Pseudokineococcus basanitobsidens sp. nov., isolated from volcanic rock

Dong Wan Lee,1,2 Min Young Park,1 Jae-Jin Kim3 and Beom Seok Kim1,*

Abstract

A novel Gram-strain-positive, non-spore-forming bacterial strain, designated SKC1-2T, was isolated from volcanic rock of the scoria cone of Seobjikoji, Jeju, Republic of Korea. Cells were aerobic, catalase-positive, oxidase-negative, motile and cocci. Colonies of cells were dark orange-coloured, circular, smooth and convex. Phylogenetic analyses based on 16S rRNA gene sequences indicated that the isolate was related to members of the genus Pseudokineococcus. Phylogenetic neighbours were P. marinus KCCM 42250T (98.2 %, 16S rRNA gene sequence similarity) and P. lusitanus DSM 23768T (98.0 %). The diagnostic diamino acid in the cell-wall peptidoglycan was meso-diaminopimelic acid. The predominant respiratory quinone was MK-9 (H2). The predominant respiratory quinone was MK-9(H2) and the major fatty acid was anteiso-C15:0. The polar lipid profile included major amounts of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two unidentified phospholipids and two unidentified phosphoglycolipids. The DNA G+C content was 74.9 mol%. DNA–DNA relatedness values between strain SKC1-2T and P. lusitanus DSM 23768T or P. marinus KCCM 42250T were 73.5–76.8 % or 45.4–46.4 %, respectively. On the basis of the phenotypic differences and DNA–DNA relatedness data, the isolate represents a new species of the genus Pseudokineococcus, for which the name Pseudokineococcus basanitobsidens sp. nov. is proposed. The type strain is SKC1-2T (=DSM 103726T=KCCM 43221T).

The genus Pseudokineococcus was first proposed by Jurado et al. [1] with Pseudokineococcus lusitanus as the type species. In addition, the species Kineococcus marinus [2] was reclassified and transferred to the genus Pseudokineococcus (as Pseudokineococcus marinus) to accommodate an aero- bic, Gram-reaction-positive, motile, coccus bacterium. The genus Pseudokineococcus was characterized chemotaxonomically by the presence of meso-diaminopimelic acid in the cell wall, MK-9(H2) as the major menaquinone, diphosphatidylglycerol and phosphatidylglycerol in the polar lipid profile, and anteiso-C15:0 as a predominant cellular fatty acid. Mycolic acids are not present [1]. During a study of microbial diversity and bioactive compounds from a scoria cone, a novel Pseudokineococcus species isolated from volcanic rock by using a polyphasic taxonomic approach is described.

Strain SKC1-2T was isolated from volcanic rock of the scoria cone of Seobjikoji, Jeju, Republic of Korea. Volcanic rock samples (1.0 g) were ground into powder with a pestle and suspended in 10 ml distilled water. Ali- quots of serial dilutions were spread onto starch-casein agar (1.0 % soluble starch, 0.03 % casein, 0.2 % KNO3, 0.2 % NaCl, 0.2 % KH2PO4, 0.002 % CaCO3, 0.005 % MgSO4, 7H2O, 0.001 % FeSO4.7H2O and 1.8 % agar; pH 7.2) supplemented with cyclohexamide (0.5 mg l−1), and the plates were incubated at 28 °C for 2 weeks. A single colony was selected and further streaked on ISP 2 medium [3] at least three times. For phenotypic and genotypic comparisons, P. lusitanus DSM 23768T and P. marinus KCCM 42250T were grown on ISP 2 medium for 3 days at 28 °C.

Growth at different temperatures 4 to 42 °C was assessed on yeast extract–malt extract agar (YE agar) and at initial pH 4.0–12.0 (interval of 1.0 unit). The pH of the medium was adjusted before sterilization using 10 mM MES (pH 4.0–6.0) or 10 mM Trizma (pH 7.0–12.0) as biological buffers and confirmed with pH paper after sterilization. Tolerance of

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Keywords: Actinobacteria; volcanic rock; polyphasic taxonomic characterization; DNA–DNA hybridization; Pseudokineococcus.

Abbreviations: Ara, arabinoside; DPG, diphosphatidylglycerol; DSM, Deutsche Sammlung von Mikroorganismen; Gal, galactose; GL, unidentified glycolipid; Glu, glucose; KCCM, Korean Culture Center of Microorganisms; PG, phosphatidylglycerol; PGL, unidentified phosphoglycolipid; PI, phosphatidylinositol; PL, unidentified phospholipid; Rha, rhamnose; Rib, ribose; TSA, trypticase soy agar; YE agar, yeast extract–malt extract agar; YMG broth, yeast extract–malt extract–glucose broth.

The GenBank accession number for the 16S rRNA gene sequence of strain SKC1-2T is KY244028.

One supplementary table and one supplementary figure are available with the online Supplementary Material.
for growth was examined on YE agar supplemented with 0–9 % (w/v) NaCl (at intervals of 1 %). The results were observed and recorded after incubation for 5 days at 28 °C. The cell morphology and colony properties were observed with cultures grown on YE agar for 3 days at 28 °C. Cell morphology and motility were observed using phase-contrast and transmission electron microscopy. To check for the presence of flagella, cells were negatively stained with 1 % (w/v) phosphotungstic acid and observed with a JEM-1010 transmission electron microscope (JEOL). Gram stain, oxidase and catalase activities were determined by using previously described methods [4]. Degradation tests were performed on YE agar supplemented with 0.5 % (w/v) casein, 0.5 % (w/v) CM-Cellulose, 0.4 % (w/v) hypoxanthine, 0.5 % (w/v) dl-tyrosine and 0.4 % (w/v) xanthine. Hydrolysis of starch and DNA was determined by using starch agar (Difco) and DNase test agar (Difco), respectively. The ability to utilize 95 individual substrates as sole carbon sources was tested using GP2 MicroPlates (Biolog) according to the manufacturer’s instructions. Cells grown on YE agar at 28 °C for 3 days were suspended in GN/GP inoculating fluid, inoculated into the microplate and incubated for 48 h at 28 °C. Other physiological and biochemical characteristics were examined using API ZYM and API 20NE systems (bioMérieux) according to the instructions of the manufacturer. Cells of strain SKC1-2T were aerobic, Gram-reaction-positive, non-spore-forming, motile cocci (1.3–1.5 µm in diameter) (Fig. 1). The results of cultural, physiological and biochemical tests are given in the species description and in Table 1.

Genomic DNA extraction, PCR-mediated amplification and sequencing of the 16S rRNA gene were performed as described by Lee and Lee [4]. The 16S rRNA gene was amplified by PCR using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGYYTACCTGTAGCAGTT-3') [5]. An almost-complete 16S rRNA gene sequence (1434 bp) of strain SKC1-2T was determined in this study. A preliminary BLAST search showed that the isolate was related to members of the genus Pseudokineococcus. The CLUSTAL_X program [6] was used to perform multiple alignments of the sequences. Phylogenetic analyses were performed using neighbour-joining [7], maximum-likelihood [8] and maximum-parsimony [9] methods. A phylogenetic tree was drawn using the neighbour-joining method and the coefficient of Jukes and Cantor [10]. The reliability of the tree topology was evaluated by using bootstrap analysis [11] of 1000 resampled datasets. In the neighbour-joining tree (Fig. 2) based on 16S rRNA gene sequences, strain SKC1-2T formed a distinct clade with Pseudokineococcus species, with a high bootstrap value of 100 %. This branching pattern was also found in maximum-parsimony and maximum-likelihood treeing algorithms. The phylogenetic neighbours were P. marinus KCCM 42250T (98.2 %, 16S rRNA gene sequence similarity) and P. lusitanus DSM 23768T (98.0 %).

Chemical characteristics of strain SKC1-2T were determined using freeze-dried biomass obtained from the cells grown in YMG (0.4 % yeast extract, 1.0 % malt extract and 0.4 % glucose, pH 7.2) broth for 3 days at 28 °C. Amino acids in the cell-wall peptidoglycan were analysed with the cell walls prepared by the method of Komagata and Suzuki [12]. The isomer of diaminopimelic acid and acyl type of the cell wall and sugar composition of whole-cell hydrolysates were analysed according to the methods of Stanek and Roberts [13]. Isoprenoid quinones were determined by high-performance liquid chromatography [14]. The analysis of polar lipids by thin-layer chromatography was performed as described previously [15]. The G+C content of the DNA was determined by the method of Mesbah et al. [16].

Strain SKC1-2T contained meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The major menaquinone profile contained MK-9(H2). The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, two unidentified phospholipids and two unidentified phosphoglycolipids (Fig. S1, available with the online Supplementary Material). No mycolic acids were detected. For total cellular fatty acid analysis, cells of strain SKC1-2T, P. lusitanus DSM 23768T and P. marinus KCCM 42250T were grown on trypticase

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**Fig. 1.** Transmission electron micrograph of strain SKC1-2T grown on YE agar for 3 days at 28 °C. Bar, 0.5 µm.
Table 1. Chemotaxonomic and phenotypic properties of strain SKC1-2\textsuperscript{T} and from the type strain of phylogenetically close Pseudokineococcus species

Strains: 1, Strain SKC1-2\textsuperscript{T}; 2, P. lusitanus DSM 23768\textsuperscript{T}; 3, P. marinus KCCM 42250\textsuperscript{T}. Carbon source utilization, temperature and tolerance of NaCl for growth for all strains were obtained from this study under same experimental conditions. Other data for reference strains were taken from Jurado et al. [1] and Lee [2]. All of the strains were positive for catalase, aesculin degradation and gelatinase, but negative for oxidase, nitrate reduction and urease. All of the strains utilized dextrin, turanose, xyitol and L-alanine. Negative reactions for all strains were not described (GP2 MicroPlates, Biolog). Ara, arabinose; Gal, galactose; Glu, glucose; Rha, rhamnose; Rib, ribose; DPG, diphasphatidyglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unidentified phospholipid; PGL, unidentified phosphoglycolipid; GL, unidentified glycolipid; +, Positive; −, Negative.

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<td>DNA G+C content (mol%)</td>
<td>74.9</td>
<td>76.9*</td>
<td>76.6†</td>
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<td>Isolation source</td>
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<td>Roof tile*</td>
<td>Marine sediment†</td>
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†Data from Jurado et al. [1].
| Data from Lee [2].

soy agar (TSA; Difco) for 3 days at 28 °C. Fatty acid methyl esters were extracted and analysed using the Microbial Identification System (version 6; MIDI) according to the manufacturer’s instructions. The fatty acids profile of strain SKC1-2\textsuperscript{T} was represented by the predominance of anteiso-C\textsubscript{15:0} (57.6 %); other cellular fatty acids detected as minor components were anteiso-C\textsubscript{17:0} (9.5 %), anteiso-C\textsubscript{17:1ω9c} (5.1 %), mixture of anteiso-C\textsubscript{17:1} B and/or iso-C\textsubscript{17:1} I (4.2 %), iso-C\textsubscript{14:0} (2.8 %), C\textsubscript{14:1ω5c} (2.4 %), iso-C\textsubscript{15:0} (1.8 %), iso-C\textsubscript{16:0ω7c} and/or C\textsubscript{16:1ω6c} (1.8 %), C\textsubscript{14:0} 2-ΟΗ (1.7 %), C\textsubscript{16:0} (1.5 %), C\textsubscript{17:1ω8c} (1.3 %) and C\textsubscript{12:0} aldehyde (1.1 %). The DNA G+C content of strain SKC1-2\textsuperscript{T} was 74.9 mol%.

DNA–DNA relatedness studies provide a reliable way of distinguishing between representatives of species that share high 16S rRNA gene sequence similarity [17]. In this study, DNA–DNA hybridization values of strain SKC1-2\textsuperscript{T} and the closest phylogenetic relatives based on 16S rRNA sequence were determined using by photobiotin-labelled DNA probes and microwell plates, as described previously [18] with modifications [19]. strain SKC1-2\textsuperscript{T} showed DNA–DNA relatedness values of 37.5–38.1 and 45.4–46.4 % with P. lusitanus DSM 23768\textsuperscript{T} and P. marinus KCCM 42250\textsuperscript{T}, respectively. These values were well below the 70 % cut-off point recommend by Wayne et al. [20] for assigning strains to the same species.

The phylogenetic analyses, morphological and chemotaxonomic characteristics including meso-diaminopimelamic acid, predominant fatty acid composition and major respiratory quinone support the characterization of strain SKC1-2\textsuperscript{T} as a member of the genus Pseudokineococcus. However, it differed from the related strains in several ways, as listed in Table 1. Also, the amount of anteiso-C\textsubscript{17:0} and anteiso-C\textsubscript{17:1ω9c} in the cellular fatty acid profiles of strain SKC1-2\textsuperscript{T} were significantly higher than other species (Table S1).

On the basis of the differences of phenotypic characteristics, phylogenetic evidence and low DNA–DNA relatedness values, strain SKC1-2\textsuperscript{T} represents a new species of the genus Pseudokineococcus, for which the name Pseudokineococcus basanitobsidens sp. nov. is proposed.

**DESCRIPTION OF PSEUDOKINEOCoccus BASANITOBSIDENS SP. NOV.**

Pseudokineococcus basanitobsidens (ba.sa.nit. ob’si.dens. N.L. neut. n. basanitum basanite, volcanic rock; L. pres. part. obsi-dens, staying, remaining, occupying; N.L. part. adj, basanitobsidens, rock-occupying).

Cells are aerobic, Gram-stain-positive, catalase-positive, oxidase-negative, non-sphere-forming, motile and cocci (1.3–1.5 μm in diameter) singly, in pairs or in clusters. Colonies are dark orange, circular, smooth and convex after incubation of 5 days on YE agar. Growth occurs at 4–37 °C (optimum at 28 °C), pH 5.0–11.0 (optimum at pH 7.0–8.0) and 0–7 % (w/v) NaCl (optimum at 0–2 %). Aesculin, casein, gelatin and starch are hydrolysed but CM-cellulose, DNA, hypoxanthine, DL-tyrosine and xanthine are not. H\textsubscript{2}S is not produced. Negative for nitrogen reduction. Tests for Esterase (C4), esterase lipase (C8), α-glucosidase, β-gluco-side, N-acetyl-β-glucosaminidase and α-mannosidase are positive but activities of alkaline phosphatase, lipase (C14), leucine arylamidase, valine arylamidase, cystine

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arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase and α-fucosidase are negative (API ZYM). Dextrin, Tween 40, arbutin, D-gluconic acid, maltotriose, D-mannitol, methyl β-D-glucoside, palatinose, D-psicose, raffinose, L-rhamnose, salicin, turanose, xylitol, acetic acid, α-hydroxy butyric acid, D-lactic acid, methyl ester, methyl pyruvate, propionic acid, D-alanine, L-alanine and adenosine are utilized as sole carbon and energy sources but α-cyclodextrin, β-cyclodextrin, glycosen, inulin, mannan, Tween 80, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, amygdalin, L-arabinose, D-arabitol, cellobiose, D-fructose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, α-D-glucose, m-inositol, lactose, lactulose, maltose, D-mannose, melezitose, melibiose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl-D-glucoside, methyl α-D-glucoside, methyl α-D-mannoside, D-ribose, sedoheptulosan, D-sorbitol, stachyose, sucrose, D-tagatose, trehalose, D-xylene, β-hydroxybutyric acid, γ-hydroxy butyric acid, α-hydroxy butyric acid, p-hydroxy phenylacetic acid, α-ketoglutaric

Fig. 2. Neighbour-joining tree showing the position of strain SKC1-2T within the radiation of the genus Pseudokineococcus, based on 1232 bp aligned positions present in the 16S rRNA gene sequences of all strains. Nocardia asteroides was used as an outgroup taxon (not shown). Asterisks represent the corresponding branches supported by both maximum-likelihood [8] and maximum-parsimony [9] methods. Bootstrap support values greater than 50% are shown on the nodes. Bar, 0.01 substitutions per nucleotide position.

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acid, α-ketovaleric acid, lactamide, L-lactic acid, 3-malic acid, L-malic acid, monomethyl succinate, pyruvic acid, succinamic acid, succinic acid, N-acetyl-L-glutamic acid, L-alaninamide, L-alanylglycine, L-asparagine, L-glutamic acid, glycyrl-1-glutamic acid, L-progloglutamic acid, L-serine, putrescine, 2,3-butanediol, glycerol, 2-deoxyadenosine, inosine, thymidine, uridine, adenosine-5'-monophosphate, thymidine-5'-monophosphate, uridine-5'-monophosphate, D-fructose-6-phosphate, α-D-glucose-1-phosphate, D-glucose-6-phosphate and D-L-α-glycerol phosphate are not (GP2 MicroPlates, Biolog). The diagnostic diamino acid of alaninamide, L-alanine, L-aspartic acid, succinamic acid, succinic acid, glycylputrescine, 2,3-butanediol, glycerol, 2-3-monophosphate, uridine-5'-monophosphate, uridine-5'-monophosphate, uridine-5'-monophosphate, uridine-5'-monophosphate, D-fructose-6-phosphate, α-D-glucose-1-phosphate, D-glucose-6-phosphate and D-L-α-glycerol phosphate are not (GP2 MicroPlates, Biolog). The diagnostic diamino acid of the peptidoglycan is meso-diaminopimelic acid. Whole-cell hydrolysates contain glucose, rhamnose and ribose as characteristic sugars. Mycolic acids are not detected. The predominant menaquinone is MK-9(H2). The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two unidentified phospholipids and two unidentified phosphoglycolipids. The major fatty acid is anteiso-C15:0. The G+C content of the DNA is 74.9 mol%.

The type strain SKC1-2T (=DSM 103726T=KCCM 43221T), was isolated from volcanic rock of the scoria cone of Seobji-koji, Jeju, Republic of Korea.

Funding information
This work was supported by a grant of National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT, and Future Planning (Grant No. NRF-2014R1A2A2A01005461) and Fisheries of Korea and the BK21 Plus program in 2013 (Project No. 21A20130012270).

Conflicts of interest
The authors declare that there are no conflicts of interest.

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