Silanimonas lenta gen. nov., sp. nov., a slightly thermophilic and alkaliphilic gammaproteobacterium isolated from a hot spring

Eun Mi Lee,[1,2] Che Ok Jeon,[1] Inpyo Choi,[1] Kyu-Seob Chang[2] and Chang-Jin Kim[1]

[1]Korea Research Institute of Bioscience and Biotechnology, 52 Oeundong, Yusong, Daejeon 305-333, Republic of Korea

[2]Department of Food Science and Technology, Chungnam National University, 220 Gung-Dong, Yusong, Daejeon 305-764, Republic of Korea

Correspondence
Chang-Jin Kim
changjin@kribb.re.kr

A moderately thermophilic aerobic bacterium, strain 25-4^T, was isolated from a hot spring at Baekdoo Mountain in Korea. The cells were Gram-negative, motile rods each having a polar flagellum. Analysis of the 16S rRNA gene sequence indicated that the strain represented a new lineage within the family '*Xanthomonadaceae*' of the '*Gammaproteobacteria*', being most closely related to the genera *Thermomonas*, *Xanthomonas*, *Luteimonas*, *Pseudoxanthomonas*, *Stenotrophomonas* and *Xylella* and having 16S rRNA gene sequence similarities to the most related species of the genera of between 92·9 and 94·4 %. The strain contained Q-8 as the major isoprenoid quinone and had a fatty acid profile with predominant iso-branched fatty acids. Growth occurred at pH 6·0–10, with an optimum at pH 9·0, and at 25–53 °C, with an optimum at 47 °C. The G+C content of the genomic DNA was 50·7 mol%. On the basis of phylogenetic analyses and its phenotypic characteristics, strain 25-4^T belongs to a new genus, *Silanimonas* gen. nov., within the '*Gammaproteobacteria*'. The sole species of this genus is *Silanimonas lenta* sp. nov. (type strain, 25-4^T=DSM 16282^T=KCTC 12236^T).

Because moderately thermophilic bacteria are abundant in neutral hot springs, composts and even mesophilic environments and have great biotechnological potential for the production of thermoactive enzymes, a large number of these organisms have been isolated from various environments during the last few decades (Niehaus *et al.*, 1999). Most of these bacteria belong to many different taxonomic groups, but the majority of the species within the phylum '*Proteobacteria*' rarely grow at temperatures that exceed 45 °C (Alves *et al.*, 2003). However, recently some members of the genera *Thermomonas* and *Pseudoxanthomonas*, belonging to the '*Proteobacteria*' and showing slightly thermophilic properties, have been isolated from hot environments such as hot springs (Alves *et al.*, 2003; Busse *et al.*, 2002; Chen *et al.*, 2002; Mergaert *et al.*, 2003). They belong to the class '*Gammaproteobacteria*' and have genomic DNA G+C contents in the range 64·7–70·1 mol%. Strain 25-4^T, a moderately thermophilic bacterium within the '*Gammaproteobacteria*' and having a relatively low DNA G+C content (50·7 %), was isolated from a hot spring at Baekdoo Mountain in Korea. Here, we report the taxonomic characterization of this strain.

Strain 25-4^T was collected from a hot spring of Baekdoo Mountain in Korea and isolated on nutrient agar (NA) after 2 days incubation at 45 °C. The isolate was routinely cultured aerobically on NA for 2 days at 47 °C. Growth was tested at different temperatures (10–55 °C) and at different pH values (5·0–11·0). Media with different pH values were prepared using appropriate biological buffers: Na_2HPO_4/NaH_2PO_4 buffer, Na_2CO_3/$NaHCO_3$ buffer and Na_2HPO_4/NaOH buffer were used for pH values below 8·0, pH values of 8·0–10·0 and pH 11·0, respectively (Bates & Bower, 1956; Gomori, 1955). Strain 25-4^T formed pale-yellow, translucent, flat, irregular, sticky colonies and grew at pH values from 6·0 to 10·0, with an optimum at pH 9·0. Growth was observed at temperatures between 25 and 53 °C, but not at 55 °C (optimum temperature 47 °C). Strain 25-4^T grew in nutrient broth containing 3 % (w/v) NaCl, but not in that

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The polar lipid content (Fig. A) and the fatty acid composition (Table A) of strain 25-4^T are available as supplementary material in IJSEM Online.

containing 5 % (w/v) NaCl. Anaerobic growth was not observed after incubation in an anaerobic chamber for 5 days at 47 °C on NA. Strain 25-4^T was tested for its susceptibility to eight antimicrobial compounds (ampicillin, 10 μg; erythromycin, 30 μg; fusidic acid, 10 μg; gentamicin, 10 μg; kanamycin, 30 μg; lincomycin, 15 μg; neomycin, 30 μg; penicillin G, 10 IU; streptomycin, 10 μg) by using a method described previously (Alves *et al.*, 2003): the strain was sensitive to all the antibiotics tested.

The cellular morphology of strain 25-4^T was examined using light microscopy and transmission electron microscopy on cells grown on NA for 2 days at 37 and 47 °C. Each agar-coated wet mount used for motility observations was prepared by placing 10 μl culture under a cover-glass on a glass slide that had been previously coated with a film consisting of 0·5 % (w/v) agarose (Cambrex). For visualization of the flagella, cells were mounted on Formvar-coated copper grids (Electron Microscopy Science) and negatively stained with 2 % (w/v) uranyl acetate for 15 s, then subjected to transmission electron microscopy (JEM-1010; JEOL). Gram staining of strain 25-4^T was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Oxidase activity was tested using a Bactident Oxidase strip (Merck), whereas catalase activity was determined by bubble production in a 3 % (v/v) hydrogen peroxide solution. Hydrolysis of casein, L-tyrosine, starch, elastin, gelatin, aesculin and urea was determined as described by Lányi (1987). Acid production, by the isolate, from various carbohydrates was characterized using the API 50 CH kit (bioMérieux) according to the manufacturer's instructions. Additional enzyme activities were tested using the API ZYM microtube system (bioMérieux), as recommended by the manufacturer. For quantitative analysis of whole-cell fatty acids, strain 25-4^T was cultivated on NA for 2 days at 37 and 47 °C. Isoprenoid quinones and polar lipids from strain 25-4^T were analysed according to the methods of Komagata & Suzuki (1987). The genomic DNA G + C composition of the isolate was determined by reversed-phase HPLC using the method of Kaneko *et al.* (1986).

The 16S rRNA gene of strain 25-4^T was amplified and its sequence analysed as described previously (DeLong, 1992; Lane, 1991). The 16S rRNA gene sequence of the strain was aligned together with those of representative members of selected genera by using the CLUSTAL W program (Thompson *et al.*, 1994). Sequence-similarity values were computed using Similarity Matrix, version 1.1 (http://rdp8.cme.msu.edu/html/; Cole *et al.*, 2003). Gaps at the 5′ and 3′ ends of the alignment were omitted from further analyses. Phylogenetic trees were constructed using three different algorithms – neighbour-joining, maximum likelihood and maximum parsimony – available in PHYLIP software, version 3.6 (Felsenstein, 2002). Evolutionary distance matrices were calculated according to the algorithm of the Kimura two-parameter model for the neighbour-joining method. A bootstrap analysis (1000 replications) was performed to evaluate the stability of the phylogenetic tree with the neighbour-joining method in the PHYLIP package. The tree constructed by the neighbour-joining method showed that strain 25-4^T formed a phyletic line that was distinct from those of the closely related genera *Thermomonas*, *Luteimonas*, *Pseudoxanthomonas*, *Stenotrophomonas*, *Xylella* and *Xanthomonas* (Fig. 1). The topologies of phylogenetic trees built using the maximum-likelihood and maximum-parsimony algorithms were similar to that of the tree constructed by using neighbour-joining analysis (data not shown). Comparative 16S rRNA gene sequence analysis indicated that the strain is a member of the family '*Xanthomonadaceae*' and has a unique taxonomic position within the class '*Gammaproteobacteria*'. Strain 25-4^T was most closely related to *Thermomonas haemolytica* DSM 13605^T, but with only 94·4 % 16S rRNA gene sequence similarity, which is above the threshold level that is generally used to define a new genus (Ludwig *et al.*, 1998).

Busse *et al.* (2002) reported that the flagellation type of *T. haemolytica* could only be visualized by staining, not by electron microscopy, and motility appeared only at higher temperatures (37 °C and above). In contrast, a polar flagellum from strain 25-4^T was observed under transmission electron microscopy, but not all cells had a flagellum. The proportion of cells with motility and flagellation was about 10–20 %. All other closely related taxa (except *Rhodanobacter lindaniclasticus* LMG 18385^T) in the phylogenetic tree shown in Fig. 1 also show polar flagellation (Nalin *et al.*, 1999). The genomic DNA G + C content of the strain was 50·7 mol%, which is much lower than those of the closely related genera *Thermomonas*, *Pseudoxanthomonas*, *Stenotrophomonas* and *Xanthomonas* within the class '*Gammaproteobacteria*', but is similar to that of the genus *Xylella* (Table 1). Despite the fact that strain 25-4^T and *T. haemolytica* have similar physiological and chemotaxonomic properties, strain 25-4^T was easily distinguishable from *T. haemolytica* by the large difference (about 18 %) in the genomic DNA G + C content.

The cellular fatty acids were analysed after growth of the strain at 37 and 47 °C. The predominant cellular fatty acids were the same at 37 and 47 °C, but the proportion of long-chain fatty acids increased somewhat at 47 °C (Table A, available as supplementary material in IJSEM Online). On NA at 47 °C, the isolate contained iso-branched fatty acids such as $C_{15:0}$ iso (36·5 %), $C_{16:0}$ iso (35·2 %), $C_{17:0}$ iso (7·6 %), $C_{11:0}$ iso 3-OH (6·6 %), $C_{17:0}$ iso *cis*9 (7·6 %) and $C_{15:0}$ iso (2·3 %) as the major fatty acids, this being similar to the corresponding data for the closely related taxa (except *Xylella fastidiosa*) (Table 1). The predominant hydroxyl fatty acid of strain 25-4^T, *T. haemolytica* and *Luteimonas mephitis* was $C_{11:0}$ iso 3-OH. However, other related taxa, i.e. *Pseudoxanthomonas broegbernensis*, *Stenotrophomonas maltophilia*, *Xylella fastidiosa* and *Xanthomonas campestris*, contained different hydroxyl fatty acids such as $C_{16:0}$ 2-OH, $C_{13:0}$ iso 3-OH and $C_{10:0}$ 2-OH that were not even detected in strain 25-4^T (Table A, IJSEM Online). The strain

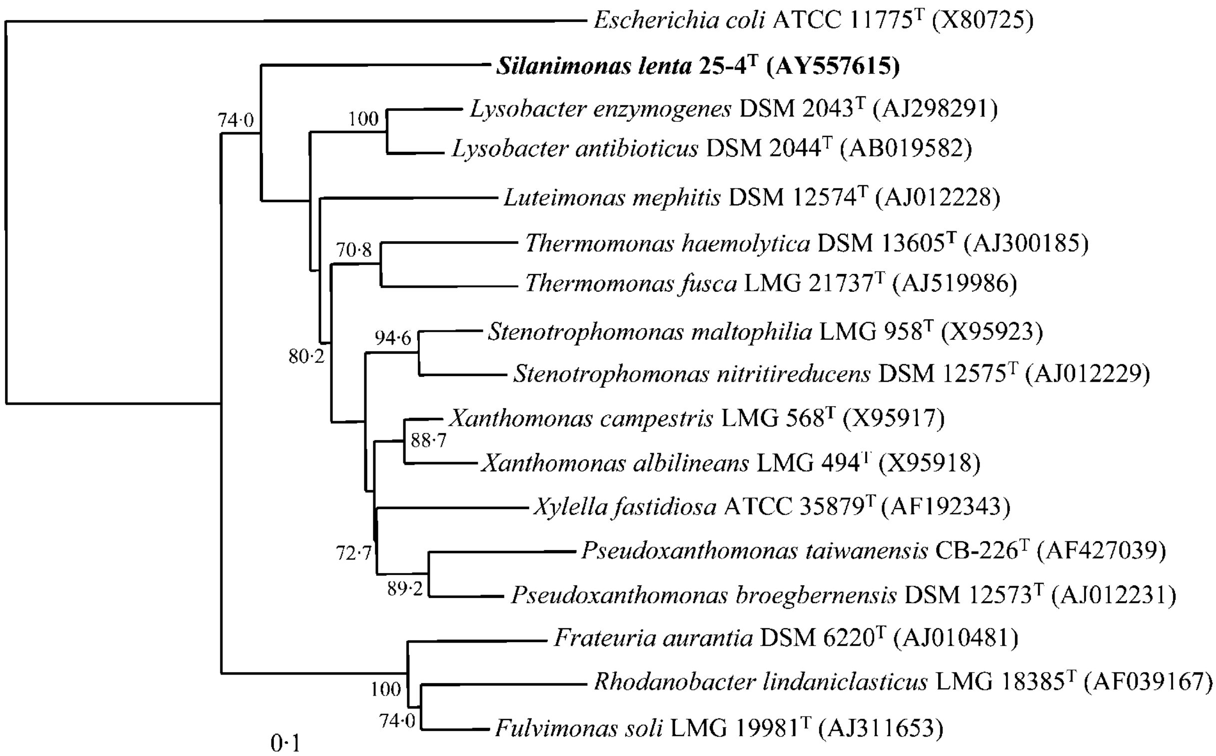

Fig. 1. Neighbour-joining tree showing the phylogenetic relationships between strain 25-4^T and related taxa. Bootstrap probabilities based on a neighbour-joining analysis of 1000 resampled datasets are indicated at the nodes; only those values greater than 50 % are indicated. The 16S rRNA gene sequence of *Escherichia coli* ATCC 11775^T was used as an outgroup. Bar, estimated number of base changes per nucleotide sequence position.

contained a large amount of an unknown phospholipid (PL1), a small amount of another unknown phospholipid (PL2) and one unidentified spot inferring the presence of glycolipids in addition to diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as the polar lipids (Fig. A, available as supplementary material in IJSEM Online). The presence of three unknown polar lipids was sufficient to distinguish the isolate from *T. haemolytica*. On the basis of its chemotaxonomic and phylogenetic properties, strain 25-4^T represents a new genus, *Silanimonas* gen. nov., and novel species, *Silanimonas lenta* sp. nov., within the family '*Xanthomonadaceae*' of the class '*Gammaproteobacteria*'.

Description of *Silanimonas* gen. nov.

Silanimonas (Si.lan.i.mo′nas. L. m. *silanus* a fountain; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Silanimonas* a monad isolated from a fountain).

Cells are strictly aerobic, Gram-negative, non-spore-forming rods. Oxidase- and catalase-positive. Nitrate is not reduced. Major isoprenoid quinone is Q-8. DNA G+C content is 50·7 mol% (HPLC). Predominant cellular fatty acids are iso-branched fatty acids such as $C_{15:0}$ iso, $C_{16:0}$ iso, $C_{17:0}$ iso and $C_{11:0}$ iso 3-OH. Phylogenetically, the genus belongs to the family '*Xanthomonadaceae*' within the class '*Gammaproteobacteria*'.

The type species is *Silanimonas lenta*.

Description of *Silanimonas lenta* sp. nov.

Silanimonas lenta (len′ta. L. fem. adj. *lenta* sticky).

Colonies are pale yellow, translucent, irregular and sticky on NA. Cells are 0·3–0·5 μm wide and 0·8–1·8 μm long. Some, but not all, cells have a polar flagellum. The pH range for growth is 6·0–10·0, with an optimum at pH 9·0. The temperature range for growth is 25–53 °C, with an optimum at 47 °C. Starch, casein, L-tyrosine, elastin and gelatin are hydrolysed, but hydrolysis of aesculin, arbutin and urea is not observed. Alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, acid phosphatase, trypsin, α-chymotrypsin and naphthol-AS-BI-phosphohydrolase are produced, but valine arylamidase, cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are not produced. Acids are produced from D-glucose, fructose, ribose, maltose, cellobiose, aesculin and mannose, but not from glycerol, D-trehalose, D-xylose, L-arabinose, rhamnose,

Table 1. Characteristics useful for differentiating strain 25-4^T from representatives of related genera

Strains: 1, *T. haemolytica*; 2, *Luteimonas mephitis*; 3, *P. broegbernensis*; 4, *Stenotrophomonas maltophilia*; 5, *Xylella fastidiosa*; 6, *Xanthomonas campestris*; 7, *Lysobacter enzymogenes*. Data for strain 25-4^T were generated in this study; all other data are taken from previous studies (Busse *et al.*, 2002; Chen *et al.*, 2002; Tóth *et al.*, 2001; Finkmann *et al.*, 2000; Vauterin *et al.*, 1995, 1996; Palleroni & Bradbury, 1993; Wells *et al.*, 1987; Palleroni, 1984; Christensen & Cook, 1978; Sullivan *et al.*, 2003). Abbreviations: v+, most of the strains are not susceptible; v−, most of the strains are susceptible; NA, not available; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PME, phosphatidylethanolamine; PL, unknown phospholipid.

Species	25-4^T	1	2	3	4	5	6	7
Temp. optimum (°C)	47	37–50	28	28	35	26–28	28	25–35
Polar flagellation	+	+	NA	+	+	+	+	−
Catalase	+	+	NA	+	+	+	+	+
Oxidase	+	+	NA	+	+	−	−	+
Nitrate reduction	−	−	−	−	+	−	−	−
Susceptibility to:								
Ampicillin (10 μg)	+	+	−	−	−	+	−	NA
Penicillin G (10 IU)	+	+	−	−	−	−	−	−
Erythromycin (30 μg)	+	+	−	−	−	NA	−	NA
Kanamycin (30 μg)	+	+	v−	−	+	+	+	NA
Neomycin (30 μg)	+	+	v+	+	−	NA	−	NA
Streptomycin (10 μg)	+	+	−	−	−	−	+	−
Major fatty acids	15 : 0 iso, 16 : 0 iso	15 : 0 iso, 16 : 0 iso,	15 : 0 iso, 17 : 1, 17 : 0 iso	15 : 0 iso, 15 : 0 anteiso, 16 : 0	15 : 0 iso, 15 : 0 anteiso	16 : 0, 16 : 1, 17 : 0	15 : 0 iso, 16 : 1, 17 : 1 iso	15 : 0 iso, 17 : 1 isoω9*c*
Major hydroxyl fatty acids	11 : 0 iso 3-OH	11 : 0 iso 3-OH	11 : 0 iso 3-OH	16 : 0 2-OH	13 : 0 iso 3-OH	10 : 0 2-OH	13 : 0 iso 3-OH	11 : 0 iso 3-OH
Major polar lipids	DPG, PE, PG, PL	DPG, PE, PG	NA	NA	DPG, PE, PG	NA	DPG, PE, PG, PME, PL	NA
DNA G+C content (mol%)	50·7	68·5	NA	70·1	62–66	51–53	65–66	69–70

lactose, adonitol, raffinose, mannitol, sucrose, arbutin, D-salicin, sorbitol, erythritol or galactose. Major isoprenoid quinone is Q-8. Predominant polar lipids are phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol and a large amount of an unknown phospholipid (PL1). The major cellular fatty acids on NA at 47 °C are $C_{15:0}$ iso (36·5 %), $C_{16:0}$ iso (35·2 %), $C_{17:0}$ iso (7·6 %), $C_{11:0}$ iso 3-OH (6·6 %), $C_{17:0}$ iso *cis*9 (7·6 %) and $C_{15:0}$ iso (2·3 %). DNA G+C content is 50·7 mol% (HPLC).

The type strain of the species is 25-4^T (=DSM 16282^T= KCTC 12236^T).

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