Reclassification of *Paenibacillus ginsengisoli* as a later heterotypic synonym of *Paenibacillus anaericanus*

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The type strains of the species *Paenibacillus ginsengisoli* (KCTC 13931^T) and *Paenibacillus anaericanus* (DSM 15890^T) were compared in order to clarify the taxonomic relationship of the two species. On the basis of 16S rRNA and *rpoB* gene sequence comparisons, the two strains shared 99.9 and 99.6 % similarity, respectively. The mean DNA–DNA relatedness value was 77 % and the genomic DNA G + C contents were 43.2 and 42.2 mol%, respectively. Phenotypic data, including fatty acid patterns and acid-production, enzyme-activity and substrate-utilization profiles, showed no pronounced differences between the type strains of the two species. These genotypic and phenotypic data suggest that the two taxa constitute a single species. According to Rules 38 and 42 of the *Bacteriological Code*, they should be united under the name *Paenibacillus anaericanus*, with the name *Paenibacillus ginsengisoli* as a later heterotypic synonym.

The genus *Paenibacillus*, type genus of the family *Paenibacillaceae* (De Vos *et al.*, 2010), accommodates facultatively anaerobic or strictly aerobic, rod-shaped bacteria that have ellipsoidal spores in swollen sporangia, anteiso-C_{15:0} as the major fatty acid and DNA G + C contents ranging from 36 to 59 mol% (Ash *et al.*, 1993; Shida *et al.*, 1997; De Vos *et al.*, 2009). *Paenibacilli* are ubiquitous in nature, especially in soil. At the time of writing, the genus *Paenibacillus* comprises 114 recognized species, of which nearly 70 have been isolated from soil environments including humus, phyllosphere, rhizosphere, soil and sediments (Daane *et al.*, 2002; Euzéby, 2010). The genus *Paenibacillus* has remained monophyletic with ever-increasing numbers of species, but some tightly clustered taxa share >99.5 % 16S rRNA gene sequence similarity, e.g. *Paenibacillus favisporus* (Velázquez *et al.*, 2004) and *Paenibacillus cineris* (Logan *et al.*, 2004), *Paenibacillus massiliensis* (Roux & Raoult, 2004) and *Paenibacillus panacisoli* (Ten *et al.*, 2006), and *Paenibacillus anaericanus* (Horn *et al.*, 2005) and *Paenibacillus ginsengisoli* (Lee *et al.*, 2007b), which has led to taxonomic re-evaluation.

P. ginsengisoli was described by Lee *et al.* (2007a) for a novel strain isolated from soil of a ginseng field, and its name was validated on Validation List no. 116 (Lee *et al.*, 2007b). According to the original description, whilst *P. ginsengisoli* formed a tight phylogenetic clade with *P. anaericanus* due to the high 16S rRNA gene sequence similarity between them, a DNA–DNA relatedness value of 62 % and several phenotypic characteristics distinguished between the two species. However, the DNA–DNA relatedness value was just below the threshold for species delineation (Wayne *et al.*, 1987) and resequencing of the 16S rRNA genes of the type strains of *P. anaericanus* and *P. ginsengisoli* revealed that they share 99.9 % sequence similarity, which raised the possibility of their relatedness at the species level. Subsequently, the taxonomic relationship between the two species was re-evaluated by examining a variety of genotypic and phenotypic criteria.

P. ginsengisoli KCTC 13931^T (=Gsoil 1638^T) and *P. anaericanus* DSM 15890^T (=MH21^T) were cultivated on tryptic soy agar (TSA) or broth (Difco) at 30 °C for 48 h, unless stated otherwise. *P. ginsengisoli* LMG 23406^T and *P. anaericanus* LMG 23658^T were used for confirmation of 16S rRNA gene sequences and phenotypic data. Genomic DNA was extracted by using an UltraClean microbial DNA isolation kit (MO BIO Laboratories).

The 16S rRNA gene was PCR-amplified using a universal bacterial primer set (27F and 1492R; Lane, 1991). The amplified PCR product was purified by using a PCR purification kit (Solgent) and sequenced directly by the fluorescent dye-terminator method using an ABI 3730XL Capillary DNA Analyzer (Applied Biosystems) at Solgent

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *P. ginsengisoli* KCTC 13931^T and LMG 23406^T and *P. anaericanus* DSM 15890^T and LMG 23658^T are FN673695, FR690143, FN673694 and FR690142, respectively. The GenBank accession numbers for the *rpoB* gene sequences of *P. ginsengisoli* KCTC 13931^T and *P. anaericanus* DSM 15890^T are FN673684 and FN673683, respectively.

A supplementary table and figure are available with the online version of this paper.

Co. Ltd (Daejeon, Korea). The full-length 16S rRNA gene sequence was compiled by using SeqMan software (DNASTAR) and aligned with published sequences retrieved from GenBank by using CLUSTAL_X (Thompson *et al.*, 1997).

Almost-complete 16S rRNA gene sequences of 1475 nt, corresponding to *Escherichia coli* positions 28–1491 with gaps, were determined and compared with published sequences. The 16S rRNA gene sequences of *P. ginsengisoli* (GenBank accession numbers FN673695, FR690143 and AB245382 for strains KCTC 13931^T, LMG 23406^T and Gsoil 1638^T, respectively) showed 100 % identity, but those of *P. anaericanus* (GenBank accession numbers FN673694 and AJ318909 for strains DSM 15890^T and MH21^T, respectively) shared only 99.7 % similarity, which was due to five mismatches (four nucleotide substitutions and one gap). This discrepancy probably arose from a sequencing error by the original submitter, as the original sequence (GenBank accession no. AJ318909) contained two substitutions and one insertion in the highly conserved region of the 16S rRNA gene, and the 16S rRNA gene sequence of *P. anaericanus* LMG 23658^T (GenBank accession no. FR690142) matched that of *P. anaericanus* DSM 15890^T perfectly. Consequently, the 16S rRNA gene sequences of the type strains of *P. ginsengisoli* and *P. anaericanus* shared 99.9 % similarity, showing only two nucleotide differences, which was slightly higher than reported previously (Lee *et al.*, 2007a).

For the measurement of G + C content, genomic DNA was extracted and purified by using a Blood & Cell Culture DNA Midi kit (Qiagen). DNA was degraded enzymically into nucleosides, and 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), using a Fluoroskan Ascent fluorescence plate reader (Thermo Life Science). The hybridization temperature was 42 °C. Reciprocal experiments were performed with five replications per sample.

According to the results obtained by Lee *et al.* (2007a) and Horn *et al.* (2005), the DNA G + C contents of *P. ginsengisoli* Gsoil 1638^T and *P. anaericanus* MH21^T were 50.7 and 42.6 mol%, respectively. However, the redetermined DNA G + C contents of the type strains of *P. ginsengisoli* and *P. anaericanus* were 43.2 ± 0.2 and 42.2 ± 0.1 mol%, respectively, which showed that the difference was less than the common range found within a species (Rosselló-Mora & Amann, 2001). Besides, results of the DNA–DNA cross-hybridization between the type strains of *P. ginsengisoli* and *P. anaericanus* yielded a relatedness of 82.8 % (reciprocal, 70.9 %), which was much higher than the value of 62 % obtained by Lee *et al.* (2007a) and indicated that the two taxa constitute a single species (Wayne *et al.*, 1987). These inconsistencies may possibly have been attributable to experimental errors by Lee *et al.*

(2007a), i.e. poor-quality DNAs, due to impurities in DNA preparations or contamination of the culture with another organism, may have yielded misleading results, in that a comparative study for analysis of DNA G + C contents was not performed, the authenticity of the type strain of *P. anaericanus* was not confirmed and the type strain of *P. anaericanus* showed an unusual fatty acid profile in the previous study (Lee *et al.*, 2007a). The apparent incongruity of the results from DNA–DNA hybridization has been reported in several recent studies dealing with taxonomic reclassification (Wang *et al.*, 2008; Dobritsa *et al.*, 2010; Chimento *et al.*, 2011).

To confirm the above results, we analysed the *rpoB* gene, a single-copy gene encoding the β -subunit of RNA polymerase. It has already been reported that sequences of this protein-encoding gene have higher discriminatory power than 16S rRNA gene sequences, and sequence comparison of this gene has proven to be helpful to describe the relationships among closely related taxa (Drancourt *et al.*, 2004; Durak *et al.*, 2006; Adékambi *et al.*, 2008; Hoshino *et al.*, 2009). PCR-mediated amplification of the *rpoB* gene and sequencing of the purified PCR product were carried out according to Wu *et al.* (2011) with some modifications: primers 1F (5'-TTGRCAGGWCAACTWGTTC-3') and 3384R (5'-GAAYGATTCCGGAACRCCTGGTTC-3'), which were designed in this study, were used instead of primers 73F and 3306R, respectively.

Almost-complete *rpoB* gene sequences of 3343 nt, corresponding to positions 21–3359 of the *Bacillus subtilis* sequence (GenBank accession no. AL009126) with gaps, were determined. The *rpoB* gene sequences of the type strains of *P. ginsengisoli* and *P. anaericanus* shared 99.6 % similarity, which was clearly above the cut-off value for species delineation (97.7 %; Adékambi *et al.*, 2008) and fell within the range of intraspecific *rpoB* gene sequence similarity (98.2–100 %; Adékambi *et al.*, 2008). Consequently, comparative sequence analysis of the *rpoB* gene could complement the DNA–DNA hybridization data.

For analysis of fatty acids, cells were grown on TSA for 3 days at the optimal growth temperature (30 °C), as well as at 25 °C for comparison with the previous data obtained by Lee *et al.* (2007a). Fatty acid methyl esters were prepared and analysed as described by Klatte *et al.* (1994) by using the standard Microbial Identification System (MIDI) for automated GC analysis (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

In contrast to data reported previously (Lee *et al.*, 2007a), the type strains of *P. ginsengisoli* and *P. anaericanus* had very similar fatty acid profiles, i.e. the fatty acid anteiso-C_{15:0} was predominant, and no significant quantitative or qualitative differences were detected in the fatty acid compositions, regardless of the conditions under which cells were grown. Members of the genus *Paenibacillus* have been described to contain anteiso-C_{15:0} as the major fatty acid (Ash *et al.*, 1993; Shida *et al.*, 1997; De Vos *et al.*, 2009). However, according to the data obtained by Lee *et al.* (2007a), the type

strain of *P. anaericanus* had iso-C_{15:0} as the major fatty acid, a finding that was not repeated in this study. Detailed fatty acid compositions are shown in Table 1 and Supplementary Table S1 (available in IJSEM Online).

The Gram reaction was performed as described by Smibert & Krieg (1994) with cells grown at 30 °C for 24 h. Cell morphology and motility were observed under a phase-contrast microscope (Eclipse 80i, Nikon; ×1000 magnification). For the observation of flagella, cells were negatively stained with 1% (w/v) uranyl acetate and examined using a model H-7600 transmission electron microscope (Hitachi). 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, 35, 37, 40 and 42 °C and in the presence of 1, 2, 3, 5 and 10% (w/v) NaCl. Hydrolysis of DNA, casein and starch was tested on DNase test agar, casein agar and starch agar (BBL), respectively. Hydrolysis of Tweens 20 and 80 was examined by using the method of Sierra (1957). Production of lecithinase was detected on egg yolk agar (McClung & Toabe, 1947). H₂S production was tested in tryptic soy broth 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 using API 50CH (with CHB/E medium), API ZYM, API 20E and API 20NE galleries according to the instructions of the manufacturer (bioMérieux). Antibiotic-susceptibility tests were performed using Sensi-Discs (6 mm; BBL) containing the following: amikacin (30 µg), ampicillin (10 µg), bacitracin (10 IU), ceftazidime (30 µg), ceftriaxone (30 µg), cephalothin (30 µg), ciprofloxacin (5 µg), colistin (10 µg), doxycycline (30 µg), gentamicin (10 µg), imipenem (10 µg), norfloxacin (10 µg), novobiocin (30 µg), oxacillin (1 µg), polymyxin B (300 IU) and tobramycin (10 µg).

Cells of the type strains of *P. ginsengisoli* and *P. anaericanus* were Gram-reaction-negative, catalase- and oxidase-positive, motile rods with peritrichous flagella (see Supplementary Fig. S1, available in IJSEM Online). The type strains of *P. ginsengisoli* and *P. anaericanus* showed identical antibiotic-susceptibility profiles: they were susceptible to amikacin, ampicillin, bacitracin, ceftazidime, ceftriaxone, cephalothin, ciprofloxacin, doxycycline, gentamicin, imipenem, norfloxacin, novobiocin, polymyxin B and tobramycin, but resistant to colistin and oxacillin. The physiological and biochemical characteristics of KCTC 13931^T and DSM 15890^T are listed in Table 2. The strains shared almost-identical characteristics, except for β-glucosidase

Table 1. Cellular fatty acid composition (%) of the type strains of *P. ginsengisoli* and *P. anaericanus* grown at 25 °C

Fatty acids are listed using standard abbreviations (no. of carbon atoms: no. of double bonds). Means ± SD are given where appropriate. ND, Not detected.

Fatty acid	<i>P. ginsengisoli</i>		<i>P. anaericanus</i>	
	This study*	Lee <i>et al.</i> (2007a)	This study*	Lee <i>et al.</i> (2007a)
Saturated straight-chain				
C _{14:0}	6.0 ± 0.5	8.5	4.7 ± 0.3	ND
C _{15:0}	5.2 ± 0.5	2.9	3.2 ± 0.8	ND
C _{16:0}	14.8 ± 1.8	25.4	11.3 ± 0.1	11.0
Saturated branched				
iso-C _{14:0}	4.3 ± 0.3	2.5	4.9 ± 0.4	ND
iso-C _{15:0}	5.2 ± 0.1	5.9	6.6 ± 0.9	37.5
iso-C _{16:0}	4.8 ± 0.1	5.8	7.2 ± 0.4	5.0
iso-C _{17:0}	0.5	2.0	1.1 ± 0.4	7.4
anteiso-C _{15:0}	57.3 ± 2.9	43.7	57.5 ± 2.7	12.6
anteiso-C _{17:0}	1.5 ± 0.1	3.3	2.5 ± 0.2	5.4
Unsaturated				
C _{16:1} ω7c alcohol	ND	ND	ND	3.9
C _{16:1} ω11c	ND	ND	ND	8.1
iso-C _{17:1} ω9c	ND	ND	ND	2.8
Summed feature 5†	0.4	ND	0.8 ± 0.1	2.6
Others‡	ND	ND	ND	3.7

*Strains KCTC 13931^T and LMG 23406^T were used for *P. ginsengisoli*, and strains DSM 15890^T and LMG 23658^T for *P. anaericanus*.

†Summed feature 5 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B, which could not be separated by the MIDI system.

‡Not defined by Lee *et al.* (2007a).

Table 2. Physiological and biochemical characteristics of the type strains of *P. ginsengisoli* and *P. anaericanus*

Strains: 1, *P. ginsengisoli* KCTC 13931^T; 2, *P. anaericanus* DSM 15890^T. Data were obtained in this study. +, Positive; (+), weakly positive; –, negative. Both strains were positive for catalase, oxidase and motility; negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, urease and indole production; and showed identical profiles in terms of acid production from carbohydrates and antibiotic susceptibility.

Characteristic	1	2
Gram reaction	–	–
Temperature range (optimum) for growth (°C)	5–40* (30–35)*	5–40 (30–35)
Growth with:		
3 % (w/v) NaCl	+	+
5 % (w/v) NaCl	–*	–
Methyl red test	–	–
Voges–Proskauer test	+	+†
H ₂ S production	–	–
Nitrate reduction	+	+†
β-Glucosidase‡	+	–
Lecithinase on egg yolk agar	–	–
Utilization of:		
<i>N</i> -Acetylglucosamine	(+)*	+
D-Fructose	–	(+)
D-Ribose	+*	+
D-Xylose	+*	+
Hydrolysis of:		
Aesculin	+*	+
Casein	–	–
DNA	–	–
Gelatin	–	–
Starch	+*	(+)
Tween 20	–	–
Tween 80	–	–

*Data not congruent with those reported by Lee *et al.* (2007a).

†Data not congruent with those reported by Horn *et al.* (2005).

‡Data from API ZYM tests.

activity and utilization of D-fructose. Strains LMG 23406^T and LMG 23658^T gave identical results to strains KCTC 13931^T and DSM 15890^T, respectively, which confirmed that there were no significant characteristics differentiating between *P. ginsengisoli* and *P. anaericanus*.

On the basis of the genotypic and phenotypic evidence described above, the species *P. ginsengisoli* and *P. anaericanus* constitute a single genomic species, without differentiation at the subspecies level. Therefore, it is proposed that the two species be united under the same name. According to the rules of priority (Rules 38 and 42 of the *Bacteriological Code*; Lapage *et al.*, 1992), the name *Paenibacillus anaericanus* should be retained for the united taxon, with *Paenibacillus ginsengisoli* as a later heterotypic synonym.

Emended description of *Paenibacillus anaericanus* Horn *et al.* 2005

The description is as given by Horn *et al.* (2005) with the following additions and modifications. Cells are motile by means of peritrichous flagella. Growth occurs in the presence of up to 3 % (w/v) NaCl. H₂S is not produced. Nitrate is reduced. The methyl red test is negative, but the Voges–Proskauer test is positive. Aesculin is hydrolysed, but casein, DNA and Tweens 20 and 80 are not. Lecithinase and ornithine decarboxylase are not produced. On the basis of the method of Kim *et al.* (2010), amygdalin, L-arabinose, D-galactose, glycogen, melibiose, D-ribose, D-salicin, trehalose, turanose and D-xylose are utilized as sole sources of carbon and energy, but L-alanine, L-arabitol, L-fucose, gentiobiose, gluconate, glycerol, L-histidine, malate, malonate, D-mannitol, melezitose, L-proline, raffinose, L-rhamnose and L-serine are not. In the API 50CH gallery, acid is produced from *N*-acetylglucosamine, aesculin, amygdalin, L-arabinose, arbutin, cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, glycerol, glycogen, inulin, lactose, maltose, D-mannose, melibiose, methyl α-D-glucoside, methyl β-D-xyloside, raffinose, L-rhamnose, D-ribose, D-salicin, starch, sucrose, trehalose, turanose and D-xylose, but not from D-adonitol, D-arabinose, D- or L-arabitol, dulcitol, erythritol, D- or L-fucose, gluconate, inositol, 2- or 5-ketogluconate, D-lyxose, D-mannitol, melezitose, methyl α-D-mannoside, D-sorbitol, L-sorbose, D-tagatose, xylitol or L-xylose. According to the results of the API ZYM test, esterase (C4), esterase lipase (C8), leucine arylamidase and β-galactosidase are present, but alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. Susceptible to amikacin, ampicillin, bacitracin, ceftazidime, ceftriaxone, cephalothin, ciprofloxacin, doxycycline, gentamicin, imipenem, norfloxacin, novobiocin, polymyxin B and tobramycin. Resistant to colistin and oxacillin. The major fatty acid is antesio-C_{15:0}. The G + C content of the DNA is 42.2–43.2 mol%.

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