNA–DNA relatedness and differences in some phenotypic characteristics. On the basis of the data presented, it is suggested that strain KSL-107^T (=KCTC 19073^T=DSM 16824^T) represents a novel *Aeromicrobium* species, for which the name *Aeromicrobium alkaliterrae* sp. nov. is proposed. An emended description of the genus *Aeromicrobium* is also proposed.

The genus *Aeromicrobium* was first described by Miller *et al.* (1991) with a single species, *Aeromicrobium erythreum*. Subsequently, *Nocardioides fastidiosus* was transferred to the genus as *Aeromicrobium fastidiosum* (Tamura & Yokota, 1994). One further species, *Aeromicrobium marinum*, was described recently (Bruns *et al.*, 2003). Phylogenetic analyses based on 16S rRNA gene sequences showed that the genus *Aeromicrobium* belongs to the family *Nocardioidaceae* (Stackebrandt *et al.*, 1997; Yoon *et al.*, 1998; Bruns *et al.*, 2003). In this study, we report on the taxonomic characterization of an *Aeromicrobium*-like strain, KSL-107^T, which was isolated from an alkaline soil in Korea.

Alkaline soil (approx. pH 10.0) collected from Kwangchun, Korea, was used as the source for the isolation of bacterial strains. Strain KSL-107^T was isolated by the standard dilution plating technique at 25 °C on 10 × diluted nutrient agar (NA; Difco) with pH adjusted to 10.0 by using Na₂CO₃. *A. erythreum* DSM 8599^T and *A. marinum* DSM 15272^T were

obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). *A. fastidiosum* KCTC 9576^T was obtained from the Korean Collection for Type Cultures (KCTC). The morphological, physiological and biochemical characteristics of strain KSL-107^T were investigated using routine cultivation on trypticase soy agar (TSA; Difco) at 25 °C. Cell morphology was examined by light microscopy (E600; Nikon) and transmission electron microscopy (TEM). The presence of flagella was examined by TEM using cells from exponentially growing cultures. The Gram-reaction was determined by using the bioMérieux Gram Stain kit according to the manufacturer's instructions. Growth at various NaCl concentrations was investigated in trypticase soy broth (TSB; Difco) and TSB lacking NaCl. Growth at various temperatures (4–40 °C) was measured on TSA. The pH range for growth was determined in TSB adjusted to various pH values (pH 4.5–12.0 at intervals of 0.5 pH units). The pH was adjusted prior to sterilization by the addition of HCl or Na₂CO₃ (below pH 10.5) and KOH (above pH 10.5). Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on TSA and TSA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Catalase and oxidase activities and hydrolysis of casein, hypoxanthine, starch, tyrosine, xanthine and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and nitrate reduction were studied according to Lanyi

Published online ahead of print on 10 June 2005 as DOI 10.1099/ijs.0.63582-0.

Abbreviation: DAP, 2,6-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KSL-107^T is AY822044.

A table detailing the cellular fatty acid composition of strain KSL-107^T is available as supplementary material in IJSEM Online.

(1987). Utilization of various substrates as sole carbon and energy sources was determined as described by Shirling & Gottlieb (1966). Enzyme activity was determined by using the API ZYM system (bioMérieux). The need for vitamins for growth was investigated using the liquid medium used for substrate utilization tests supplemented with 0.4% (w/v) glucose as the sole carbon and energy sources. Vitamins were added to the medium at the following concentrations (per litre): biotin (2 mg), thiamin hydrochloride (2 mg) and nicotinic acid (2 mg). Other physiological and biochemical tests were performed with the API

20E system (bioMérieux). Morphological, cultural, physiological and biochemical properties of strain KSL-107^T are shown in Table 1 or are given in the species description (see below).

Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996) with the exception that RNase T1 was applied in combination with RNase A. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and

Table 1. Differential phenotypic characteristics of *Aeromicrobium* species

Species: 1, *A. alkaliterrae* sp. nov.; 2, *A. erythreum* (data from Miller *et al.*, 1991); 3, *A. fastidiosum* (Collins & Stackebrandt, 1989; Tamura & Yokota, 1994); 4, *A. marinum* (Bruns *et al.*, 2003). All species are aerobic, positive for Gram stain, esterase (C4), esterase lipase (C8) and leucine arylamidase. All species are negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. +, Positive; –, negative; w, weakly positive; ND, not determined.

Characteristic	1	2	3	4
Cell morphology	Rods, cocci	Irregular rods, cocci	Rods, cocci	Rods
Catalase	+	+	ND	+
Urease	–	–	–	ND
Nitrate reduction	–	–	ND	–
Motility	–	–	+	–
Optimal temperature for growth (°C)	25	35	25	25
Growth at 10 °C	+	–	+	+
Biotin and thiamin required for growth	–	+	+	ND
Hydrolysis of:				
Aesculin	–	+	ND	ND
Casein	+	+	+	–
Cellulose	–	+	–	–
Gelatin	+	+	+	ND
Starch	–	+	+	–
Utilization of:				
L-Arabinose	+	+	+	–
Citrate	–	ND	+	–
D-Fructose	–	+	+	–
D-Glucose	+	+	ND	–
Maltose	+	–	–	–
D-Mannose	–	–	+	–
Pyruvate	–	ND	+	+
Salicin	+	–	ND	ND
Sucrose	+	+	ND	–
D-Trehalose	+	+	ND	+
D-Xylose	–	+	ND	–
API ZYM:*				
Acid phosphatase	+	+	+	–
Alkaline phosphatase	–	–	+	–
α -Glucosidase	+	+	+	–
Naphthol-AS-BI-phosphohydrolase	+	w	w	w
DNA G + C content (mol%)	71.5	71–73	71–72	70.6

*Data from the type strains obtained in this study.

phylogenetic analysis were performed according to Yoon *et al.* (2004). An almost-complete 16S rRNA gene sequence of strain KSL-107^T was determined in this study and comprised 1473 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences revealed that strain KSL-107^T falls within the cluster with *Aeromicrobium* species (Fig. 1). The same tree topologies were found using the maximum-likelihood and maximum-parsimony algorithms (data not shown). Similarity values between the 16S rRNA gene sequences of strain KSL-107^T and the type strains of *Aeromicrobium* species ranged from 97.9 % (with *A. erythreum* and *A. fastidiosum*) to 98.2 % (with *A. marinum*). Sequence similarities to other species used in the phylogenetic analysis were lower than 93.5 %. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded and the means of the remaining three values were quoted as DNA–DNA relatedness values. Mean DNA–DNA relatedness levels between strain KSL-107^T and the type strains of three *Aeromicrobium* species with validly published names were in the range of 11–17 %, indicating that strain KSL-107^T represents a distinct genomic species.

Cell biomass for isoprenoid quinone and cell-wall analyses and for DNA extraction was obtained from cultures grown in TSB at 25 °C. The isomer type of the diamino acid in the cell-wall peptidoglycan was determined using TLC according to the method described by Komagata & Suzuki (1987). Isoprenoid quinones were analysed according to Komagata & Suzuki (1987) using reverse-phase HPLC. For fatty acid methyl ester (FAME) analysis, a loop of cell mass was harvested after cultivation for 6 days on NA and TSA at 25 °C. FAMES were extracted and prepared according to

the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G + C content was determined by the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed and the resultant nucleotides were analysed using reverse-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. Strain KSL-107^T contained LL-2,6-diaminopimelic acid (LL-DAP) as the diagnostic diamino acid in the cell-wall which is characteristic of wall chemotype I *sensu* Lechevalier & Lechevalier (1970). The isolate contained a tetrahydrogenated menaquinone with nine isoprene units [MK-9(H₄)] as the predominant isoprenoid quinone. This menaquinone type was different from that found in the genera *Nocardioide*s, *Marmoricola* and *Actinopolymorpha* (Park *et al.*, 1999; Urzì *et al.*, 2000; Wang *et al.*, 2001). When strain KSL-107^T was cultivated on TSA and NA, the fatty acid profiles were characterized by a common core of straight-chain, unsaturated, hydroxy and 10-methyl fatty acids in varying amounts (see Supplementary Table S1 in IJSEM Online). When cultivated on TSA, the fatty acids (>1.0 %) detected were C_{16:0} (34.1 %), C_{16:0} 2-OH (17.6 %), 10-methyl C_{18:0} (16.3 %), C_{18:1ω9c} (5.7 %), 10-methyl C_{17:0} (5.0 %), 10-methyl C_{16:0} (4.0 %), C_{17:0} (3.5 %), C_{16:1ω7c} and/or iso-C_{15:0} 2-OH (3.4 %), C_{15:0} (3.2 %), C_{17:1ω8c} (2.4 %), C_{18:0} (1.2 %) and C_{14:0} (1.0 %). No significant differences in the fatty acid profiles were found between strain KSL-107^T and *Aeromicrobium* species (Park *et al.*, 1999; Bruns *et al.*, 2003). The fatty acid profiles of strain KSL-107^T were different from those of members of the genus *Nocardioide*s, the closest phylogenetic neighbour (Park *et al.*, 1999; Bruns *et al.*, 2003). Branched fatty acids, particularly iso-C_{16:0}, which are major components in *Nocardioide*s species, were minor components in strain KSL-107^T and *Aeromicrobium* species as determined by most authors (Park *et al.*, 1999; Urzì *et al.*, 2000; Bruns *et al.*, 2003; Yi & Chun, 2004; Yoon *et al.*, 2004). Fatty acid C_{16:0}, a minor component in *Nocardioide*s species, was a major component in strain KSL-107^T and *Aeromicrobium* species. The tuberculostearic acid (10-methyl C_{18:0}) content of strain KSL-107^T was greater than that of *Nocardioide*s species in most experiments (Park *et al.*, 1999; Bruns *et al.*, 2003; Yi & Chun, 2004; Yoon *et al.*, 2004). The DNA G + C content of strain KSL-107^T was 71.5 mol%.

Comparative 16S rRNA gene sequence analyses showed that strain KSL-107^T is most closely related to the genus *Aeromicrobium* of the family *Nocardioideaceae* (Fig. 1). Chemotaxonomic properties support the monothetic phylogenetic classification of strain KSL-107^T as a member of the genus *Aeromicrobium*. The phenotypic differentiation and phylogenetic and genetic distinctiveness are sufficient to categorize strain KSL-107^T as a species that is distinct from the recognized *Aeromicrobium* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain KSL-107^T should be placed in the genus *Aeromicrobium* as a member of a novel species, for which the name *Aeromicrobium alkaliterrae* sp. nov. is proposed.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain KSL-107^T and representatives of some related taxa. Bootstrap values (1000 replications) are shown as percentages at each node. Bar, 0.01 substitutions per nucleotide position.

Description of *Aeromicrobium alkaliterrae* sp. nov.

Aeromicrobium alkaliterrae (al.ka.li.ter'rae. N.L. n. *alkali* alkali; L. gen. n. *terrae* of the soil or earth; N.L. gen. n. *alkaliterrae* of alkaline soil).

Cells are rods ($0.3\text{--}0.5 \times 0.8\text{--}1.4\ \mu\text{m}$) or cocci. Gram-positive. Non-motile. Non-endospore-forming. Colonies are circular, convex, smooth, glistening, cream-coloured and $1.0\text{--}1.5\ \text{mm}$ in diameter after 7 days cultivation at 25°C on NA. Aerial mycelium is not formed. Growth occurs at 4°C , but not at 36°C . Optimal initial pH for growth is $7.0\text{--}7.5$; growth occurs at initial pH 6.0 and 11.0 , but not at initial pH 5.5 and 11.5 . Optimal growth occurs in the absence of NaCl; growth does not occur in the presence of $>9\%$ (w/v) NaCl. Biotin, thiamin and nicotinic acid are not required for growth. Growth does not occur under anaerobic conditions on TSA or on TSA supplemented with nitrate. Oxidase-negative. Tweens 20, 40, 60 and 80 are hydrolysed. Hypoxanthine, tyrosine and xanthine are not hydrolysed. H_2S and indole are not produced. Voges-Proskauer reaction is negative. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. D-Galactose, D-cellobiose, succinate and L-malate are utilized. D-Ribose, acetate, benzoate and formate are not utilized. The cell-wall peptidoglycan contains LL-DAP as the diagnostic diamino acid. The predominant menaquinone is MK-9(H_4). The major fatty acids are $\text{C}_{16:0}$, $\text{C}_{16:0}\ 2\text{-OH}$ and 10-methyl $\text{C}_{18:0}$. The DNA G+C content is $71.5\ \text{mol}\%$ (determined by HPLC). Other phenotypic properties are given in Table 1.

The type strain, KSL-107^T (= KCTC 19073^T = DSM 16824^T), was isolated from an alkaline soil in Kwangchun, Korea.

Emended description of the genus *Aeromicrobium* Miller et al. 1991

The emended description is based on the original description given by Miller et al. (1991), the data compiled by Collins & Stackebrandt (1989), Tamura & Yokota (1994) and Bruns et al. (2003) and those generated in this study. Cells are aerobic, non-endospore-forming, Gram-positive, cultures contain rods and cocci or rods. Non-motile or motile. Mesophilic; optimal temperature for growth is about $25\text{--}35^\circ\text{C}$. Heterotrophic; a wide range of carbon sources are used for growth and various organic compounds are hydrolysed. The cell-wall peptidoglycan contains LL-DAP as the diagnostic diamino acid. The predominant menaquinone is MK-9(H_4). The major fatty acids are 10-methyl $\text{C}_{18:0}$, $\text{C}_{16:0}$ and both or either of $\text{C}_{18:1}\omega 9\text{c}$ and $\text{C}_{16:0}\ 2\text{-OH}$. The DNA G+C content is $70.6\text{--}73\ \text{mol}\%$. Members of this genus form a coherent group within the radiation of the family *Nocardioidaceae* on the basis of 16S rRNA gene sequence data. Members of the genus occur in various habitats, including soils, herbage and sea water. The type species is *Aeromicrobium erythreum*.

Acknowledgements

This work was supported by the 21C Frontier program of Microbial Genomics and Applications (grant MG02-0401-001-1-0-0) from the Ministry of Science and Technology (MOST) of the Republic of Korea.

References

- Bruns, A., Philipp, H., Cypionka, H. & Brinkhoff, T. (2003). *Aeromicrobium marinum* sp. nov., an abundant pelagic bacterium isolated from the German Wadden Sea. *Int J Syst Evol Microbiol* **53**, 1917–1923.
- Collins, M. D. & Stackebrandt, E. (1989). Molecular taxonomic studies on some LL-diaminopimelic acid-containing coryneforms from herbage: description of *Nocardioides fastidiosa* sp. nov. *FEMS Microbiol Lett* **57**, 289–294.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Komagata, K. & Suzuki, K. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Lechevalier, M. P. & Lechevalier, H. A. (1970). A critical evaluation of the genera of aerobic actinomycetes. In *The Actinomycetales*, pp. 393–405. Edited by H. Prauser. Jena: Gustav Fischer Verlag.
- Miller, E. S., Woese, C. R. & Brenner, S. (1991). Description of the erythromycin-producing bacterium *Arthrobacter* sp. strain NRRL B-3381 as *Aeromicrobium erythreum* gen. nov., sp. nov. *Int J Syst Bacteriol* **41**, 363–368.
- Park, Y.-H., Yoon, J.-H., Shin, Y. K., Suzuki, K.-I., Kudo, T., Seino, A., Kim, H.-J., Lee, J.-S. & Lee, S. T. (1999). Classification of '*Nocardioides fulvus*' IFO 14399 and *Nocardioides* sp. ATCC 39419 in *Kribbella* gen. nov., as *Kribbella flavida* sp. nov. and *Kribbella sandramycini* sp. nov. *Int J Syst Bacteriol* **49**, 743–752.
- Sasser, M. (1990). *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*. Newark: MIDI Inc.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Tamura, T. & Yokota, A. (1994). Transfer of *Nocardioides fastidiosa* Collins & Stackebrandt 1989 to the genus *Aeromicrobium* as *Aeromicrobium fastidiosum* comb. nov. *Int J Syst Bacteriol* **44**, 608–611.
- Urzi, C., Salamone, P., Schumann, P. & Stackebrandt, E. (2000). *Marmoricola aurantiacus* gen. nov., sp. nov., a coccoid member of the family *Nocardioidaceae* isolated from a marble statue. *Int J Syst Evol Microbiol* **50**, 529–536.

- Wang, Y. M., Zhang, Z. S., Xu, X. L., Ruan, J. S. & Wang, Y. (2001).** *Actinopolymorpha singaporensis* gen. nov., sp. nov., a novel actinomycete from the tropical rainforest of Singapore. *Int J Syst Evol Microbiol* **51**, 467–473.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987).** International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Yi, H. & Chun, J. (2004).** *Nocardioides ganghwensis* sp. nov., isolated from tidal flat sediment. *Int J Syst Evol Microbiol* **54**, 1295–1299.
- Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996).** Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.
- Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998).** Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rDNA gene sequences. *Int J Syst Bacteriol* **48**, 187–194.
- Yoon, J.-H., Kim, I.-G., Kang, K. H., Oh, T.-K. & Park, Y.-H. (2004).** *Nocardioides aquiterrae* sp. nov., isolated from groundwater in Korea. *Int J Syst Evol Microbiol* **54**, 71–75.