Niastella koreensis gen. nov., sp. nov. and *Niastella yeongjuensis* sp. nov., novel members of the phylum *Bacteroidetes*, isolated from soil cultivated with Korean ginseng

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Two novel strains, GR20-10^T and GR20-13^T, were isolated from soil using R2A medium. The soil sample was collected from a field in the Yeongju region of Korea that was cultivated with Korean ginseng. Phylogenetic analysis based on 16S rRNA gene sequences indicated that these strains formed a cluster with several uncultured bacterial clones and with *Flexibacter filiformis*, *Flexibacter sancti*, *Flexibacter japonensis*, *Cytophaga arvensicola* and *Flavobacterium ferrugineum* (recently reclassified as *Terrimonas ferruginea*) in the phylum *Bacteroidetes*. The level of 16S rRNA gene sequence similarity between the two novel strains was 98.9%. Isolates GR20-10^T and GR20-13^T showed the highest sequence similarities to *Flexibacter japonensis* IFO 16041^T (91.8 and 91.9%, respectively) and *T. ferruginea* ATCC 13524^T (90.4 and 90.6%, respectively). The whole-cell fatty acid profiles of the two isolates were similar and their major fatty acids were 15:0 iso, 17:0 iso 3-OH and 15:1 iso G. The major isoprenoid quinone of both strains was MK-7. The G + C contents of GR20-10^T and GR20-13^T were 45.8 and 44.3 mol%, respectively. DNA–DNA hybridization (57% DNA–DNA hybridization value) and phenotypic data indicated that strains GR20-10^T and GR20-13^T each belong to a separate species. On the basis of phenotypic and phylogenetic data and genomic distinctiveness, strains GR20-10^T and GR20-13^T represent two novel species in a novel genus in the phylum *Bacteroidetes*; the names *Niastella koreensis* gen. nov., sp. nov. (the type species; type strain GR20-10^T = KACC 11465^T = DSM 17620^T) and *Niastella yeongjuensis* sp. nov. (type strain GR20-13^T = KACC 11466^T = DSM 17621^T) are proposed.

Within the phylum *Bacteroidetes*, the genera *Flavobacterium*, *Flexibacter* and *Cytophaga* are taxonomically heterogeneous. The taxonomic relationships amongst species within these genera have been largely resolved by phylogenetic analysis (Sly *et al.*, 1999; Nakagawa & Yamasato, 1993; Nakagawa *et al.*, 2002). However, it is obvious that some species, for example *Flexibacter filiformis*, *Flexibacter sancti*, *Flexibacter japonensis* and *Cytophaga arvensicola*, form distinct lineages in the phylum *Bacteroidetes* (Sly *et al.*, 1999) and thus

remain misclassified at the genus level. *Flavobacterium ferrugineum*, recently reclassified as *Terrimonas ferruginea* (Xie & Yokota, 2006), was similarly misclassified.

In the course of a study of the bacterial diversity in fields cultivated with Korean ginseng (*Panax ginseng* C. A. Meyer), two novel strains, GR20-10^T and GR20-13^T, were isolated in the Yeongju region, Korea. On the basis of phylogenetic analysis, these strains formed a distinct cluster closely related to *Flexibacter filiformis*, *Flexibacter sancti*, *Flexibacter japonensis*, *Cytophaga arvensicola* and *T. ferruginea*. The phenotypic and genotypic characterization of these two novel strains is described in this report.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains GR20-10^T and GR20-13^T are DQ244077 and DQ244076, respectively.

For observation of cell morphology by transmission electron microscopy (model 912AB; LEO) and phase-contrast microscopy (AXIO; Zeiss), cells were grown on R2A agar (Difco). To investigate basic physiological and biochemical characteristics, the methods of Smibert & Krieg (1994) were used for the following tests: Gram staining, catalase, oxidase, indole production and hydrolysis of agar, casein, DNA and starch. Carboxymethylcellulose (Sigma) (0.1 %) and Whatman powder CF11 (0.1 %) were used for the cellulase test; hydrolysis of alginic acid (0.5 %, w/v), chitin from crab shells (1 %, w/v), pectin (0.5 %, w/v) and tyrosine (0.5 %, w/v) was also tested. Urease was determined as described by MacFaddin (2000). Flexirubin pigments were examined by the method of Fautz & Reichenbach (1980). Congo red adsorption was tested by directly flooding colonies on agar plates with 0.01 % aqueous Congo red solution. Motility was examined in 1/10 strength R2A medium and gliding motility was observed by oil-immersion phase-contrast microscopy of the edge of colonies in exponential phase. Temperature tolerance was tested by growing cells at 5, 10, 15, 20, 25, 30, 33, 35, 37 and 40 °C. Tolerance of different salinity levels was tested by growing cells in R2A broth supplemented with 0, 1, 2, 3, 5 and 7 % (w/v) NaCl. The pH range (pH 4.0–10.0 at intervals of 1.0 pH unit) for growth was determined in R2A broth that was buffered with citrate-phosphate or Tris/HCl buffers (Breznak & Costilow, 1994). Tests in the commercial systems API 20NE, API 50CH, API ID 32 GN and API ZYM (bioMérieux) were generally performed according to the manufacturer's instructions in duplicate. The API ZYM tests were read after 4 h incubation at 37 °C and the other API tests were read after at least 48 h at 28 °C.

Isoprenoid quinones were identified by an HPLC method as described previously (Groth *et al.*, 1996). Fatty acid methyl esters were prepared and extracted from cells grown on R2A medium for 48 h at 28 °C, according to the standard protocol of the Microbial Identification System (MIDI; Microbial ID). The DNA G+C contents were determined according to Mesbah *et al.* (1989) using a reverse-phase column (Supelcosil LC-18-S; Supelco).

DNA–DNA hybridization was carried out as described by Seldin & Dubnau (1985). Probe labelling was conducted using the non-radioactive DIG-High prime system (Roche) and hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified using a densitometer (Bio-Rad).

The 16S rRNA gene sequence was determined by PCR amplification (Kwon *et al.*, 2003) and direct sequencing (Hiraishi, 1992). A BLAST search (Altschul *et al.*, 1997) revealed that the two novel isolates were closely related to several uncultured soil and marine bacterial clones. The 16S rRNA gene sequences retrieved from GenBank were multiply aligned with those of the two novel isolates, approximately 1440 bases, using the program MEGALIGN (DNASTAR). The sequence similarity between the two novel strains was 98.9 %. The two strains showed highest sequence

similarity (96.8 and 96.3 %, respectively) to uncultured bacterial clone E26 (GenBank accession no. AM085477). The closest similarities among the type strains of all known species studied were obtained with *Flexibacter japonensis* IFO 16041^T (GenBank accession no. AB078055; 91.8 and 91.9 %, respectively) and *T. ferruginea* ATCC 13524^T (GenBank accession no. M62798; 90.4 and 90.6 %, respectively); sequences from other type strains showed <90 % sequence similarity. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.0 (Kumar *et al.*, 2004), clustering was determined using the neighbour-joining and maximum-parsimony algorithms and bootstrap analysis (1000 replications) was made to determine the stability of the clusters. The neighbour-joining tree is shown in Fig. 1. A similar result (not shown) was obtained using the maximum-parsimony algorithm. The two novel isolates formed a cluster with three uncultured bacterial clones and *T. ferruginea*. The uncultured clones originated from a sea-sediment core (clone E26), soil (clone OF70; LaMontagne *et al.*, 2003) and air (clone AKIW428). This cluster was related to another compact cluster that was composed of *Flexibacter japonensis*, *Flexibacter sancti*, *Flexibacter filiformis* and *Chitinophaga pinensis*. From the phylogenetic tree, it is clear the two novel isolates were affiliated with the phylum *Bacteroidetes*, but their phylogenetic position indicated that they were distinct from species with validly described names. Moreover, the strains clustered with several other species that are misclassified at the genus level (Nakagawa & Yamasato, 1993; Sly *et al.*, 1999). With their unique phylogenetic position shown in Fig. 1, considering the threshold value of 3 % 16S rRNA gene sequence difference to define a novel genus and species (Stackebrandt & Goebel, 1994), the low similarity of the two novel isolates with respect to any other known bacterial species demonstrates that they represent a novel genus.

Both strains grew well on R2A and nutrient agar (NA; Difco), but did not grow on tryptic soy agar (TSA; Difco) or MacConkey agar (Difco). Cells of both strains were filamentous (Fig. 2), but lacked flagella (data not shown). Whereas strain GR20-10^T formed light-yellow-coloured colonies, those of strain GR20-13^T were milky-coloured. Colonies of both strains were irregular. In the API 50CH tests, neither isolate gave any positive reactions, except for aesculin hydrolysis. The phenotypic differences between the two novel strains and other related genera or species are summarized in Table 1. The differential phenotypic characteristics of GR20-10^T, GR20-13^T and the most closely related species, *T. ferruginea*, are shown in Table 2. In the case of disparity between the results of conventional tests and API kit tests, conventional test results were recorded. The DNA–DNA hybridization value between GR20-10^T and GR20-13^T was 57 %.

The fatty acid profiles of GR20-10^T and GR20-13^T were very similar. The major fatty acids of both strains were 15:0 iso (26.8 and 30.6 % in GR20-10^T and GR20-13^T, respectively), 17:0 iso 3-OH (29.4 and 27.1 %) and 15:1 iso G (15.6 and

Fig. 1. Neighbour-joining tree showing the phylogenetic relationships of 16S rRNA gene sequences (*Escherichia coli* positions 50–1483) of strains GR20-10^T and GR20-13^T and their closest relatives in the DNA data-bases. *E. coli* (strain unknown) was used as an outgroup to root the tree. Numbers at nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets. Bootstrap values below 50 % are not indicated. Bar, 5 substitutions per 100 nt.

14.7 %) (Table 3). *T. ferruginea* DSM 30193^T could be differentiated from the two novel isolates by the presence of the unknown 13.565 (4.7 %). The major isoprenoid quinone of both strains was MK-7. The DNA G + C contents of GR20-10^T and GR20-13^T were 45.8 and 44.3 mol%, respectively.

On the basis of our results, it is proposed that isolates GR20-10^T and GR20-13^T represent two separate, novel species in a new genus, *Niastella koreensis* gen. nov., sp. nov. and *Niastella yeongjuensis* sp. nov., respectively.

Description of *Niastella* gen. nov.

Niastella (Ni.as.tel'la. L. dim. suff. -ella; N.L. fem. n. *Niastella* arbitrary name after NIAST, the National Institute of Agricultural Science and Technology, where taxonomic studies of this taxon were conducted).

Fig. 2. Phase-contrast micrograph of cells of strain GR20-10^T after growth for 2 days at 28 °C on R2A medium. Bar, 10 μm.

Cells are Gram-negative, aerobic, non-flagellated, gliding and filamentous. Colonies are irregular. Grow on R2A and NA, but not on TSA or MacConkey agar. Flexirubin pigments are not formed, Congo red is not absorbed and nitrates are not reduced. Positive for hydrolysis of chitin, carboxymethylcellulose, casein, gelatin and tyrosine. Negative for hydrolysis of starch, agar, urea and DNA. Cellular fatty acids include large amounts of 15:0 iso, 17:0 iso 3-OH and 15:1 iso G. The major respiratory quinone is MK-7. Phylogenetically, the genus *Niastella* is a member of the phylum *Bacteroidetes*. The type species is *Niastella koreensis*.

Description of *Niastella koreensis* sp. nov.

Niastella koreensis (ko.re.en'sis. N.L. fem. adj. *koreensis* of Korea, where the type strain was isolated).

Cells are 0.4–0.5 μm in diameter and 10–50 μm in length. Colonies are light yellow in colour on R2A. Growth occurs at 10–37 °C and pH 5–8. Physiological and biochemical properties are listed in Table 1. The fatty acid profile is given in Table 3. Positive for aesculin hydrolysis and negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatin hydrolysis (API 20NE). Positive for alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase, weakly positive for cystine arylamidase and α-galactosidase and negative for esterase (C4), esterase lipase (C8), lipase (C14), trypsin, α-chymotrypsin, β-glucuronidase, α-mannosidase and α-fucosidase (API ZYM). None of the substrates included in API 20NE and API ID 32 GN (D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, 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, salicin, D-melibiose, L-fucose, D-sorbitol, propionic acid, valeric acid, trisodium citrate, L-histidine,

Table 1. Phenotypic properties of strains GR20-10^T and GR20-13^T and related species

Taxa: 1, strain GR20-10^T; 2, strain GR20-13^T; 3, *T. ferruginea* DSM 30193^T; 4, *Chitinophaga pinensis*; 5, *Flexibacter japonensis*; 6, *Flexibacter filiformis*; 7, *Flexibacter sancti*; 8, *Cytophaga arvensicola*; 9, *Flavobacterium aquatile*; 10, *Flexibacter flexilis*; 11, *Cytophaga hutchinsonii*. Data for *T. ferruginea* are from this study with the exception of the major menaquinone (from Xie & Yokota, 2006) and G+C content (from Holmes *et al.*, 1984). Data for other reference taxa are from Bernardet *et al.* (1996), Fujita *et al.* (1996), Holmes *et al.* (1984), Nakagawa & Yamasato (1993), Reichenbach (1989a, b), Sangkhobol & Skerman (1981), Sly *et al.* (1998), Vandamme *et al.* (1994) and Xie & Yokota (2006). +, 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
Catalase/oxidase	–/–	–/+	+*/+	+/-	+/+	+/+	–/ND	+/+	+/+	–/+	–/+
Flexirubin reaction	–	–	–	ND	+	+	+	+	–	–	+
Gliding motility	+	+	–	+	+	ND	ND	V	+	+	+
Colony colour†	LY	M	OR	Y	OY	OY	GY	YO	LY	O	BY
Hydrolysis of:											
Starch	–	–	+	–	–	–	+	V	V	–	–
Chitin	+	+	–	+	–	–	–	ND	–	–	–
Carboxymethylcellulose	+	+	–	–	–	ND	+	ND	–	–	+
Casein	+	+	–	+	–	–	ND	ND	+	–	ND
Gelatin	+	+	+	+	+	W	+	V	V	+	+
Urea	–	–	–	+	ND	ND	ND	–	–	ND	+
DNA	–	–	–	–	+	+	ND	+	–	–	–
Nitrate reduction	–	–	–‡	–	+	+	+	V	V	–	ND
Major menaquinone	MK-7	MK-7	MK-7	ND	MK-7	MK-7	MK-7	MK-7	MK-6	ND	MK-7
G+C content (mol%)	45.8	44.3	48.6	42–46	49.8	46–47	46–47	43–46	32	40–43	40

*Reported as weak for strain IAM 15098^T by Xie & Yokota (2006).

†BY, Bright yellow; GY, golden yellow; LY, light yellow; M, milky; O, orange; OR, orange to red; OY, orange–yellow; Y, yellow; YO, yellow–orange.

‡Reported as positive for strain IAM 15098^T by Xie & Yokota (2006).

Table 2. Differential phenotypic characteristics of strains GR20-10^T and GR20-13^T and *T. ferruginea*

Strains: 1, GR20-10^T; 2, GR20-13^T; 3, *T. ferruginea* DSM 30193^T. All strains are positive for aesculin hydrolysis, alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl- β -glucosaminidase. All strains are negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esterase lipase (C8), lipase (C14), trypsin, β -glucuronidase and α -fucosidase.

Characteristic	1	2	3
API 20NE*			
Gelatin hydrolysis	–	+	+
API ZYM			
Esterase (C4)	–	–	+
Cystine arylamidase	W	W	+
α -Chymotrypsin	–	+	–
α -Galactosidase	W	W	–
β -Galactosidase	+	+	–
α -Glucosidase	+	W	+
β -Glucosidase	+	W	W
α -Mannosidase	–	–	W

*Results were recorded after 48 h incubation.

Table 3. Cellular fatty acid composition (%) of strains GR20-10^T and GR20-13^T and *T. ferruginea*

Strains: 1, GR20-10^T; 2, GR20-13^T; 3, *T. ferruginea* DSM 30193^T. Only fatty acids that represent more than 1% of total fatty acids are indicated; –, not detected.

Fatty acid	1	2	3
14:0	–	–	1.4
15:0 anteiso	4.9	3.3	1.6
15:0 iso	26.8	30.6	36.5
15:0 iso 3-OH	1.3	1.9	2.1
15:1 iso G	15.6	14.7	20.7
16:0	2.6	2.9	3.8
16:0 3-OH	1.3	2.3	1.7
16:0 iso	1.0	–	–
16:0 iso 3-OH	–	–	1.0
16:1 ω 5c	–	1.2	–
16:1 ω 7c/15:0 iso 2-OH	4.3	5.3	8.2
17:0 2-OH	3.5	2.6	–
17:0 3-OH	1.2	–	–
17:0 iso	1.6	–	–
17:0 iso 3-OH	29.4	27.1	11.3
Unknown 13:565	–	–	4.7
Unknown 16:582	1.4	1.6	1.2

potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid and L-proline) can be assimilated.

The type strain is GR20-10^T (=KACC 11465^T=DSM 17620^T), isolated from soil cultivated with Korean ginseng. The DNA G+C content of the type strain is 45.8 mol% (as determined by HPLC).

Description of *Niastella yeongjuensis* sp. nov.

Niastella yeongjuensis (ye.ong.ju.en'sis. N.L. fem. adj. *yeongjuensis* pertaining to Yeongju, a city in Korea, where the organism was first isolated).

Cells are 0.4–0.6 µm in diameter and 10–40 µm in length. Colonies are milky in colour on R2A. Growth occurs at 15–33 °C and pH 5–8. Physiological and biochemical properties are listed in Table 1. The fatty acid profile is given in Table 3. Positive for aesculin hydrolysis and gelatin hydrolysis and negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase and urease (API 20NE). Positive for alkaline phosphatase, leucine arylamidase, valine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase and N-acetyl-β-glucosaminidase, weakly positive for cystine arylamidase, α-galactosidase, α-glucosidase and β-glucosidase and negative for esterase (C4), esterase lipase (C8), lipase (C14), trypsin, β-glucuronidase, α-mannosidase and α-fucosidase (API ZYM). None of the substrates included in API 20NE and API ID 32 GN (see above) can be assimilated.

The type strain is GR20-13^T (=KACC 11466^T=DSM 17621^T), isolated from soil cultivated with Korean ginseng. The DNA G+C content of the type strain is 44.3 mol% (as determined by HPLC).

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