Segetibacter koreensis gen. nov., sp. nov., a novel member of the phylum *Bacteroidetes*, isolated from the soil of a ginseng field in South Korea

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A novel strain, designated Gsoil 664^T, isolated from the soil of a ginseng field in South Korea, was characterized by a polyphasic approach to clarify its taxonomic position. The isolate was Gram-negative, strictly aerobic, heterotrophic, non-motile, non-spore-forming and possessed rod-shaped cells. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the novel isolate formed a cluster with several uncultured bacterial clones and with the established genera *Terrimonas*, *Niastella* and *Chitinophaga* in the phylum *Bacteroidetes*. However, the isolate was clearly separated from these genera: the gene sequence similarities with respect to the type strains of recognized species from closely related genera ranged from 86.7 to 90.7 %. The G+C content of the genomic DNA was 40.4 mol%. The predominant isoprenoid quinone was MK-7. The major fatty acids were iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{15:1} and C_{16:1}ω5c. The results of physiological and biochemical tests allowed the genotypic and phenotypic differentiation of strain Gsoil 664^T from recognized species of related genera. On the basis of the polyphasic evidence, Gsoil 664^T represents a novel genus and species, for which the name *Segetibacter koreensis* gen. nov., sp. nov. is proposed. The type strain of *S. koreensis* is Gsoil 664^T (=KCTC 12655^T=DSM 18137^T).

Bacteria constituting the *Cytophaga*–*Flavobacterium*–*Bacteroides* group, also referred to as the phylum *Bacteroidetes* (Garrrity & Holt, 2001), are widely distributed over a diverse range of ecological niches. Although they are dominant in marine environments (Bowman *et al.*, 1997), they are also found in soil, fresh water, plants and in the air (Buczolits *et al.*, 2002). During the course of a study on the culturable bacterial community of soil from a ginseng field in South Korea, a large number of novel bacterial strains were isolated (Im *et al.*, 2005). One of these isolates, Gsoil 664^T, was found to be a member of the phylum *Bacteroidetes* and was subjected to further taxonomic investigation. On the basis of the results obtained in this study, we propose that strain Gsoil 664^T represents a new genus and novel species.

Strain Gsoil 664^T was isolated from soil from a ginseng field in Pocheon Province, South Korea. The soil sample was thoroughly suspended with 50 mM phosphate buffer (pH 7.0) and the suspension was then spread on one-fifth-strength modified R2A agar plates (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, 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 K₂HPO₄, 0.05 g MgSO₄, 0.05 g CaCl₂ and 15 g agar) after serial dilution with 50 mM phosphate buffer (pH 7.0). The plates were incubated at 30 °C for 1 month. Single colonies on the plates were purified by transferring them onto new plates for further incubation on full-strength modified R2A or one-half-strength modified R2A. The purified colonies were tentatively identified using partial sequences of the 16S rRNA gene (Im *et al.*, 2005). As strain Gsoil 664^T could grow well both on full-strength modified R2A agar and on commercial R2A agar (Difco), it was routinely cultured on R2A agar or one-half-strength R2A agar (Difco) at 30 °C and maintained as a glycerol suspension (20 %, w/v) at –70 °C.

The Gram-stain reaction was performed using the non-staining method, as described by Buck (1982). Cell morphology was observed at ×1000 magnification with a light microscope (Nikon), using cells grown for 3 days at 30 °C on R2A agar. Catalase activity was determined by assessing bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) tetramethyl-*p*-phenylenediamine. Carbon-source utilization and enzyme activities

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 664^T is AB267478.

were tested by using the API 20NE, API ID 32GN and API ZYM test kits (bioMérieux). The ability to use nitrate as an alternative terminal electron acceptor was tested in serum bottles by adding sodium thioglycolate (1 g l^{-1}) to R2A broth and substituting the upper airspace with nitrogen gas. The anaerobic nitrate-reduction test for determining the final electron acceptor was performed in serum bottles by adding sodium thioglycolate (1 g l^{-1}) and 10 mM KNO_3 to R2A broth, under nitrogen gas. Tests for the degradation of DNA [performed by flooding DNase agar (Scharlau) plates with 1 M HCl], casein, chitin, starch (Atlas, 1993), xylan and cellulose (Ten *et al.*, 2004) were performed and evaluated after 10 days. Growth at various temperatures (4, 15, 20, 25, 30, 37 and 42°C) and pH values (pH 5.0–10.0, in increments of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested on R2A agar supplemented with 1–10% (w/v) NaCl after 5 days incubation. Growth on nutrient agar, trypticase soy agar (TSA) and MacConkey agar was also evaluated at 30°C .

Strain Gsoil 664^T was strictly aerobic, Gram-negative, heterotrophic and comprised non-motile, non-spore-forming, rod-shaped cells. The strain did not grow on nutrient agar, MacConkey agar or TSA, whereas it grew weakly on one-tenth-strength TSA. The ability to reduce nitrate was absent. Other physiological characteristics of Gsoil 664^T are summarized in the species description and comparisons of selective characteristics with those of closely related type strains are shown in Table 1.

For measurement of the G + C content of the chromosomal DNA, genomic DNA of the novel strain was extracted and purified, as described by Moore & Dowhan (1995), and enzymically degraded into nucleosides. The G + C content was then determined as described by Mesbah *et al.* (1989), using reversed-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), evaporated under vacuum conditions and then re-extracted in n-hexane/water (1 : 1, v/v). The crude n-hexane-quinone solution was purified using Sep-Pak Vac silica cartridges (Waters) and was subsequently analysed by HPLC as described previously (Hiraishi *et al.*, 1996). The cellular fatty acid profile was determined from biomass harvested from one-tenth-strength TSA (Difco) after 3 days incubation. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). Fatty acids were analysed with a GC (model 6890; Hewlett Packard) and identified by using the Microbial Identification software package (Sasser, 1990).

The G + C content of the genomic DNA of strain Gsoil 664^T was 40.4 mol%, a value that is lower than those of the most phylogenetically related genera (47.2–48.9 mol% for the genus *Terrimonas* and 44.3–45.8 mol% for the genus *Niastella*). The major respiratory quinone was MK-7. As shown in Table 2, the major fatty acids of this strain were iso-C_{15:0} (23.4%), iso-C_{17:0} 3-OH (14.5%), iso-C_{15:1}

(14.4%) and C_{16:1}ω5c (12.5%). The presence of iso-C_{13:0} 3-OH, C_{18:0} and summed feature 4, and the absence of iso-C_{15:0} 3-OH, C_{16:0} 3-OH, and an unknown fatty acid with an equivalent chain-length of 16.582, together with some quantitative differences in the fatty acid composition, served to distinguish strain Gsoil 664^T from other recognized species in phylogenetically related genera (Table 2).

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 the CLUSTAL_X program (Thompson *et al.*, 1997), gaps were edited in the BioEdit program (Hall, 1999) and evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed using a neighbour-joining method (Saitou & Nei, 1987) with the MEGA3 program (Kumar *et al.*, 2004); bootstrap percentages were based on 1000 replications (Felsenstein, 1985).

An almost-complete 16S rRNA gene sequence (1479 bp) for strain Gsoil 664^T was determined and subjected to comparative analysis. A phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate formed a cluster with several uncultured bacterial clones and with the established genera *Terrimonas*, *Niastella* and *Chitinophaga* (phylum *Bacteroidetes*). However, it was difficult to allocate the isolate to any of these previously established genera (Fig. 1). The levels of 16S rRNA gene sequence similarity between strain Gsoil 664^T and the type strains of recognized species of these genera ranged from 86.7 to 90.7%. The unique phylogenetic position of the novel isolate, shown in Fig. 1, and the low levels of gene sequence similarity with respect to any other recognized related bacterial species demonstrate that strain Gsoil 664^T represents a novel genus.

On the basis of the data and observations described above, it is appropriate to conclude that strain Gsoil 664^T represents a new genus and novel species of the phylum *Bacteroidetes*, for which the name *Segetibacter koreensis* gen. nov., sp. nov. is proposed.

Description of *Segetibacter* gen. nov.

Segetibacter (Se.ge'ti.bac'ter. L. n. *seges* -etis soil; N.L. n. *bacter* a rod; N.L. masc. n. *Segetibacter* rod from soil).

Gram-negative, oxidase-positive and catalase-positive. Strictly aerobic and chemoheterotrophic. Nitrate is not reduced to nitrite. The major fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{15:1} and C_{16:1}ω5c. MK-7 is the predominant respiratory quinone. Positioned phylogenetically in the phylum *Bacteroidetes*. The type species is *Segetibacter koreensis*.

Table 1. Biochemical characteristics of strain Gsoil 664^T and related taxa

Strains: 1, Gsoil 664^T; 2, *Terrimonas lutea* DY^T; 3, *Terrimonas ferruginea* ATCC 13524^T; 4, *Niastella koreensis* GR20-10^T; 5, *Niastella yeongjuensis* GR20-13^T; 6, *Chitinophaga japonensis* IFO 16041^T; 7, *Chitinophaga arvensicola* IAM 12650^T; 8, *Chitinophaga sancti* IFO 15057^T; 9, *Chitinophaga filiformis* IFO 15056^T; 10, *Chitinophaga skermanii* CC-SG1B^T; 11, *Chitinophaga pinensis* UQM 2034^T. Data in columns 2–11 are from Xie & Yokota (2006), Weon *et al.* (2006) and Kämpfer *et al.* (2006). All of the strains are negative for the assimilation of inositol. +, Positive; –, negative; w, weak reaction; v, variable among studies; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Pigment	Yellow	Red or yellow	Yellowish orange	Light yellow	Milky	Yellowish orange	Yellowish orange	Golden yellow	Golden yellow	Yellow	Yellow
Cell length (µm)	1–2	1–2	0.6–4	10–50	10–40	2–18	0.6–4	2–15	30–80	1–2	<40
Gliding movement	–	–	–	+	+	+	–	+	+	–	+
Major fatty acids (>20 %)	iso-C _{15:0}	iso-C _{15:0} , iso-C _{15:1}	iso-C _{15:0} , iso-C _{15:1}	iso-C _{15:0} , iso-C _{17:0} 3-OH	iso-C _{15:0} , iso-C _{17:0} 3-OH	iso-C _{15:0} , C _{16:1} ω5c	iso-C _{15:0} , C _{16:1} ω5c	iso-C _{15:0}	iso-C _{15:0} , C _{16:1} ω5c	iso-C _{15:0} , C _{16:1} ω5c	iso-C _{15:0} , C _{16:1} ω5c
Oxidase	+	+	+	–	+	+	+	ND	+	+	+
Catalase	+	w	+	–	–	+	+	–	+	+	–
Hydrolysis of:											
Casein	–	ND	–	+	+	–	ND	ND	–	ND	+
Chitin	–	–	–	+	+	–	ND	–	–	ND	+
DNA	–	ND	–	–	–	+	+	ND	+	ND	–
Gelatin	–	+	–	+	+	+	v	+	w	+	+
Starch	–	ND	+	–	–	–	v	+	–	ND	–
Urea	+	–	–	–	–	ND	–	ND	ND	–	+
Growth at 37 °C	–	+	+	+	–	+	–	–	+	+	+
Highest NaCl conc. (% w/v) tolerated	3.0	1.0	1.0	ND	ND	2.0	2.0	1.0	0.3	ND	ND
Assimilation of:											
N-Acetyl-D-glucosamine	+	+	–	–	–	+	+	–	–	+	+
L-Arabinose	–	+	–	–	–	+	+	+	–	–	+
Gluconate	–	–	–	–	–	–	–	+	–	–	–
D-Mannose	+	+	+	–	–	+	+	+	+	+	+
Maltose	+	+	+	–	–	+	+	+	+	+	+
α-D-Melibiose	w	+	–	–	–	+	+	+	+	+	–
L-Rhamnose	+	–	+	–	–	+	+	–	–	–	+
D-Ribose	–	–	–	–	–	–	+	–	–	–	–
Sucrose	–	+	–	–	–	+	+	–	+	–	+
Salicin	+	–	–	–	–	+	+	–	+	+	+
Nitrate reduction	–	–	–	–	–	+	v	+	+	ND	–
Major menaquinone	MK-7	MK-7	MK-7	MK-7	MK-7	MK-7	MK-7	MK-7	MK-7	MK-7	ND
DNA G+C content (mol%)	40.4	47.2	48.9	45.8	44.3	49.8	43–46	46–47	46–47	40.7	42–46

Table 2. Fatty acid profiles of strain Gsoil 664^T and related taxa

Strains: 1, Gsoil 664^T; 2, *T. lutea* DY^T; 3, *T. ferruginea* ATCC 13524^T; 4, *N. koreensis* GR20-10^T; 5, *N. yeongjuensis* GR20-13^T; 6, *C. japonensis* IFO 16041^T; 7, *C. arvensicola* IAM 12650^T; 8, *C. sancti* IFO 15057^T; 9, *C. filiformis* IFO 15056^T; 10, *C. skermanii* CC-SG1B^T; 11, *C. pinensis* UQM 2034^T. Data for taxa 2–3, 4–5 and 6–11 are from Xie & Yokota (2006), Weon *et al.* (2006) and Kämpfer *et al.* (2006), respectively. Fatty acids that account for <1.0 % of the total are not shown. Major fatty acids are shown in bold type.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
Saturated:											
C _{14:0}	1.0						1.4			1.8	
C _{15:0}	1.1		2.7								
C _{16:0}	8.5	1.1	1.7	2.6	2.9	3.0	5.1	4.2	4.3	3.5	4.2
C _{18:0}	1.9										
Unsaturated:											
C _{16:1} ω5c	12.5				1.2	22.2	33.6	13.5	25.5	24.4	33.2
C _{16:1} ω11c						1.0					1.9
Branched-chain:											
iso-C _{13:0} 3-OH	1.6										
iso-C _{14:0}		4.3	1.1								
iso-C _{15:0}	23.4	34.8	28.4	26.8	30.6	40.0	35.3	44.0	37.3	47.3	30.4
iso-C _{15:0} 3-OH		2.3	2.2	1.3	1.9	2.4	3.0	3.4	3.3	2.6	3.1
iso-C _{15:1}	14.4	21.8	26.2	15.6	14.7						
iso-C _{16:0}			1.4	1.0			0.3				
iso-C _{17:0}				1.6							
iso-C _{17:0} 3-OH	14.5	14.2	15.3	29.4	27.1	13	5.9	9.1	11.8	5	11.5
iso-C _{17:1} ω9c								1.1			
anteiso-C _{15:0}	3.5			4.9	3.3						
Hydroxy:											
C _{16:0} 2-OH						3.0			1.1		
C _{16:0} 3-OH		3.3	2.5	1.3	2.3		1.7		1.2	1.6	1.2
C _{17:0} 2-OH				3.5	2.6						
C _{17:0} 3-OH				1.2							
Unknown:*											
ECL 13.565	2.1	3.2	1.3			3.1	3.6	3.2	2.5	4.4	2.6
ECL 16.582		1.8	1.3	1.4	1.6	1.1		1.3	0.9		1.1
Summed features:											
3	6.6	14.9	11.2	4.3	5.3	3.3	3.7	16.0	11.2	3.5	7.7
4	3.9					1.3					

*Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified; equivalent chain-lengths are given. Summed feature 3 comprises any combination of iso-C_{15:0} 2-OH and/or C_{16:1}ω7c and summed feature 4 comprises any combination of iso-C_{17:1} I and/or anteiso-C_{17:1} B.

Description of *Segetibacter koreensis* sp. nov.

Segetibacter koreensis (ko.re.en'sis. N.L. masc. adj. *koreensis* of Korea, from where the novel organism was isolated).

Possesses the following properties in addition to those given in the genus description. Cells are 1.0–1.3 × 1.6–2.0 µm in size. Colonies on R2A agar plates at 30 °C are circular, convex, non-luminescent and yellow. On R2A agar medium, the strain is able to grow at 15–30 °C, but not at 4 or 37 °C. Growth occurs at pH 5.5–8.5 and at NaCl concentrations of up to 3%; growth is optimal at pH 7.0 in the absence of NaCl. *N*-acetyl-D-glucosamine, L-fucose, glucose, histidine, maltose, mannose, D-melibiose, L-proline, rhamnose and salicin are assimilated. Positive for

the hydrolysis of aesculin, urea and arginine dihydrolase. Negative reactions are observed for β-galactosidase activity, indole production, glucose fermentation and the assimilation of acetate, adipate, L-alanine, L-arabinose, caprate, citrate, gluconate, glycogen, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, inositol, itaconate, 2-ketogluconate, 5-ketogluconate, lactate, malate, malonate, mannitol, phenylacetate, propionate, D-ribose, L-serine, D-sorbitol, suberate, sucrose and valerate. According to the API ZYM gallery, gives a positive reaction in tests for *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, α-galactosidase and leucine arylamidase; weakly positive for α-glucosidase, β-glucosidase and α-fucosidase and negative for chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8),

Fig. 1. Rooted phylogenetic tree based on 16S rRNA gene sequences of strain Gsoil 664^T and related bacteria in the phylum *Bacteroidetes*. This tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) with a two-parameter distance matrix Kimura (1983) and pairwise deletion. Bootstrap percentages (of 1000 replications) greater than 70% are shown at the branch points. Bar, 20 nucleotide substitutions per 1000 nucleotides.

β -galactosidase, β -glucuronidase, lipase (C14), α -mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase. The DNA G+C content is 40.4 mol%.

The type strain, Gsoil 664^T (=KCTC 12655^T=DSM 18137^T), was isolated from the soil of a ginseng field in Pocheon Province, South Korea.

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