Cohnella yongneupensis sp. nov. and *Cohnella ginsengisoli* sp. nov., isolated from two different soils

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Two aerobic, Gram-positive, rod-shaped bacterial strains, 5YN10-14^T and GR21-5^T, were isolated from the Yongneup wetland and ginseng soil in Korea, respectively. The two strains formed ellipsoidal or oval spores positioned centrally or paracentrally in swollen sporangia. On the basis of 16S rRNA gene sequence analysis, these strains were related to members of the genus *Cohnella*. 16S rRNA gene sequence similarity between strains 5YN10-14^T and GR21-5^T was 95.9%. Strains 5YN10-14^T and GR21-5^T showed, respectively, 94.3 and 95.2% 16S rRNA gene sequence similarity to *Cohnella thermotolerans* CCUG 47242^T, 94.6 and 94.4% to *Cohnella hongkongensis* HKU3^T, 94.7 and 94.7% to *Cohnella laeviribosi* RI-39^T, and 95.4 and 94.8% to *Cohnella phaseoli* GSPC1^T. The major fatty acids of strain 5YN10-14^T were anteiso-C_{15:0} (51.1%), iso-C_{16:0} (18.5%) and C_{16:0} (13.2%), and the major fatty acids of strain GR21-5^T were anteiso-C_{15:0} (48.9%), iso-C_{16:0} (15.0%) and iso-C_{15:0} (12.2%). The two strains contained menaquinone with seven isoprene units (MK-7) as the predominant quinone, and diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as major polar lipids; however, strain 5YN10-14^T also contained lysylphosphatidylglycerol as a major polar lipid, whereas strain GR21-5^T had an unknown aminophospholipid as another major polar lipid. The DNA G + C contents of strains 5YN10-14^T and GR21-5^T were 58.8 and 61.3 mol%, respectively. Based on the results of the phylogenetic and phenotypic data presented, it was concluded that the two strains represent two novel species of the genus *Cohnella*, for which the names *Cohnella yongneupensis* sp. nov. (type strain 5YN10-14^T=KACC 11768^T=DSM 18998^T) and *Cohnella ginsengisoli* sp. nov. (type strain GR21-5^T=KACC 11771^T=DSM 18997^T) are proposed.

The genus *Cohnella* was created to accommodate two strains, one isolated by means of hygiene control checks at a starch-producing company and the other a '*Paenibacillus hongkongensis*' strain. Members of the genus *Cohnella* differ from those of the genus *Paenibacillus* on the basis of 16S rRNA gene sequence analysis, polar lipid patterns and fatty acid composition (Kämpfer *et al.*, 2006). At present, the genus *Cohnella* comprises four recognized species, namely *Cohnella thermotolerans* (the type species), *C. hongkongensis* (Kämpfer *et al.*, 2006), *C. laeviribosi* (Cho *et al.*, 2007)

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 5YN10-14^T and GR21-5^T are EF368008 and EF368010, respectively.

Two-dimensional thin-layer chromatograms of polar lipids of strains 5YN10-14^T and GR21-5^T are available as supplementary material with the online version of this paper.

and *C. phaseoli* (García-Fraile *et al.*, 2008). The present study describes two novel *Cohnella*-like strains.

Strain 5YN10-14^T was isolated from soil of the Yongneup wetland, Republic of Korea (38° 12' 53" N 128° 07' 30" E), located at 1200–1280 m above sea level. Strain GR21-5^T was isolated from ginseng soil in the Youngju region of Korea. Both of the soil samples were serially diluted with 0.85% NaCl (w/v) and dilutions were plated on R2A agar (Reasoner & Geldreich, 1985). Petri dishes were incubated at 30 °C for 3 days, and colonies were purified by using the streak plate method. Purified colonies were tentatively identified from partial 16S rRNA gene sequences. The pure culture was transferred to R2A medium for further analysis.

Cell morphology was examined by using phase-contrast microscopy on cells grown on R2A for 2 days. Spore

formation was checked after growth on R2A agar medium including 0.05 % MnSO_4 . Motility tests were performed on one-tenth-strength R2A medium including 0.1 % agar. The tube was incubated at 20 and 30 °C for 5 days. Growth at different temperatures and pH was tested at 5–55 °C (in increments of 5 °C) and pH 3–10 (in increments of 1 pH unit) on R2A medium. Tolerance to 1, 2, 3, 5 and 7 % NaCl (w/v) was assessed on R2A medium. Gram staining was performed by using a Difco Gram-stain kit. Catalase activity was tested with a 3 % (v/v) H_2O_2 solution. Oxidase activity, and hydrolysis of casein, chitin, starch, tyrosine and carboxymethylcellulose were tested on R2A agar medium according to the methods of Smibert & Krieg (1994). Growth and biochemical tests were also carried out by using API 20NE, API ID 32GN and API ZYM commercial kits (bioMérieux).

The two novel strains were motile, Gram-positive and rod-shaped. They formed ellipsoidal or oval spores positioned centrally or paracentrally in swollen sporangia after growth for 24 h. The strains grew on R2A and nutrient agar (Difco) but not on MacConkey agar (Difco). Strain GR21-5^T grew on trypticase soy agar (TSA; Difco) whereas strain 5YN10-14^T did not. The two strains could be differentiated from each other on the basis of several physiological and biochemical properties (Table 1).

Cellular fatty acid methyl esters were prepared and analysed by GC according to the instructions of the Microbial Identification System (MIDI). Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). Extraction and analysis of polar lipids by two-dimensional TLC were performed according to Minnikin *et al.* (1984). The major fatty acids of strains GR21-5^T and 5YN10-14^T were anteiso- $\text{C}_{15:0}$ (48.9–51.1 %) and iso- $\text{C}_{16:0}$ (15.0–18.5 %), consistent with data for recognized *Cohnella* species (Table 2). Characteristically, strain 5YN10-14^T did not contain iso- $\text{C}_{15:0}$, whereas strain GR21-5^T had a relatively high proportion (12.2 %) of this fatty acid. Strains 5YN10-14^T and GR21-5^T contained menaquinone 7 (MK-7; 91 and 93 %, respectively) as the predominant quinone, and had MK-6 (9 and 7 %, respectively) as a minor component. The predominant polar lipids of the two strains were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine (see Supplementary Fig. S1, available in IJSEM Online). However, strain 5YN10-14^T contained lysylphosphatidylglycerol as a fourth major polar lipid, indicating that the polar lipid pattern of strain 5YN10-14^T was similar to those of *C. thermotolerans* and *C. hongkongensis* (Kämpfer *et al.*, 2006). Strain GR21-5^T did not contain lysylphosphatidylglycerol, similar to data for *C. phaseoli* (García-Fraile *et al.*, 2008). DNA G+C contents were determined by HPLC of deoxyribonucleosides as described by Mesbah *et al.* (1989), by using a reverse-phase column (Supelcosil LC-18 S; Supelco). The DNA G+C contents of strains 5YN10-14^T and GR21-5^T were 58.8 and 61.3 mol%, respectively.

Genomic DNA was isolated by using the method of Ausubel *et al.* (1987). The 16S rRNA gene was amplified

and sequenced as described by Weon *et al.* (2005). Alignment of 16S rRNA gene sequences was performed with the CLUSTAL W program (Thompson *et al.*, 1994). Phylogenetic analysis was performed by using MEGA version 3.1 (Kumar *et al.*, 2004). Distances with options according to the Kimura two-parameter model (Kimura, 1983) and clustering according to the neighbour-joining and maximum-parsimony methods were determined by using bootstrap values based on 1000 replicates.

The almost-complete 16S rRNA gene sequences (approximately 1385 bp) of the two novel isolates were used for phylogenetic analyses. The neighbour-joining tree (Fig. 1) revealed that strains 5YN10-14^T and GR21-5^T and the type strains of all recognized *Cohnella* species formed one compact cluster with 100 % bootstrap support. The grouping of the two novel strains within the genus *Cohnella* was also well supported in the maximum-parsimony tree (data not shown). 16S rRNA gene sequence similarity between strains 5YN10-14^T and GR21-5^T was 95.9 %. Strains 5YN10-14^T and GR21-5^T showed levels of 16S rRNA gene sequence similarity, respectively, of 94.3 and 95.2 % to *C. thermotolerans* CCUG 47242^T, 94.6 and 94.4 % to *C. hongkongensis* HKU3^T, 94.7 and 94.7 % to *C. laevis* RI-39^T and 95.4 and 94.8 % to *C. phaseoli* GSPC1^T. However, the two strains showed 16S rRNA gene sequence similarities of less than 93 % to the type strains of all recognized species of the genus *Paenibacillus* included in the phylogenetic analysis.

On the basis of 16S rRNA gene sequence analysis, the two novel strains were clearly affiliated with the genus *Cohnella*. According to the bacterial species concept and that strains showing less than 97 % 16S rRNA gene sequence similarity and levels of DNA–DNA relatedness below 70 % can thus be considered as members of different species (Wayne *et al.*, 1987), strains 5YN10-14^T and GR21-5^T can be considered to represent two separate species within the genus *Cohnella*. Phenotypically, strains 5YN10-14^T and GR21-5^T could be clearly differentiated from each other and from other recognized species of the genus *Cohnella* based on carbon assimilation patterns, enzyme activities, polar lipid patterns and fatty acid compositions (Tables 1 and 2). In particular, strain 5YN10-14^T was able to assimilate only a few of the test substrates.

On the basis of the data from the present polyphasic taxonomic study, strains 5YN10-14^T and GR21-5^T are considered to represent two novel species of the genus *Cohnella*, for which the names *Cohnella yongneupensis* sp. nov. and *Cohnella ginsengisoli* sp. nov., respectively, are proposed.

Description of *Cohnella yongneupensis* sp. nov.

Cohnella yongneupensis (yong.neup.en'sis. N.L. fem. adj. *yongneupensis* pertaining to Yongneup, an upland wetland of the Republic of Korea, from where the type strain was isolated).

Table 1. Phenotypic comparison between strains 5YN10-14^T and GR21-5^T and the type strains of recognized *Cohnella* species

Strains: 1, 5YN10-14^T (*C. yongneupensis* sp. nov.); 2, GR21-5^T (*C. ginsengisoli* sp. nov.); 3, *C. thermotolerans* KACC 11643^T; 4, *C. hongkongensis* KACC 116448^T; 5, *C. laeviribosi* KACC 13447^T; 6, *C. phaseoli* KACC 13436^T. Data were obtained in the present study unless indicated otherwise. All strains are positive for aesculin hydrolysis, β -galactosidase, esterase (C4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase. All strains are negative for indole production, glucose fermentation, arginine dihydrolase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, β -glucuronidase, *N*-acetyl- β -glucosaminidase and α -mannosidase. All are unable to assimilate capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, propionic acid, valeric acid, L-histidine, 3-hydroxybutyric acid, 4-hydroxybenzoic acid and L-proline. +, Positive; (+), weakly positive; –, negative.

Characteristic	1	2	3	4	5	6
Catalase/oxidase	(+)/+	+/+	+/+	(+)/+	+/-	(+)/+
Nitrate reduction	–	+	–	+	–	–
Urease	–	–	–	–	+	–
Gelatin hydrolysis	–	–	–	–	(+)	–
Assimilation of (API 20NE and API ID 32GN):						
D-Glucose	–	+	+	+	+	+
L-Arabinose	–	+	+	+	+	+
D-Mannose	–	+	+	+	+	+
D-Mannitol	–	+	+	+	+	–
<i>N</i> -Acetylglucosamine	–	–	–	+	–	+
Maltose	–	+	+	+	+	+
Potassium gluconate	–	–	+	–	–	–
L-Rhamnose	–	–	+	+	+	–
D-Ribose	–	–	+	+	+	+
Inositol	–	–	+	+	–	–
Sucrose	–	–	+	+	+	+
Glycogen	–	–	+	–	+	+
Salicin	–	+	+	+	–	+
Melibiose	–	+	+	+	+	+
L-Fucose	–	–	+	+	+	+
D-Sorbitol	–	–	+	+	+	–
Potassium 2-ketogluconate	–	–	+	–	–	+
Enzyme activities (API ZYM)						
Alkaline phosphatase	+	–	+	+	–	–
Leucine arylamidase	–	–	+	+	(+)	(+)
Acid phosphatase	+	–	–	–	–	–
α -Galactosidase	+	–	+	–	+	–
α -Glucosidase	+	–	+	–	+	+
β -Glucosidase	+	–	+	+	+	+
α -Fucosidase	+	–	–	+	–	–
DNA G + C content (mol%)	58.8	61.3	59*	60.9†	51‡	60.3§

*Data from Kämpfer *et al.* (2006).

†Data from Teng *et al.* (2003).

‡Data from Cho *et al.* (2007).

§Data from García-Fraile *et al.* (2008).

Cells are motile, Gram-positive rods ($0.7 \times 2.5\text{--}3.5\ \mu\text{m}$) with ellipsoidal or oval spores positioned centrally or paracentrally in swollen sporangia. Growth occurs at 10–40 °C (optimum, 30 °C), at pH 4.0–9.0 (optimum, pH 7.0) and in the presence of 0–1 % (w/v) NaCl. Catalase- and oxidase-positive. Carboxymethylcellulose and starch are hydrolysed, but casein, chitin and tyrosine are not. Positive for aesculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation,

arginine dihydrolase, urease and gelatin hydrolysis (API 20NE test strips). Does not assimilate any substrates included in the API 20NE and API ID 32GN test strips. The predominant menaquinone is MK-7. The major fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and C_{16:0}. The predominant polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and lysylphosphatidylglycerol. The DNA G+C content of the type strain is 58.8 mol% (HPLC).

Table 2. Cellular fatty acid compositions of strains 5YN10-14^T and GR21-5^T and the type strains of recognized *Cohnella* species

Strains: 1, 5YN10-14^T (*C. yongneupensis* sp. nov.); 2, GR21-5^T (*C. ginsengisoli* sp. nov.); 3, *C. thermotolerans* KACC 11643^T; 4, *C. hongkongensis* KACC 11644^T; 5, *C. laeviribosi* KACC 13447^T; 6, *C. phaseoli* KACC 13436^T. All data were obtained in the present study. All strains were cultivated on R2A medium at 30 °C for 3 days before harvesting cell mass. Values are percentages of the total fatty acids. —, Not detected or <1 %.

Fatty acid	1	2	3	4	5	6
iso-C _{12:0}	—	—	—	1.0	—	—
C _{13:0}	—	—	—	1.5	—	—
anteiso-C _{13:0}	2.2	—	—	3.3	—	2.2
C _{14:0}	3.2	3.1	2.0	6.0	1.7	1.7
iso-C _{14:0}	2.4	8.2	4.1	9.1	7.7	5.1
anteiso-C _{15:0}	51.1	48.9	35.2	35.6	28.6	46.0
iso-C _{15:0}	—	12.2	2.7	1.4	7.2	3.4
C _{16:0}	13.2	6.7	5.6	12.6	6.2	8.9
iso-C _{16:0}	18.5	15.0	30.6	17.6	40.9	17.0
C _{16:1} ω11c	—	—	1.2	1.9	—	2.3
C _{16:1} ω7c alcohol	—	—	1.6	3.3	—	4.0
C _{17:0}	—	—	—	—	—	2.1
anteiso-C _{17:0}	4.2	1.8	5.7	1.7	3.7	3.4
C _{17:1} ω6c	—	—	2.7	—	—	—
C _{18:1} ω7c	—	—	5.9	—	—	—
Summed feature 3*	1.0	—	—	1.3	—	—
Summed feature 4*	—	1.7	—	—	—	—
Summed feature 5*	—	—	1.3	—	—	—

*Summed features represent groups of two fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 comprises C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; summed feature 4 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B; summed feature 5 comprises anteiso-C_{18:0} and/or C_{18:2}ω6,9c.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of strains 5YN10-14^T and GR21-5^T and the type strains of closely related species. Numbers at nodes indicate the percentage of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; values below 50 % are not shown. Bar, 0.01 substitutions per nucleotide position.

The type strain, 5YN10-14^T (=KACC 11768^T=DSM 18998^T), was isolated from the Yongneup wetland in the Republic of Korea.

Description of *Cohnella ginsengisoli* sp. nov.

Cohnella ginsengisoli (gin.sen.gi.so'li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of the soil of a ginseng field, the source of the type strain).

Cells are motile, Gram-positive rods (1.0 × 2.0–3.0 µm) with ellipsoidal or oval spores positioned centrally or paracentrally in swollen sporangia. Growth occurs at 10–40 °C (optimum, 30 °C), at pH 5.0–9.0 (optimum, pH 7.0) and in the presence of 0–2 % (w/v) NaCl. Catalase- and oxidase-positive. Starch is hydrolysed, but casein, carboxymethyl-cellulose, chitin and tyrosine are not. Positive for nitrate reduction and aesculin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatin hydrolysis (API 20NE test strips). Assimilates D-glucose, L-arabinose, D-mannose, D-mannitol, maltose, salicin and melibiose, but not N-acetylglucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, inositol, sucrose, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid or L-proline (API 20NE and API ID 32GN test strips). The predominant menaquinone is MK-7. The major fatty acids are anteiso-C_{15:0}, iso-C_{16:0} and iso-C_{15:0}. The predominant polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unknown aminophospholipid. The DNA G + C content of the type strain is 61.3 mol% (HPLC).

The type strain, GR21-5^T (=KACC 11771^T=DSM 18997^T), was isolated from ginseng soil in the Youngju region of the Republic of Korea.

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