Emticicia ginsengisoli sp. nov., a species of the family 'Flexibacteraceae' isolated from soil of a ginseng field

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A taxonomic study was carried out on Gsoil 085^T, a bacterial strain isolated from a soil sample from a ginseng field in Pocheon Province (South Korea). Comparative 16S rRNA gene sequence studies showed a clear affiliation of this bacterium to the family 'Flexibacteraceae' and indicated that the closest relative was *Emticicia oligotrophica* GPTSA100-15^T (94.6 % sequence similarity). Lower sequence similarities (<83.0 %) were found with respect to all other recognized species of the family 'Flexibacteraceae'. Strain Gsoil 085^T was found to be Gram-negative, strictly aerobic, non-motile and rod-shaped. It grew well on half-strength R2A medium but did not utilize a broad range of carbon sources. The G + C content of the genomic DNA was 40.5 mol%. The major fatty acids were summed feature 4 (iso-C_{15:0} 2-OH and/or C_{16:1ω7c}, 45.4 %), iso-C_{15:0} (13.9 %), C_{16:1ω5c} (8.6 %) and C_{16:0} (8.2 %). On the basis of evidence from the polyphasic study, strain Gsoil 085^T represents a novel species of the genus *Emticicia*, for which the name *Emticicia ginsengisoli* sp. nov. is proposed. The type strain is Gsoil 085^T (=KCTC 12588^T =LMG 23396^T).

The *Cytophaga*–*Flavobacterium*–*Bacteroidetes* group, which is also known as the phylum *Bacteroidetes* (Ludwig & Klenk, 2001), is considered to comprise organisms associated with the degradation of organic compounds such as complex polysaccharides (Höfle, 1982; Pinhassi *et al.*, 1999). This group of organisms is an important component of marine microbial communities (Glöckner *et al.*, 1999). In recent years, there has been a surge in the number of publications describing novel genera within the phylum *Bacteroidetes*, mainly concerning organisms within the family *Flavobacteriaceae*, including *Aquimarina* (Nedashkovskaya *et al.*, 2005), *Epilithonimonas* (O'Sullivan *et al.*, 2006), *Formosa* (Ivanova *et al.*, 2004), *Gillisia* (Van Trappen *et al.*, 2004), *Kordia* (Sohn *et al.*, 2004), *Persicivirga* (O'Sullivan *et al.*, 2006) and *Ulvibacter* (Nedashkovskaya *et al.*, 2004). Recently, some novel genera outside the family *Flavobacteriaceae*, including *Adhaeribacter* (Rickard *et al.*, 2005), *Hongiella* (Yi & Chun, 2004), *Arcicella* (Nikitin *et al.*, 2004) and *Belliella* (Brettar *et al.*, 2004), have also been described. In addition, a novel family within the phylum

Bacteroidetes was recently described: the family *Cryomorphaceae* contains five new genera, *Brumimicrobium*, *Cryomorpha*, *Crocinitomix*, *Owenweeksia* and *Fluviicola* (Bowman *et al.*, 2003; Lau *et al.*, 2005; O'Sullivan *et al.*, 2005). Most of these newly described bacteria were isolated from marine environments and hypersaline lakes, but a few bacterial strains were isolated from other habitats, such as soil (Reichenbach, 1989).

The genus *Emticicia* was recently described by Saha & Chakrabarti (2006) and currently comprises only one species, *Emticicia oligotrophica*. It was isolated on nutritionally poor medium from warm spring water. It was characterized as a Gram-negative, strictly aerobic, chemo-organotrophic bacterium comprising non-motile, rod- or coccus-shaped cells. The major fatty acid was iso-C_{15:0}, which is also present in many members of the family 'Flexibacteraceae'. On the basis of the 16S rRNA gene sequence, the genus *Emticicia* is affiliated to the family 'Flexibacteraceae' in the phylum *Bacteroidetes*.

During the course of a study on the culturable aerobic and facultatively anaerobic bacterial community in soil from a ginseng field in Pocheon Province, South Korea, a large

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain Gsoil 085^T is AB245370.

number of bacteria were isolated (Im *et al.*, 2005). One of these isolates, strain Gsoil 085^T, was identified as belonging to the family 'Flexibacteraceae' within the phylum *Bacteroidetes* and was the subject of further taxonomic investigation in order to establish its phylogenetic affiliation. Here we report on its taxonomic position, including its genotypic, chemotaxonomic and classical phenotypic characteristics, and propose that strain Gsoil 085^T represents a novel species of the genus *Emticicia*.

Strain Gsoil 085^T was originally isolated from soil from a ginseng field in Pocheon Province, South Korea, as described by Liu *et al.* (2006), using modified R2A agar plates incubated under aerobic conditions. The strain was routinely cultured on R2A agar at 30 °C and maintained as a glycerol suspension (20 %, w/v) at –70 °C.

For the phylogenetic analysis of strain Gsoil 085^T, DNA was extracted using a genomic DNA extraction kit (Core Biosystem), the 16S rRNA gene was amplified with a PCR and sequencing of the purified PCR product was carried out according to Kim *et al.* (2005). The almost-complete 16S rRNA gene sequence was compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database and multiple alignments were created using the CLUSTAL_X program (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were constructed with the neighbour-joining method (Saitou & Nei, 1987) using

the MEGA3 program (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

The length of the almost-complete 16S rRNA gene sequence of strain Gsoil 085^T was 1463 bp (base position 9–1505 with respect to the *Escherichia coli* numbering system). Preliminary sequence comparisons against the 16S rRNA gene sequences deposited in GenBank indicated that the isolate belonged to the family 'Flexibacteraceae' within the phylum *Bacteroidetes*. On the basis of 16S rRNA gene similarities, the closest cultured relative of strain Gsoil 085^T was *E. oligotrophica* GPTSA100-15^T (94.6 %). Lower sequence similarities (<83.0 %) were found with respect to other member species of the family. This relationship between our isolate and other members of the family 'Flexibacteraceae' was also evident in the phylogenetic tree (Fig. 1). DNA–DNA hybridization between strain Gsoil 085^T and its nearest phylogenetic neighbours was not attempted since strains differing by >3.0 % at the 16S rRNA gene level are unlikely to exhibit >70 % relatedness at the whole-genome level (Stackebrandt & Goebel, 1994).

A Gram reaction was performed using the non-staining method, as described by Buck (1982). Cell morphology was observed under a Nikon light microscope at ×1000 magnification, using cells grown for 3 days at 25 °C on R2A agar. Catalase and oxidase tests were performed as outlined by Cappuccino & Sherman (2002). The defined agar medium (containing basal salt media) used for single-carbon-source assimilation studies contained the following (g l^{–1}): K₂HPO₄, 1.8 g; KH₂PO₄, 1.08 g; NaNO₃, 0.5 g;

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strain Gsoil 085^T and its nearest neighbours. Bootstrap percentages (based on 1000 replications) >70 % are shown at branch points. Bar, 5 substitutions per 100 nucleotide positions.

Table 1. Differential characteristics of strain Gsoil 085^T and the nearest relative, *E. oligotrophica* MTCC6937^T

Data for *E. oligotrophica* MTCC6937^T were taken from Saha & Chakrabarti (2006). Both strains were Gram-negative aerobes consisting of non-spore-forming, non-motile, rod-shaped cells. Both strains showed NaCl tolerance of <1 % (w/v) and were positive for the following: nitrate reduction to nitrite; production of catalase and oxidase; growth on R2A, 1/100 LB agar, 1/100 NA, 1/100 TSA, 1/100 MacConkey agar, R3A, 1/100 TSIA and 1/100 MHA; assimilation of raffinose; sensitivity to chloramphenicol, erythromycin, neomycin, novobiocin, streptomycin and tetracycline. Both strains were negative for the following: β -galactosidase activity; degradation of xylan, cellulose and chitin; acid production from mannitol, sorbitol, amygdalin and arabinose; assimilation of D-fucose, ethanol, D-adonitol, dulcitol, D-mannitol, D-sorbitol and xylitol; growth on LB agar, MacConkey agar and TSIA. +, Positive; w, weakly positive; –, negative; R, resistant; S, sensitive; NA, data not available from the sources used.

Characteristic	Gsoil 085 ^T	<i>E. oligotrophica</i> MTCC6937 ^T
Colony colour	Orange	Light pink
Urease activity	–	+
Temperature range for growth (°C)	4–30	15–42
pH range for growth	5.0–8.5	5.0–11.0
Response to antibiotics		
Ampicillin (10 µg)	R	S
Gentamicin (10 µg)	R	S
Kanamycin (30 µg)	R	S
Lincomycin (2 µg)	R	S
Penicillin G (10 U)	R	S
Cephalothin (20 µg)	R	NA
Carbenicillin (6 µg)	R	NA
Utilization as sole carbon sources		
D-Galactose	–	w
D-Glucose	–	w
Starch	–	+
D-Fructose	–	w
D-Arabinose	–	w
L-Arabinose	–	w
Cellobiose	–	+
Sucrose	+	w
Amygdalin	–	+
Glycerol	–	w
Acid production from glucose, sucrose and melibiose	–	+
Growth on NA, TSA and MHA	+	–
DNA G + C content (mol%)	40.5	36.9

NH₄Cl, 0.5 g; KCl, 0.1 g; MgSO₄, 0.1 g; CaCl₂, 0.05 g. A vitamin solution (Widdel & Bak, 1992), a trace element solution (SL-10; Widdel *et al.*, 1983) and a selenite/tungstate solution (Tschech & Pfennig, 1984) were added to the medium, and the pH was adjusted to 6.8. Filter-sterilized carbon sources (each at 0.1 %, w/v) were added to this medium. Plates were incubated for 7 days at 25 °C and inspected visually. Negative control media did not contain any added carbon source. A positive control culture was grown on R2A medium. Some physiological characteristics were determined with API 20E galleries according to the instructions of the manufacturer (bioMérieux). Anaerobic growth was performed in serum bottles by adding thioglycolate (1 g l⁻¹) to R2A broth and replacing the upper air layer with nitrogen gas. Nitrate- and

nitrite-reduction tests were performed in serum bottles containing R2A broth supplemented with KNO₃ (10 mM) or NaNO₂ (10 mM), respectively. Tests for the degradation of DNA [by flooding DNase agar (Scharlau) plates with 1 M HCl], casein, chitin, starch (Atlas, 1993), lipid (Kouker & Jaeger, 1987), xylan and cellulose (Ten *et al.*, 2004) were performed and were evaluated after 10 days. Growth at different temperatures (4, 15, 20, 25, 30, 37 and 42 °C) and pH (pH 5.0–10.0, in increments of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested on R2A medium supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Growth on nutrient agar (NA), trypticase soy agar (TSA) and MacConkey agar was also evaluated at 30 °C. Growth on NA, TSA, MacConkey agar, R3A agar (Reasoner & Geldreich, 1985), triple-sugar–iron

agar (TSIA), Mueller–Hinton agar (MHA), Luria–Bertani (LB) agar and diluted media, all of which were made nutritionally poor by diluting them with distilled water and solidifying them with 1.5 % agarose, was also evaluated at 25 °C. Sensitivity to antibiotics was tested on R2A plates, using antibiotic discs containing the following amounts (µg, unless indicated otherwise): streptomycin, 2, 5, 10 and 20; penicillin, 2, 5, 10 and 20 U; chloramphenicol, 5, 10, 30 and 50; ampicillin, 2, 5, 10 and 20; cephalothin, 2, 6, 10 and 20; gentamicin, 2, 5, 10 and 20; novobiocin, 10, 30, 50 and 100; tetracycline, 1, 3, 5 and 10; kanamycin, 5, 10, 30 and 50; lincomycin, 1, 2, 5 and 10; oleandomycin, 0.6, 1, 3 and 6; neomycin, 10, 20, 30 and 50; carbenicillin, 0.5, 1.5, 4 and 6; erythromycin, 5, 10, 15 and 30.

Cells of strain Gsoil 085^T were Gram-negative, rod-shaped, strictly aerobic, non-spore-forming and non-motile. Colonies grown on R2A agar plates for 3 days were circular, smooth, convex, glossy, orange-coloured and 2–4 mm in diameter. On R2A agar, strain Gsoil 085^T was able to grow at 4–30 °C but not at 37 °C; the optimal growth temperature was 20–25 °C. Physiological characteristics of strain Gsoil 085^T are summarized in the species description and a comparison of selective characteristics with those of its nearest phylogenetic neighbour is given in Table 1.

To measure the G+C content of the chromosomal DNA, genomic DNA of the novel strain was extracted and purified as described by Moore & Dowhan (1995), enzymically degraded into nucleosides and then determined as described by Mesbah *et al.* (1989) using reversed-phase HPLC. Cellular fatty acid profiles were determined for strains grown on R2A plates for 4 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 by gas chromatography (6890; Hewlett Packard) and identified with the Microbial Identification software package (Sasser, 1990).

The G+C content of the genomic DNA of strain Gsoil 085^T was 40.5 mol%. The following cellular fatty acids were present (at more than 1.0 %) in strain Gsoil 085^T: C_{14:0} (1.1 %), C_{15:0} (2.0 %), C_{16:0} (8.2 %), iso-C_{15:0} (13.9 %), iso-C_{15:0} 3-OH (3.7 %), iso-C_{15:1} G (3.8 %), iso-C_{17:0} 3-OH (3.4 %), anteiso-C_{15:0} (1.8 %), C_{16:0} 3-OH (1.2 %), C_{16:1}ω5c (8.6 %), summed feature 3 (C_{14:0} 3-OH and/or iso-C_{16:1} I, 1.1 %), summed feature 4 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c, 45.4 %) and an unknown fatty acid (equivalent chain length of 13.566, 1.6 %). Some qualitative and quantitative differences in fatty acid content could be observed between strain Gsoil 085^T and its phylogenetically closest relative, *E. oligotrophica*, perhaps because of differences in cultivation conditions (Table 2).

On the basis of the data and observations described above, strain Gsoil 085^T should be assigned to the genus *Emticicia* as the type strain of a novel species, for which the name *Emticicia ginsengisoli* sp. nov. is proposed.

Table 2. Comparison of fatty acid compositions (%) of strain Gsoil 085^T and *E. oligotrophica* MTCC6937^T

Data for *E. oligotrophica* MTCC6937^T were taken from Saha & Chakrabarti (2006). –, Not detected.

Fatty acid	Gsoil 085 ^T	<i>E. oligotrophica</i> MTCC6937 ^T
C _{16:0}	8.2	–
iso-C _{15:0}	13.9	42.9
iso-C _{15:0} 3-OH	3.7	5.6
iso-C _{17:0} 3-OH	3.4	12.5
anteiso-C _{15:0}	1.8	12.2
C _{15:1} ω6c	–	5.5
C _{16:1} ω5c	8.6	3.8
Summed feature 4*	45.4	11.3

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 contained iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

Description of *Emticicia ginsengisoli* sp. nov.

Emticicia ginsengisoli (gin.seng.i.so'li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of soil from a ginseng field, the source of the type strain).

Cells are Gram-negative, strictly aerobic, non-spore-forming, non-motile, rod-shaped, 0.3–0.4 µm in diameter and 3–5 µm in length. Grows at temperatures between 4 and 30 °C, with optimum growth at 20–25 °C. The pH growth range is between pH 5.0 and 8.5, with an optimum between pH 6.5 and 7. Growth occurs in the absence of NaCl and in the presence of 1.0 % (w/v) NaCl, but not with 2.0 % (w/v) NaCl. No growth occurs on LB agar, MacConkey agar or TSIA, but can occur on R2A, NA, TSA, MHA, R3A, 1/100 LB agar, 1/100 NA, 1/100 MacConkey agar, 1/100 TSA, 1/100 TSIA, 1/10 MHA and 1/100 MHA. Oxidase- and catalase-positive and reduces nitrate and nitrite under aerobic conditions. Negative for hydrolysis of DNA, casein, collagen, chitin, starch, CM-cellulose, xylan and aesculin. The following substrates are utilized for growth: L-xylose, D-lactose, maltose, melibiose, sucrose, trehalose, raffinose and inulin. The following substrates are not utilized for growth: D-arabinose, L-arabinose, D-glucose, D-galactose, D-mannose, D-fructose, D-xylose, D-fucose, ethanol, L-rhamnose, L-sorbose, D-lyxose, D-ribose, cellobiose, pyruvate, formate, acetate, propionate, 3-hydroxybutyrate, valerate, capric acid, malate, fumarate, phenylacetate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, citrate, lactate, malate, malonate, succinate, glutarate, tartrate, itaconate, adipate, suberate, oxalate, gluconate, dulcitol, inositol, D-adonitol, D-mannitol, D-sorbitol, xylitol, methanol, glycerol, salicin, amygdalin, glycogen, N-acetyl-D-glucosamine, dextran, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, L-histidine, glycine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline,

L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine. In API 20E tests, positive results are obtained in the Voges–Proskauer test and for gelatinase activity, but negative results are obtained for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, hydrogen sulphide production and indole production. Acid is not produced from D-glucose, melibiose, amygdalin, L-arabinose, D-mannitol, inositol, D-sorbitol, L-rhamnose or sucrose. Susceptible to chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (20 µg), lincomycin (5 µg), neomycin (30 µg), novobiocin (30 µg), penicillin G (20 U), streptomycin (5 µg) and tetracycline (1 µg). The major fatty acids are iso-C_{15:0} 2-OH and/or C_{16:1}ω7c, iso-C_{15:0} 3-OH, C_{16:1}ω5c and C_{16:0}. The G+C content of genomic DNA of the type strain is 40.5 mol%.

The type strain, Gsoil 085^T (=KCTC 12588^T =LMG 23396^T), was isolated from soil from a ginseng field in Pocheon Province, South Korea.

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