Chitinophaga ginsengisegetis sp. nov. and *Chitinophaga ginsengisoli* sp. nov., isolated from soil of a ginseng field in South Korea

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Two novel strains belonging to the phylum *Bacteroidetes* [formerly the *Cytophaga–Flexibacter–Bacteroides* (CFB) group], designated Gsoil 040^T and Gsoil 052^T, were isolated from the soil of a ginseng field in Pocheon province, South Korea. A polyphasic approach was used to characterize the taxonomic position of the novel strains. Both strains were Gram-negative, aerobic, non-motile, non-spore-forming and rod-shaped. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the novel isolates belong to the genus *Chitinophaga* but are clearly separated from the recognized species of this genus; gene sequence similarities between the novel isolates and type strains of recognized species ranged from 91.2 to 96.5 %. One exception was found; strain Gsoil 052^T and the type strain of *Chitinophaga filiformis* had a gene sequence similarity of 99.6 % but had a DNA–DNA relatedness value of 38 %. Phenotypic and chemotaxonomic data (major menaquinone, MK-7; major fatty acids, iso-C_{15:0} and C_{16:1ω5c}; major hydroxy fatty acid, iso-C_{17:0} 3-OH and major polyamine, homospermidine) supported the affiliation of both strains Gsoil 040^T and Gsoil 052^T to the genus *Chitinophaga*. The results of physiological and biochemical tests enabled the genotypic and phenotypic differentiation of the novel strains from the other recognized species of the genus *Chitinophaga*. Therefore, it is suggested that the new isolates represent two novel species, for which the names *Chitinophaga ginsengisegetis* sp. nov. [type strain Gsoil 040^T (=KCTC 12654^T=DSM 18108^T)] and *Chitinophaga ginsengisoli* sp. nov. [type strain Gsoil 052^T (=KCTC 12592^T=DSM 18017^T)] are proposed.

The genus *Chitinophaga* was originally described by Sangkhobol & Skerman (1981) to include strains of filamentous, chitinolytic, gliding bacteria which transform into spherical bodies on ageing. Recently, Kämpfer *et al.* (2006) proposed *Chitinophaga skermanii* sp. nov. and reclassified [*Flexibacter*] *filiformis*, [*Flexibacter*] *sancti*, [*Cytophaga*] *arvensicola* and [*Flexibacter*] *japonensis* as members of the genus *Chitinophaga*. At the time of writing, the genus *Chitinophaga* contains six species with validly published names; *Chitinophaga pinensis* (the type species), *Chitinophaga filiformis*, *Chitinophaga sancti*, *Chitinophaga*

arvensicola, *Chitinophaga japonensis* and *Chitinophaga skermanii*.

In this paper, a polyphasic taxonomic approach was used to characterize novel bacterial strains that were isolated from the soil of a ginseng field in South Korea. Two strains, Gsoil 040^T and Gsoil 052^T, were found to represent two novel species of the genus *Chitinophaga*.

Strains Gsoil 040^T and Gsoil 052^T were originally isolated from the soil of a ginseng field from Pocheon province, South Korea. This soil sample was thoroughly suspended with 50 mM phosphate buffer (pH 7.0) and the suspension was spread on one-fifth-strength modified R2A agar [containing (l⁻¹) 0.25 g tryptone, 0.25 g peptone, 0.25 g yeast extract, 0.125 g malt extract, 0.125 g beef extract,

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains Gsoil 040^T and Gsoil 052^T are AB264798 and AB245374, respectively.

0.25 g Casamino acids, 0.25 g Soytone, 0.5 g glucose, 0.3 g soluble starch, 0.2 g xylan, 0.3 g sodium pyruvate, 0.3 g K_2HPO_4 , 0.05 g $MgSO_4$, 0.05 g $CaCl_2$ and 15 g agar] plates after serial dilution with 50 mM phosphate buffer (pH 7.0). The plates were incubated at 30 °C for one month. Single colonies on the plates were purified by transferring them onto new plates and were then incubated again on full-strength modified R2A or half-strength modified R2A. The purified colonies were tentatively identified by partial sequences of the 16S rRNA gene (Im *et al.*, 2005). They were routinely cultured on R2A agar at 30 °C and maintained as a glycerol suspension (20 %, w/v) at –70 °C.

The Gram reaction was performed by the non-staining method as described by Buck (1982). Cell morphology was observed under a light microscope (Nikon) at $\times 1000$, with cells grown for 3 days at 30 °C on R2A agar. Catalase activity was determined by assessing bubble production in 3 % (v/v) H_2O_2 and oxidase activity was determined using 1 % (w/v) tetramethyl-*p*-phenylenediamine. Carbon source utilization and enzyme activities were tested by using the API 20 NE, API ID 32 GN and API ZYM test kits (bioMérieux). The ability to grow under anaerobic conditions was tested in serum bottles by adding sodium thioglycolate (1 g l^{-1}) to R2A broth and substituting the upper air layer with nitrogen gas. Tests for the degradation of DNA, in which DNase agar (Scharlau) plates were flooded with 1 M HCl, casein, chitin, starch (Atlas 1993), lipid (Kouker & Jaeger, 1987), xylan and cellulose (Ten *et al.*, 2004), were performed and then evaluated after 10 days. Growth at different temperatures (4, 15, 20, 25, 30, 37 and 42 °C) and various pH values (pH 5.0–10.0 at intervals of 0.5 pH units) was assessed after 10 days incubation. Salt tolerance was tested on R2A agar supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Growth on nutrient agar and trypticase soy agar (TSA) was also evaluated at 30 °C. Flexirubin-like pigments were observed by flooding the plates with 20 % (w/v) potassium hydroxide (Fautz & Reichenbach, 1980).

Extraction of genomic DNA was performed with a commercial genomic DNA extraction kit (Solgent) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed with CLUSTAL_X (Thompson *et al.*, 1997). Gaps were edited with BioEdit (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using a neighbour-joining method (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) using the MEGA3 software package (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

For the measurement of G + C content of the chromosomal DNA, genomic DNA of the novel strains was extracted and purified as described by Moore & Dowhan (1995) and was

then enzymically degraded into nucleosides. DNA G + C content was determined as described by Mesbah *et al.* (1989) using a reversed-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), evaporated under vacuum conditions, and reextracted in *n*-hexane/water (1 : 1, v/v). The crude *n*-hexane–quinone solution was purified using silica Sep-Pak Vac cartridges (Waters) and subsequently analysed by HPLC as described previously (Hiraishi *et al.*, 1996). Cellular fatty acid profiles were determined for strains grown on TSA (Difco) for 2 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids were analysed using a gas chromatograph (6890; Hewlett Packard) and identified by the Microbial Identification software package (Sasser, 1990). Polyamines were extracted and analysed according to Busse & Auling (1988) and Schenkel *et al.* (1995), respectively.

Cells of strains Gsoil 040^T and Gsoil 052^T were aerobic, Gram-negative, non-motile and non-spore-forming rods. When grown on R2A agar plates for three days, colonies of both strains were smooth, circular, yellowish and 1.5–4.0 mm in diameter. Both isolates grew well on nutrient agar and TSA. Tests for flexirubin pigmentation were positive for both strains. The presence of flexirubin pigments was demonstrated by a fast shift of colony colour from yellow to reddish brown after the addition of 20 % KOH and the retention of the original colour upon the addition of HCl. Other physiological characteristics of strains Gsoil 040^T and Gsoil 052^T are summarized in the species descriptions and comparisons of selective characteristics with closely related type strains are shown in Table 1.

Almost complete 16S rRNA gene sequences (1521 and 1525 bp, respectively) for strains Gsoil 040^T and Gsoil 052^T were determined and were subjected to comparative analysis. Phylogenetic analysis based on 16S rRNA gene sequences indicated that both novel strains belong to the genus *Chitinophaga* (Fig. 1). However, the 16S rRNA gene sequence similarity between strains Gsoil 040^T and Gsoil 052^T was 93.3 %, suggesting that they represent separate species (Stackebrandt & Goebel, 1994). Strain Gsoil 040^T showed highest 16S rRNA gene sequence similarity to *C. arvensicola* IAM 12650^T (96.5 %), followed by *C. japonensis* IFO 16041^T (94.5 %), *C. sancti* IFO 15057^T (93.4 %), *C. filiformis* IFO 15056^T (93.4 %), *C. skermanii* CC-SG1B^T (92.0 %) and *C. pinensis* UQM 2034^T (91.9 %). These values (<97 %) were low enough, according to Stackebrandt & Goebel (1994), to place strain Gsoil 040^T as a novel species in the genus *Chitinophaga*. Strain Gsoil 052^T showed highest 16S rRNA gene sequence similarity with *C. filiformis* IFO 15056^T (99.6 %), followed by *C. pinensis* UQM 2034^T (95.9 %), *C. sancti* IFO 15057^T (94.8 %), *C. japonensis* IFO 16041^T (94.2 %), *C. skermanii* CC-SG1B^T (94.1 %) and *C. arvensicola* IAM 12650^T (91.2 %). Strain Gsoil 052^T and *C. filiformis* shared a low DNA–DNA relatedness value of 38 %, which is well below the threshold (70 %) recommended for

Table 1. Phenotypic characteristics of strains Gsoil 040^T, Gsoil 052^T and related type strains

Strains: 1, *C. ginsengisegetis* sp. nov. Gsoil 040^T; 2, *C. ginsengisoli* sp. nov. Gsoil 052^T; 3, *C. arvensicola* DSM 3695^T; 4, *C. japonensis* DSM 13484^T; 5, *C. sancti* DSM 784^T; 6, *C. filiformis* CCUG 12809^T; 7, *C. skermanii* CC-SG1B^T; 8, *C. pinensis* DSM 2588^T. All the strains were isolated from soil. None of the strains showed acid production from glucose or H₂S production. All strains gave positive results for the assimilation of D-mannose and D-maltose. Data for taxa 3–8 are taken from Kämpfer *et al.* (2006). The carbon assimilation tests were read after 48 h incubation. +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8
Pigment	Yellow	Yellow	Yellow–orange	Yellow–orange	Golden yellow	Golden yellow	Yellow	Yellow
Cell length (µm)	1.1–1.3	1.2–1.6	0.6–4	2–18	2–15	30–80	1–2	<40
Gliding movement	–	–	–	+	+	+	–	+
Oxidase	–	+	+	+	ND	+	+	+
Catalase	–	+	+	+	–	–	+	+
Growth at 37 °C	+	+	–	+	–	+	+	+
Highest NaCl tolerance (% w/v)	2.0	<1.0	2.0	2.0	1.0	0.3	ND	ND
Urease	+	+	–	ND	ND	ND	–	+
Gelatin liquefaction	+	+	–	+	+	+	+	+
Chitin degradation	–	+	–	–	–	+	ND	+
Assimilation of:								
<i>N</i> -Acetyl-D-glucosamine	+	+	+	+	–	–	+	+
L-Arabinose	+	+	+	+	+	–	–	+
Gluconate	+	+	–	–	+	–	–	–
α-D-Melibiose	+	+	+	+	+	+	+	–
L-Rhamnose	+	+	+	+	–	–	–	+
D-Ribose	–	–	+	–	–	–	–	–
Sucrose	+	+	+	+	–	+	–	+
Salicin	+	+	+	+	–	+	+	+
Inositol	+	+	–	–	–	–	–	–
DNA G + C content (mol%)	47.1	48.4	46.0	49.8	43.3	45.0	40.7	45.2

*Positive after 7 days incubation.
†Positive after 2 weeks incubation.

the delineation of bacterial species (Wayne *et al.*, 1987). It is clear from DNA–DNA hybridization experiments that strain Gsoil 052^T belongs to a separate genomic species in the genus *Chitinophaga*.

The DNA G + C contents of strains Gsoil 040^T and Gsoil 052^T were 47.1 and 48.4 mol%, respectively. The major respiratory quinone was MK-7. The major polyamine of both strains was homospermidine, as is the case for the other recognized species of the genus *Chitinophaga* (Kämpfer *et al.*, 2006). As shown in Table 2, the major fatty acids of both strains were iso-C_{15:0}, C_{16:1}ω5c and the hydroxy fatty acids were iso-C_{17:0} 3-OH and iso-C_{15:0} 3-OH, which are consistent with those found in other species of the genus *Chitinophaga* (Kämpfer *et al.*, 2006). However, qualitative and quantitative differences in the fatty acid contents could be used to distinguish strains Gsoil 040^T and Gsoil 052^T from the other species in the genus *Chitinophaga* (Table 2).

On the basis of these data and the observations described above, both strains Gsoil 040^T and Gsoil 052^T should be assigned to the genus *Chitinophaga* as representing two novel species, for which the names *Chitinophaga*

ginsengisegetis sp. nov. and *Chitinophaga ginsengisoli* sp. nov. are proposed.

Description of *Chitinophaga ginsengisegetis* sp. nov.

Chitinophaga ginsengisegetis (gin.seng.i.se.ge.ti's. N.L. n. *ginsengum* ginseng; L. n. *seges segetis* a field; N.L. gen. n. *ginsengisegetis* of a ginseng field, the source of the organism).

Cells are Gram-negative, aerobic, non-motile and rod-shaped. Cells are 0.4–0.6 µm in width and 1.1–1.3 µm in length after 3 days culture on R2A agar. Colonies grown on R2A agar for 3 days are smooth, circular, convex, transparent and yellowish. Grows well at temperatures of 15–42 °C and at pH 5.5–8.5, but grows slowly at 4 °C. Growth occurs in the absence of NaCl and in the presence of 2.0 % (w/v) NaCl, but growth does not occur at 3 % (w/v) NaCl. Does not reduce nitrate. Anaerobic growth does not occur. Does not degrade DNA, chitin or xylan. Substrate utilization, enzyme production and other physiological characteristics are shown in Table 1. In addition to the data in Table 1, also able to utilize *N*-acetyl-D-glucosamine,

Fig. 1. Phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of *Chitinophaga ginsengisegetis* sp. nov. Gsoil 040^T and *Chitinophaga ginsengisoli* sp. nov. Gsoil 052^T with other related species. This tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) with a Kimura (1983) two-parameter distance matrix and pairwise deletion. Bootstrap values (expressed as percentages of 1000 replications) greater than 60% are shown at the branch points. Bar, 0.02 substitutions per 1 nucleotide position.

arabinose, L-fucose, glucose, maltose, mannose, D-melibiose, L-proline, rhamnose, salicin, D-sorbitol and sucrose as sole carbon sources. Positive results for the utilization of L-alanine, gluconate, histidine, inositol and L-serine are found after 7 days of incubation. Utilization of 5-ketogluconate and malate is positive after 14 days of incubation. Does not utilize acetate, adipate, caprate, citrate, glycogen, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, 2-ketogluconate, itaconate, L-lactate, malonate, mannitol, phenylacetate, propionate, D-ribose, D-sorbitol, suberate or valerate. According to the API ZYM gallery, produces *N*-acetyl- β -glucosaminidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), α -fucosidase, α -galactosidase, α -glucosidase, leucine arylamidase, trypsin and valine arylamidase, but not chymotrypsin, esterase lipase (C8), β -galactosidase, β -glucosidase, β -glucuronidase, lipase (C14), α -mannosidase or naphthol-AS-BI-phosphohydrolase. According to the API 20 NE gallery, gives positive results for arginine dihydrolase, β -galactosidase and urease and is weakly positive for glucose fermentation and gelatin hydrolysis. Negative results for nitrate reduction and tryptophan degradation. Flexirubin pigmentation is positive. MK-7 is the predominant

menaquinone and iso-C_{15:0} and C_{16:1} ω 5c are the predominant cellular fatty acids. The G+C content of the genomic DNA is 47.1 mol%.

The type strain, Gsoil 040^T (=KCTC 12654^T=DSM 18108^T) was isolated from the soil of a ginseng field in Pocheon province, South Korea.

Description of *Chitinophaga ginsengisoli* sp. nov.

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

Cells are Gram-negative, aerobic, non-motile and rod-shaped. Cells are 0.6–0.8 μ m in diameter and 1.2–1.6 μ m in length after 3 days culture on R2A agar. Colonies grown on R2A agar for 3 days are smooth, circular, convex, transparent and yellowish. Grows well at temperatures of 15–42 °C and at pH 5.5–8.5. Growth occurs in the absence of NaCl and in the presence of <1.0% (w/v) NaCl. Does not reduce nitrate. Anaerobic growth does not occur. Able to degrade chitin, but not DNA or xylan. Substrate utilization,

Table 2. Cellular fatty acid profiles of strains Gsoil 040^T, Gsoil 052^T and related type strains of species of the genus *Chitinophaga*

Strains: 1, *C. ginsengisegetis* sp. nov. Gsoil 040^T; 2, *C. ginsengisoli* sp. nov. Gsoil 052^T; 3, *C. arvensicola* DSM 3695^T; 4, *C. japonensis* DSM 13484^T; 5, *C. sancti* DSM 784^T; 6, *C. filiformis* CCUG 12809^T; 7, *C. skermanii* CC-SG1B^T; 8, *C. pinensis* DSM 2588^T. Data for taxa 3–8 are from Kämpfer *et al.* (2006). Summed feature 2 comprises any combination of C_{12:0} aldehyde, C_{14:0} 3-OH and/or iso-C_{16:1}. Summed feature 3 comprises any combination of iso-C_{15:0} 2-OH and/or C_{16:1}ω7c. Summed feature 4 comprises any combination of iso-C_{17:1} I and/or anteiso-C_{17:1} B. Values in bold type denote the major cellular fatty acids.

	1	2	3	4	5	6	7	8
Unknown 13.565		2.1	3.6	3.1	3.2	2.5	4.4	2.6
C _{14:0}	2.1	0.7	1.4	0.5	0.7	0.9	1.8	0.7
C _{15:0} 2-OH			0.3					
iso-C _{15:0} 3-OH	3.7	2.8	3.0	2.4	3.4	3.3	2.6	3.1
iso-C _{15:0}	42.5	38.1	35.3	40.0	44.0	37.3	47.3	30.4
anteiso-C _{15:0}	0.7	0.7	0.6	0.5				
Unknown 11.543			0.5				0.8	
C _{15:0}			0.4				0.4	0.4
Unknown 14.959			0.3				0.4	0.3
Summed feature 2		0.6	0.4	0.5			0.4	
iso-C _{17:1} ω9c			0.3		1.1			
Summed feature 3	3.1	6.9	3.7	3.3	16.0	11.2	3.5	7.7
Summed feature 4				1.3	0.8		0.4	
C _{16:0} 10 methyl			0.4	0.9				
C _{16:1} ω11c			0.5	1.0	0.9		0.4	1.9
C _{16:1} ω5c	29.4	29.0	33.6	22.2	13.5	25.5	24.4	33.2
C _{16:0} 2-OH				3.0	0.7	1.1		0.7
C _{16:0}	5.6	4.8	5.1	3.0	4.2	4.3	3.5	4.2
iso-C _{16:0}			0.3					
iso-C _{16:0} 3-OH			0.5	0.4			0.4	0.4
C _{16:0} 3-OH	2.1	1.5	1.7	0.8	0.5	1.2	1.6	1.2
anteiso-C _{17:0}		0.4		0.4				
C _{17:0} 2-OH			0.4	0.4				
iso-C _{17:0}		0.7	0.4	1.8	0.6		0.6	0.4
Unknown 16.582	0.3	0.4	0.8	1.1	1.3	0.9	0.7	1.1
iso-C _{17:0} 3-OH	8.5	11.3	5.9	13.0	9.1	11.8	5.0	11.5
C _{18:0}	1.2							
C _{18:3} ω6c	0.9							

enzyme production and other physiological characteristics are indicated in Table 1. In addition to the data in Table 1, can also utilize *N*-acetyl-D-glucosamine, arabinose, glucose, maltose, mannose, D-melibiose, L-proline, salicin, D-sorbitol and sucrose as sole carbon sources. Positive results are also found for gluconate, inositol and rhamnose after 7 days incubation. After 14 days incubation, gives a positive result for L-fucose and 5-ketogluconate. Does not utilize acetate, adipate, L-alanine, caprate, citrate, glycogen, histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, itaconate, 2-ketogluconate, L-lactate, malate, malonate, mannitol, phenylacetate, propionate, D-ribose, L-serine, D-sorbitol, suberate or valerate. According to the API ZYM gallery, gives positive results for *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8),

α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, leucine arylamidase, trypsin and valine arylamidase and is weakly positive for chymotrypsin and β-glucosidase. Negative for β-glucuronidase, lipase (C14), naphthol-AS-BI-phosphohydrolase and α-mannosidase. According to the API 20NE gallery, gives positive results for arginine dihydrolase, β-galactosidase, gelatin hydrolysis, glucose fermentation and urease, but is negative for nitrate reduction and tryptophan degradation. Flexirubin pigmentation is positive. MK-7 is the predominant menaquinone and iso-C_{15:0} and C_{16:1}ω5c are the predominant cellular fatty acids. The G + C content of the genomic DNA is 48.4 mol%.

The type strain, Gsoil 052^T (=KCTC 12592^T=DSM 18017^T) was isolated from the soil of a ginseng field in Pocheon province, South Korea.

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