Patulibacter ginsengiterrae sp. nov., isolated from soil of a ginseng field, and an emended description of the genus *Patulibacter*

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A novel actinobacterial strain, designated P4-5^T, was isolated from soil of a ginseng field located in Geumsan County, Republic of Korea. Cells of strain P4-5^T were Gram-stain-positive, oxidase- and catalase-positive, motile, short rods and the strain produced creamy white colonies on trypticase soy agar. The isolate contained demethylmenaquinone 7 (DMK-7) as the predominant isoprenoid quinone, C_{18:1}ω₉c and anteiso-C_{15:0} as major fatty acids, diphosphatidylglycerol, phosphatidylglycerol and several unknown lipids in the polar lipid profile, galactose, glucose, mannose, arabinose, xylose (trace) and rhamnose as cell-wall sugars, and *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The DNA G + C content of strain P4-5^T was 74.6 mol%. Phylogenetic analysis based on 16S rRNA gene sequencing showed that strain P4-5^T was related most closely to *Patulibacter minatonensis* KV-614^T and *Patulibacter americanus* CP177-2^T (98.4 and 98.2 % similarity, respectively) and that it formed a separate lineage in the genus *Patulibacter*. Combined phenotypic and DNA–DNA hybridization data supported the conclusion that strain P4-5^T represents a novel species of the genus *Patulibacter*, for which the name *Patulibacter ginsengiterrae* sp. nov. is proposed. The type strain is P4-5^T (=KCTC 19427^T =CECT 7603^T). An emended description of the genus *Patulibacter* is also provided.

The genus *Patulibacter* was created by Takahashi *et al.* (2006) based on the description of a single species, *Patulibacter minatonensis*, for a novel actinobacterium belonging to the order *Rubrobacterales*, subclass *Rubrobacteridae*. The description of the genus was emended by Reddy & Garcia-Pichel (2009) with the description of a second species, *Patulibacter americanus*, that differed mainly with respect to its respiratory quinone. At the time of writing, the genus *Patulibacter* comprises only the two species mentioned above, members of which are Gram-stain-positive, aerobic, non-endospore-forming, rod-shaped soil bacteria that have demethylmenaquinone 7 (DMK-7) or MK-7(H₂) as the predominant isoprenoid quinone, C_{18:1}ω₉c as the major fatty acid, *meso*-diaminopimelic acid (*meso*-DAP) as the diagnostic diamino acid in the cell-wall peptidoglycan and a DNA G + C content above 70 mol%.

Strain P4-5^T was recovered during the course of an investigation of the culturable aerobic bacterial community in soil from a ginseng field. On the basis of preliminary

16S rRNA gene sequence comparisons, strain P4-5^T was found to belong to the genus *Patulibacter* (>98 % sequence similarity). However, its cell and colony morphology were distinct from those of related taxa, which led to a polyphasic investigation designed to elucidate its taxonomic position.

A soil sample was collected from a ginseng field located in Geumsan County, Republic of Korea, and diluted serially in sterile distilled water, and samples of each serial dilution were spread on one-tenth-strength nutrient agar (Difco) and incubated at 25 °C for 4 weeks. A pinpoint colony, designated P4-5^T, was isolated, purified by subculturing on the same medium and maintained as a glycerol/water suspension (20 %, v/v) at –70 °C. Because of delayed growth on the above medium, the isolate was cultivated on various complex media. Consequently, it was grown routinely on trypticase soy agar (TSA; Difco). For most experiments, strain P4-5^T was cultivated on TSA or in trypticase soy broth (TSB; Difco) at 25 °C for 3 days, unless stated otherwise. For comparative purposes, *Patulibacter americanus* KCTC 19770^T and *Patulibacter minatonensis* KCTC 19436^T were grown under identical conditions.

Gram staining was performed by using Hucker's modification of the standard technique (Lányi, 1987). Cell morphology and motility were observed under a phase-

Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain P4-5^T is EU710748.

Two supplementary figures are available with the online version of this paper.

contrast microscope (Eclipse 80i; Nikon). Motility was tested with the hanging-drop technique (Skerman, 1967). For the observation of flagella, cells were grown on TSA at 25 °C for 48 h, negatively stained with 1 % (w/v) uranyl acetate and examined with a model CM-20 transmission electron microscope (Philips). Oxidase activity was tested by using oxidase reagent (bioMérieux) and catalase activity was determined by production of bubbles after the addition of a drop of 3 % H₂O₂. Growth was investigated at 5, 10, 15, 20, 25, 30, 37, 42 and 45 °C, in the presence of 1, 2, 3, 5, 6.5 and 10 % NaCl and at pH 5–10 (in increments of 0.5 pH units). The following biological buffers were used to adjust pH: Na₂HPO₄/NaH₂PO₄ below pH 9 and Na₂CO₃/NaHCO₃ for pH 9–10 (Gomori, 1955). Hydrolysis of DNA was tested on DNase test agar (BBL). Hydrolysis of casein and starch (Atlas, 1993), cellulose (Teather & Wood, 1982) and Tweens 20 and 80 (Sierra, 1957) was also investigated. The production of lecithinase was detected on egg yolk agar (McClung & Toabe, 1947). H₂S production was tested in TSB supplemented with 0.01 % (w/v) cysteine, with a strip of lead acetate paper (Fluka) as an indicator. The methyl red and Voges–Proskauer tests were performed in MR-VP broth (BBL). Single carbon source assimilation tests were performed as described by Kim *et al.* (2010). Acid production tests, enzyme activity tests and additional phenotypic tests were performed by using API 50CH (with CHB/E medium), API ZYM, API 20E and API 20NE galleries according to the manufacturer's instructions (bioMérieux). Susceptibility to antibiotics was tested by the diffusion agar method (Bauer *et al.*, 1966), with Sensi-Discs (6 mm; BBL) containing the following: amikacin (30 µg), ampicillin (10 µg), aztreonam (30 µg), bacitracin (10 U), carbenicillin (100 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), colistin (10 µg), doxycycline (30 µg), erythromycin (15 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), norfloxacin (10 µg), novobiocin (30 µg), oxacillin (1 µg), polymyxin B (300 U), rifampicin (5 µg), streptomycin (10 µg), tetracycline (30 µg), tobramycin (10 µg) and vancomycin (30 µg).

For analysis of fatty acids, cells of strain P4-5^T and reference strains were grown on TSA at 25 °C for 3 days. Fatty acid methyl esters were prepared according to the classical protocol of the Microbial Identification System (MIS; Microbial ID Inc.) and identified by using MIDI (Sasser, 1990) via the TSBA database version 4.02. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) and purified by using TLC on Kiesegel 60 F₂₅₄ plates (20 × 20 cm, 0.25 mm thickness; Merck) with petroleum ether/diethyl ether (9:1, v/v) as the solvent. Quinones were identified by using reversed-phase HPLC as described by Shin *et al.* (1996). Purified cell-wall preparations were obtained as described by Schleifer & Kandler (1972). Amino acids in cell-wall hydrolysates were analysed by two-dimensional TLC on cellulose plates by using the solvent systems of Harper & Davis (1979). Cell-wall sugars were analysed according to the procedures of

Staneck & Roberts (1974). The presence of mycolic acids was determined by TLC (Minnikin *et al.*, 1975). The murein acyl type was determined by using the colorimetric method of Uchida *et al.* (1999). Polar lipids were extracted, examined by two-dimensional TLC and identified by using published procedures (Minnikin *et al.*, 1977).

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Lee *et al.* (2011). The 16S rRNA gene sequence was compiled by using SeqMan software (DNASTAR) and aligned with published sequences retrieved from GenBank/EMBL/DDBJ via CLUSTAL X (Thompson *et al.*, 1997); the resulting multiple alignment was edited manually by using the program BioEdit (Hall, 1999) according to the 16S rRNA gene secondary structure obtained from the Ribosomal Database Project II (RDP; release 10). The neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) algorithms were used in phylogenetic tree-building. Phylogenetic dendrograms were constructed by using the DNADIST, NEIGHBOR, DNAPARS and DNAML programs in the PHYLIP package (Felsenstein, 2009). The distance matrix was produced on the basis of the Jukes–Cantor model (Jukes & Cantor, 1969). Bootstrap analysis (Felsenstein, 1985) was done with 1000 replicates. For the measurement of G + C content, genomic DNA was extracted and purified by using a Blood & Cell Culture DNA Midi kit (Qiagen). The DNA was degraded enzymically into nucleosides and the G + C content was determined by using reversed-phase HPLC, as described by Tamaoka & Komagata (1984). DNA–DNA hybridization was carried out with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989), by using a Fluoroskan Ascent Fluorescent plate reader (Thermo Life Science). The hybridization temperature was 54 °C. Reciprocal experiments were performed with five replications per sample and results were expressed as means ± SD.

Strain P4-5^T formed visible colonies (about 1–2 mm in diameter) within 3 days on TSA incubated at 25 °C. Good growth occurred at 20–30 °C. Colonies were creamy white, raised, semi-translucent, smooth and circular with undulate margins. Cells were Gram-stain-positive, oxidase- and catalase-positive, motile rods with a single polar flagellum (Supplementary Fig. S1, available in IJSEM Online). Other physiological and biochemical characteristics are summarized in the species description.

The almost-complete 16S rRNA gene sequence (1455 nt) of strain P4-5^T was determined and compared with those of the type strains of all known species of the order *Rubrobacterales*. Strain P4-5^T showed highest 16S rRNA gene sequence similarity to *P. minatonensis* KV-614^T and *P. americanus* CP177-2^T (98.4 and 98.2 %, respectively) and showed a similarity of 93.3 % or less to other representative members of the order *Rubrobacterales*. The phylogenetic tree showed that strain P4-5^T formed a robust cluster with the two recognized *Patulibacter* species, this being supported by

three tree-making algorithms (neighbour-joining, maximum-parsimony and maximum-likelihood) and a bootstrap value of 100 % (Fig. 1), but occupied a distinct position in the genus *Patulibacter*. DNA–DNA hybridization experiments revealed low levels of DNA–DNA relatedness between strain P4-5^T and *P. minatonensis* KCTC 19436^T and *P. americanus* KCTC 19770^T (30 ± 2 and 26 ± 3 %, respectively), which confirmed that the novel strain deserves separate species status (Wayne *et al.*, 1987).

The physiological and biochemical characteristics that serve to differentiate strain P4-5^T from closely related taxa are listed in Table 1. Strain P4-5^T could be clearly distinguished from the type strains of *P. americanus* and *P. minatonensis* based on differences in colony morphology, flagellar arrangement, oxidase activity, growth at 5 and 37 °C, growth with 3 % NaCl, and arginine dihydrolase, urease and lecithinase activities as well as by differences in antibiotic susceptibility, carbon source assimilation and enzyme activity (from API ZYM tests) profiles. In particular, strain P4-5^T showed oxidase activity, differing from the generic characteristics listed for *Patulibacter* (Reddy & Garcia-Pichel, 2009). Data for the type strain of *P. americanus* determined here were not congruent with those reported by Reddy & Garcia-Pichel (2009) in terms of growth at 37 °C, with 3 % NaCl and on D-fructose, and data for the type strain of *P. minatonensis* were not congruent with those reported by Takahashi *et al.* (2006) in terms of oxidase and lipase (C14) activity.

Strain P4-5^T contained DMK-7 as the only respiratory quinone. HPLC of the quinone extract of strain P4-5^T revealed a single peak, which had the same retention time as the peak detected in the quinone extract of *P. minatonensis* KCTC 19436^T. The polar lipid profile of strain P4-5^T consisted of phosphatidylglycerol, an unknown phospho-

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences, reconstructed with the maximum-likelihood method, showing the position of strain P4-5^T among related taxa within the order *Rubrobacterales*. Numbers at nodes are percentages of bootstrap support (>70 %) based on 1000 resamplings. Filled circles indicate nodes that are also found with the neighbour-joining and maximum-parsimony algorithms, and open circles indicate nodes also found with the maximum-parsimony algorithm. The sequence of *Bacillus subtilis* DSM 10^T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Table 1. Physiological and biochemical characteristics that differentiate strain P4-5^T from type strains of the genus *Patulibacter*

Strains: 1, P4-5^T; 2, *P. americanus* KCTC 19770^T; 3, *P. minatonensis* KCTC 19436^T. (+), Weakly positive. Data were obtained in this study unless indicated. All strains were positive for Gram staining, catalase and hydrolysis of Tweens 20 and 80. All were negative for indole and H₂S production, methyl red and Voges–Proskauer reactions, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase and hydrolysis of aesculin, DNA, ONPG, gelatin and starch.

Characteristic	1	2	3
Colony morphology	Raised	Convex	Flat
Colony colour*	CW	PK	PY
Flagellar arrangement†	P	None	Pe‡
Oxidase	+	–	(+)
Growth at/with:			
5 °C	+	–	–
37 °C	(+)	+	–
3 % NaCl	+	(+)	–
Arginine dihydrolase	+	–	–
Urease	+	–	–
Lecithinase on egg yolk agar	+	–	–
Growth on:			
L-Alanine	+	–	–
Citrate	+	–	–
D-Fructose	–	(+)	+
Glycogen	+	–	–
Trehalose	+	–	–
Susceptibility to:			
Bacitracin	–	+	+
Chloramphenicol	–	+	+
Tetracycline	–	+	+
Enzyme activity§			
Alkaline phosphatase	–	+	+
Lipase (C14)	+	–	–
Cystine arylamidase	+	–	–

*CW, Creamy white; PK, pink; PY, pale yellow.

†P, Polar; Pe, peritrichous.

‡Data from Takahashi *et al.* (2006) and Reddy & Garcia-Pichel (2009).

§Data from API ZYM tests.

glycolipid (PGL) and three unknown phospholipids (PL1, PL2 and PL3) as major components, plus moderate amounts of diphosphatidylglycerol and two unknown phospholipids (PL4 and PL5), similar to the profile of *P. americanus* KCTC 19770^T. However, the latter strain contained diphosphatidylglycerol as a major component, had additional components, namely two unknown phospholipids (PL6 and PL7), an unknown aminolipid (AL1) and two unknown lipids (UL1 and UL2), and lacked PL4 and PL5 (Supplementary Fig. S2). In contrast to the data reported by Reddy & Garcia-Pichel (2009) for *P. americanus*, phosphatidylcholine was not detected in this study. The polar lipid profile of *P. minatonensis* KCTC 19436^T was distinct, containing five

unknown glycolipids (GL1, GL2, GL3, GL4 and GL5), two unknown phospholipids (PL8 and PL9), an unknown aminolipid (AL2) and an unknown lipid (UL3) that were not present in the profiles of the other two strains and lacked PGL (Supplementary Fig. S2). The fatty acid profile of strain P4-5^T was similar to those of closely related taxa, i.e. almost all components were common in all taxa and C_{18:1}ω9*c* was the predominant component, although some minor quantitative differences in fatty acid content could be observed. The cell-wall sugar pattern of strain P4-5^T was also similar to those of closely related taxa, i.e. galactose, glucose, mannose, rhamnose and xylose were common in all taxa, although xylose was detected as a trace component in strain P4-5^T. The only difference was that strain P4-5^T had an additional sugar component, arabinose. In addition, strain P4-5^T contained *meso*-DAP as the diagnostic diamino acid in the cell-wall peptidoglycan and lacked mycolic acids, consistent with data reported for the genus. Detailed chemotaxonomic characteristics of strain P4-5^T and closely related taxa are listed in Table 2.

On the basis of the genotypic and phenotypic data presented herein, strain P4-5^T merits recognition as a representative of

a novel species within the genus *Patulibacter*, for which the name *Patulibacter ginsengiterrae* sp. nov. is proposed.

Emended description of the genus *Patulibacter* Takahashi *et al.* 2006 emend. Reddy and Garcia-Pichel 2009

The description is as given by Takahashi *et al.* (2006) and emended by Reddy & Garcia-Pichel (2009) with the following further amendments. Cells are oxidase-variable. The fatty acid profile is dominated by C_{18:1}ω9*c*. The DNA G + C content is in the range 72–75 mol%.

Description of *Patulibacter ginsengiterrae* sp. nov.

Patulibacter ginsengiterrae (gin.sen.gi.ter'rae. N.L. n. *ginsengum* ginseng; L. n. *terra* soil; N.L. gen. n. *ginsengiterrae* of soil from a ginseng field).

Cells are aerobic, Gram-stain-positive, non-spore-forming, short rods (0.4–0.6 × 0.8–1.0 μm) that are motile by means

Table 2. Chemotaxonomic characteristics of strain P4-5^T and type strains of the genus *Patulibacter*

Strains: 1, P4-5^T; 2, *P. americanus* KCTC 19770^T; 3, *P. minatonensis* KCTC 19436^T. Data were obtained in this study unless indicated. All three strains contained *meso*-DAP as the cell-wall diamino acid.

Characteristic	1	2	3
DNA G + C content (mol%)	74.6	71.9	72.3
Cell-wall sugars*	Gal, Glc, Man, Ara, Xyl (trace), Rha	Gal, Glc, Man, Xyl, Rha	Gal, Glc, Man, Xyl, Rha
Isoprenoid quinone	DMK-7	MK-7(H ₂)†	DMK-7†
Polar lipids‡	DPG, PG, PGL, PLs	DPG, PG, PGL, PLs, AL, Ls	DPG, PG, PLs, GLs, AL, Ls
Fatty acids (% of total)§			
anteiso-C _{15:0}	21.8	10.0	8.5
iso-C _{16:0}	0.5	0.3	1.2
C _{16:1} ω9 <i>c</i>	ND	1.0	0.3
C _{16:1} ω7 <i>c</i>	0.9	1.1	1.2
C _{16:0}	3.7	4.4	4.4
anteiso-C _{17:0}	6.2	1.2	9.0
C _{17:1} ω8 <i>c</i>	ND	1.3	ND
C _{17:0}	ND	0.6	ND
10-Methyl C _{17:0}	0.8	ND	1.3
C _{18:3} ω6 <i>c</i> (6,9,12)	1.4	1.8	ND
iso-C _{18:0}	0.6	0.5	1.6
C _{18:1} ω9 <i>c</i>	55.8	69.5	64.5
C _{18:1} ω7 <i>c</i>	0.9	1.7	1.2
C _{18:0}	4.3	5.1	4.1
anteiso-C _{19:0}	1.7	1.4	2.0
Summed feature 5	1.4	ND	0.8

*Ara, Arabinose; Gal, galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Xyl, xylose.

†Data from Takahashi *et al.* (2006) and Reddy & Garcia-Pichel (2009).

‡DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; AL, unknown aminolipid; GL, unknown glycolipid; PGL, unknown phosphoglycolipid; PL, unknown phospholipid; L, unknown lipid.

§Fatty acids are listed using standard abbreviations (number of carbon atoms: number of double bonds). Summed feature 5 comprises anteiso-C_{17:1} B and/or iso-C_{17:1} I. ND, Not detected.

of a single polar flagellum. Forms semi-translucent, circular, creamy white colonies on TSA. Oxidase- and catalase-positive. Growth occurs at 5–37 °C (optimum 25 °C) and at pH 5.5–9.0 (optimum pH 7.0). Growth occurs in the presence of 3 % NaCl, but not with 5 % NaCl. Indole and H₂S are not produced. Nitrate is not reduced. The methyl red and Voges–Proskauer tests are negative. Hydrolyses cellulose and Tweens 20 and 80, but not aesculin, casein, DNA, ONPG, gelatin or starch. Produces arginine dihydrolase and urease, but not lysine decarboxylase, ornithine decarboxylase or phenylalanine deaminase. On egg yolk agar, lecithinase activity is present. Utilizes acetate, L-alanine, L-arabinose, citrate, L-fucose, gluconate, D-glucose, glycerol, glycogen, *myo*-inositol, DL-lactate, malate, maltose, D-mannitol, D-mannose, melibiose, L-proline, propionate, L-rhamnose, D-salicin, L-serine, D-sorbitol, sucrose, trehalose and D-xylose as sole sources of carbon and energy, but not *N*-acetylglucosamine, amygdalin, L-arabitol, cellobiose, D-fructose, D-galactose, gentiobiose, L-histidine, lactose, malonate, melezitose, raffinose, D-ribose, turanose or xylitol. In the API 50CH gallery, acid is not produced from any of the carbohydrates tested. According to API ZYM test results, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are present, but alkaline phosphatase, valine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are absent. Susceptible to amikacin, ampicillin, carbenicillin, ceftriaxone, cephalothin, doxycycline, gentamicin, imipenem, kanamycin, polymyxin B, rifampicin, streptomycin, tobramycin and vancomycin. Resistant to aztreonam, bacitracin, ceftazidime, chloramphenicol, ciprofloxacin, colistin, erythromycin, nalidixic acid, norfloxacin, novobiocin, oxacillin and tetracycline. The cell-wall peptidoglycan contains *meso*-DAP as the diagnostic diamino acid and alanine and glutamic acid. The peptidoglycan is of the acetyl type. The only isoprenoid quinone is DMK-7. The fatty acid profile is dominated by C_{18:1}ω9c and anteiso-C_{15:0}. Mycolic acids are absent. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, an unknown phosphoglycolipid and five unknown phospholipids (PL1–5). The DNA G + C content of the type strain is 74.6 mol%.

The type strain, P4-5^T (=KCTC 19427^T =CECT 7603^T), was isolated from soil of a ginseng field.

Acknowledgements

This work was supported by the KRIBB Research Initiative Program and by the Ministry of Education, Science and Technology (MEST) of the Republic of Korea (grant no. M10437010001).

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