Chitinophaga niabensis sp. nov. and *Chitinophaga niastensis* sp. nov., isolated from soil

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Two yellow-coloured bacterial strains, designated JS13-10^T and JS16-4^T, were isolated from soil from Jeju Island, Republic of Korea. On the basis of 16S rRNA gene sequence analysis, the strains were found to be affiliated with members of the genus *Chitinophaga*. Phenotypically, the novel strains were identified as being different from each other and from recognized species of the genus *Chitinophaga*. DNA–DNA hybridization tests between the two novel strains and closely related *Chitinophaga* reference strains produced DNA relatedness values that were significantly lower (<36%) than those generally accepted as the highest threshold for the phylogenetic definition of a species. On the basis of their distinct taxonomic characteristics, these strains represent two novel species of the genus *Chitinophaga*, for which the names *Chitinophaga niabensis* sp. nov. (type strain JS13-10^T=KACC 12952^T=JCM 15440^T) and *Chitinophaga niastensis* sp. nov. (type strain JS16-4^T=KACC 12954^T=JCM 15441^T) are proposed.

The genus *Chitinophaga* was proposed by Sangkhobol & Skerman (1981) for strains of filamentous, chitinolytic, gliding bacteria. Later, *Cytophaga arvensicola*, *Flexibacter filiformis*, *Flexibacter japonensis* and *Flexibacter sancti* were reclassified into the genus *Chitinophaga*, *Chitinophaga skermanii* was described (Kämpfer *et al.*, 2006) and *Chitinophaga arvensicola* was redefined by Pankratov *et al.* (2006). Recently, Kim & Jung (2007) described a novel strain (KP01^T) as also representing a novel species of the genus *Chitinophaga* and the name *Chitinophaga terrae* has been validly published. At the same time, two further strains, Gsoil 219 (=KACC 12833) and Gsoil 238 (=KACC 12832), were described by An *et al.* (2007) and proposed as '*Chitinophaga soli*' and '*Chitinophaga terrae*', respectively, but these names were not validly published. At the time of writing, there are nine species with validly published names in the genus *Chitinophaga*.

Soil samples were collected on Jeju Island, Republic of Korea. Soil (1 g) was suspended in 9 ml 0.85% NaCl (w/v) and mixed in a shaker for 30 min. After serial dilution, aliquots of serial diluents were spread on R2A agar plates (Reasoner & Geldreich, 1985) and incubated at 28 °C for 5 days. Two yellow-coloured isolates, designated strains JS13-10^T and JS16-4^T, were recovered. The following 11 *Chitinophaga* strains were used as reference strains in API

(bioMérieux) tests, DNA–DNA hybridizations and fatty acid analyses: *Chitinophaga filiformis* DSM 527^T, *C. japonensis* DSM 13484^T, *C. pinensis* DSM 2588^T and *C. sancti* DSM 784^T from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany); *C. ginsengisegetis* KACC 12723^T, *C. ginsengisoli* KACC 12724^T, *C. terrae* KACC 12755^T and strains KACC 12833 and KACC 12832 from the Korean Agricultural Culture Collection (Suwon, Republic of Korea); *C. arvensicola* KCTC 2943^T from the Korean Collection for Type Cultures (Daejeon, Republic of Korea) and *C. skermanii* CIP 109140^T from the collection of the Pasteur Institute (Paris, France).

To investigate basic physiological and biochemical characteristics, the methods of Smibert & Krieg (1994) were used to test for the following: Gram stain, catalase activity, oxidase activity and hydrolysis of casein, DNA, hypoxanthine, starch and xanthine. Carboxymethylcellulose (Sigma) (0.1%) was used for the cellulase test. The hydrolysis of chitin [from crab shells (1%, w/v)], pectin (0.5%, w/v) and tyrosine (0.5%, w/v) were also tested. To investigate cell morphology using transmission electron microscopy (model LEO 912AB; Zeiss) and phase-contrast microscopy (Axio; Zeiss), cells were grown on R2A agar (Difco). Temperature tolerance was tested by growing cells at 5, 10, 15, 20, 25, 30, 35, 40 and 45 °C. Tolerance of different salinity levels was tested by growing cells in R2A broth (LAB) supplemented with 0, 1, 2, 3 and 5% (w/v)

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains JS13-10^T and JS16-4^T are EU714259 and EU714260, respectively.

NaCl. The pH range (pH 4–10, using increments of 1 pH unit) for growth was determined in R2A broth buffered with citrate-phosphate or Tris/HCl buffers (Breznak & Costilow, 1994). Tests with the commercial systems API 20NE, API ID 32GN 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 7 days at 28 °C.

Genomic DNA was extracted using a DNeasy Tissue kit (Qiagen) by following the manufacturer's instructions. Sequencing of the 16S rRNA gene was performed as described by Kwon *et al.* (2003). Multiple alignments were performed using the CLUSTAL W program (Thompson *et al.*, 1994). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed with neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods (Fitch, 1971), using the MEGA3 program (Kumar *et al.*, 2004), with bootstrap values based on 1000 replications (Felsenstein, 1985). The 16S rRNA gene sequences of strains JS13-10^T and JS16-4^T were based on a continuous stretch of 1402 bp.

DNA G+C contents were determined by means of HPLC analysis of deoxyribonucleosides, as described by Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18 S; Supelco). Isoprenoid quinones were analysed by using HPLC as described previously (Groth *et al.*, 1996). After growth of the cells on R2A for 2 days at 28 °C, fatty acid methyl esters were extracted and prepared using the standard protocol of the Microbial Identification System (MIDI; Microbial ID). DNA–DNA hybridization was carried out as described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG-High Prime DNA labelling and detection starter kit II (Roche Molecular Biochemicals). The hybridized DNA was visualized using a DIG luminescent detection kit (Roche).

Strains JS13-10^T and JS16-4^T were Gram-negative, aerobic, rod-shaped and catalase- and oxidase-positive. Strain JS13-10^T produced clear, round colonies whereas strain JS16-4^T produced smeared growth. The phenotypic characteristics of strains JS13-10^T and JS16-4^T are summarized and compared with those of type or reference strains from the genus *Chitinophaga* in Table 1.

Phylogenetically, strains JS13-10^T and JS16-4^T were found to be members of the genus *Chitinophaga* (Fig. 1). The 16S rRNA gene sequence similarity between strains JS13-10^T and JS16-4^T was 94.6%. Strain JS13-10^T showed sequence similarities in the range 91.3–98.1% with reference strains of the genus *Chitinophaga*, revealing a high level (>97%) of sequence similarity (98.1%) with '*C. soli*' Gsoil 219. Strain JS16-4^T showed sequence similarities of 92.4–98.1% with the reference strains of the genus *Chitinophaga*, having a high level (>97%) of sequence similarity (98.1%) with *C. arvensicola* DSM 3695^T and *C. ginsengisegetis* Gsoil 040^T.

The G+C contents of the DNA of strains JS13-10^T and JS16-4^T were 47 and 43 mol%, respectively. Both strains contained a menaquinone with seven isoprene units (MK-7) as the major isoprenoid quinone. The fatty acid profile of strain JS13-10^T was dominated by iso-C_{15:0} (38.2%), C_{16:1}ω5c (19.6%) and iso-C_{17:0} 3-OH (17.2%) (Table 2). DNA–DNA hybridization tests indicated that the relatedness between strain JS13-10^T and '*C. soli*' KACC 12833 was 32% and that the relatedness values between strain JS16-4^T, *C. arvensicola* KCTC 2943^T and *C. ginsengisegetis* KACC 12723^T were 36 and 30%, respectively.

The phenotypic differences found between strains JS13-10^T and JS16-4^T were in relation to maximum growth temperature, hydrolysis of urea, and in the assimilation, enzymic and fatty acid profiles. Strain JS13-10^T could be differentiated from its closest relative, '*C. soli*' KACC 12833, on the basis of growth at 37 °C, differences in the assimilation and enzymic profiles and by variation in fatty acid composition. Strain JS16-4^T could be separated from its closest relative, *C. arvensicola*, on the basis of nitrate reduction, growth at 37 °C and the assimilation, enzymic and fatty acid profiles. In addition to these phenotypic differences, the DNA–DNA hybridization values (which were considerably less than 70%) obtained with respect to closely related species indicated that strains JS13-10^T and JS16-4^T represent novel species of the genus *Chitinophaga*, for which the names *Chitinophaga niabensis* sp. nov. and *Chitinophaga niastensis* sp. nov. are proposed, respectively.

Description of *Chitinophaga niabensis* sp. nov.

Chitinophaga niabensis [ni.ab.en'sis. N.L. fem. adj. *niabensis* pertaining to NIAB, the National Institute of Agricultural Biotechnology (Suwon, Republic of Korea), where taxonomic studies were conducted].

Cells are Gram-negative, aerobic, non-motile, rod-shaped, 0.5–0.7 µm wide and 1–7 µm long. Oxidase- and catalase-positive. Colonies are yellowish, circular and convex with clear margins. Grows at temperatures between 5 and 40 °C, with optimum growth at 28–30 °C. The pH range for growth is 6.0–9.0, with an optimum at pH 7.0. Grows in the absence of NaCl and in the presence of 2.0% (w/v) NaCl, but not with 3.0% (w/v) NaCl. Grows on R2A, nutrient agar and trypticase soy agar, but not on MacConkey agar. Hydrolyses casein and tyrosine, but not chitin, carboxymethylcellulose, DNA, hypoxanthine, pectin, starch, Tween 80 or xanthine. Other physiological properties are shown in Table 1. The major isoprenoid quinone is MK-7. The major fatty acids (>10%) are iso-C_{15:0}, C_{16:1}ω5c and iso-C_{17:0} 3-OH.

The type strain, JS13-10^T (=KACC 12952^T=JCM 15440^T), was isolated from soil from Jeju Island, Republic of Korea. The DNA G+C content of the type strain is 47 mol% (by HPLC).

Table 1. Differential phenotypic characteristics of strain JS13-10^T, strain JS16-4^T and other members of the genus *Chitinophaga*

Strains: 1, JS13-10^T; 2, JS16-4^T; 3, *C. arvensicola* DSM 3695^T and IFO 12650^T (Reichenbach, 1989; Kämpfer *et al.*, 2006; Pankratov *et al.*, 2006); 4, *C. filiformis* DSM 527^T (Reichenbach, 1989; Kämpfer *et al.*, 2006); 5, *C. ginsengisegetis* KACC 12723^T (Lee *et al.*, 2007); 6, *C. ginsengisoli* KACC 12724^T (Lee *et al.*, 2007); 7, *C. japonensis* DSM 13484^T (Fujita *et al.*, 1996; Kämpfer *et al.*, 2006); 8, *C. pinensis* DSM 2588^T (Reichenbach, 1989; Kämpfer *et al.*, 2006); 9, *C. sancti* DSM 784^T (Reichenbach, 1989; Kämpfer *et al.*, 2006); 10, *C. skermanii* CIP 109140^T (Kämpfer *et al.*, 2006); 11, *C. terrae* KACC 12755^T (Kim & Jung, 2007); 12, '*C. soli*' KACC 12833 (An *et al.*, 2007); 13, KACC 12832 (An *et al.*, 2007). According to the API 20NE test strips, all strains are positive for aesculin hydrolysis, gelatin hydrolysis and β -galactosidase activity, but are negative for indole production, glucose fermentation and arginine dihydrolase. According to the API 20NE and API ID 32GN test strips, all strains assimilate D-glucose, D-mannose, N-acetylglucosamine, maltose, sucrose and melibiose, but do not assimilate D-mannitol, capric acid, adipic acid, trisodium citrate, phenylacetic acid, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, D-sorbitol, propionic acid, valeric acid, potassium 2-ketogluconate, 3-hydroxybutyric acid or 4-hydroxybenzoic acid. According to the API ZYM test strips, all strains are positive for alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -glucosidase and N-acetyl- β -glucosaminidase, but negative for lipase C14 and β -glucuronidase. +, Positive; (+), weakly positive; -, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Colony colour*	Y	Y	Y	GY	Y	Y	YO	Y	GY	Y	Y	Y	Y
Catalase/oxidase	+/+	+/+	+/+	-/+	-/-	+/+	+/+	+/-	-/ND	+/+	+/+	+/+	+/+
Growth at 37 °C	+	-	+	+	+	+	+	+	-	+	+	-	-
Cell length (µm)	1-7	1-13	1.5-40	30-80	1.1-1.3	1.2-1.6	2-18	<40	2-15	1-2	0.6-0.8	1.0-1.3	0.9-1.4
Max. NaCl (% w/v) tolerated	2.0	2.0	3.0	0.3	2.0	<1.0	2.0	<2.0	1.0	ND	ND	1.0	3.0
Chitin hydrolysis	-	-	-	+	-	+	-	+	-	ND	-	-	-
Nitrate reduction†	-	-	+‡	+	-	-	+	+	+	-	+	-	+
Urease†	-	(+)	(+)‡	-	(+)	(+)	+	-	-	(+)	+	-	-
Assimilation of:†													
L-Arabinose	+	-	+‡	+	+	+	+	+	+	-	+	+	+
Potassium gluconate	-	-	-‡	-	-	(+)	-	(+)	(+)	(+)	-	-	-
Malic acid	-	-	-‡	-	-	-	-	-	-	+	-	-	-
L-Rhamnose	+	-	+‡	+	+	+	+	+	+	-	+	+	-
D-Ribose	-	-	+‡	-	-	-	-	-	-	-	(+)	-	-
Inositol	-	-	(+)‡	-	-	-	-	-	-	-	(+)	(+)	-
L-Serine	-	-	(+)‡	-	-	-	-	-	-	(+)	-	(+)	-
Salicin	(+)	+	+‡	(+)	(+)	+	-	+	+	+	+	+	+
L-Fucose	(+)	-	+‡	-	+	-	+	(+)	-	(+)	+	(+)	(+)
L-Histidine	-	(+)	(+)‡	-	(+)	-	-	-	(+)	(+)	-	(+)	-
L-Proline	-	-	+‡	(+)	+	(+)	-	(+)	(+)	+	(+)	(+)	-
Enzyme activities:†													
Esterase C4	+	-	(+)‡	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Esterase lipase C8	+	-	-‡	-	-	-	-	(+)	-	(+)	-	(+)	(+)
Cystine arylamidase	(+)	(+)	(+)‡	-	(+)	(+)	(+)	+	(+)	+	+	+	+
Trypsin	+	-	+‡	+	+	+	+	+	+	+	+	+	+
α -Chymotrypsin	+	-	-‡	-	-	-	-	+	-	-	+	+	+
α -Galactosidase	+	-	+‡	+	+	+	+	+	+	+	+	-	+
α -Glucosidase	+	+	+‡*	+	+	+	+	+	+	+	+	+	+
α -Mannosidase	-	-	(+)‡	+	(+)	-	+	(+)	-	-	-	-	-
α -Fucosidase	+	+	+‡	+	+	+	+	+	+	-	+	+	+
DNA G + C content (mol%)	47.0	43.0	42.8-46.4	45.0	47.1	48.4	49.8	45.2	43.3	40.7	46.3	43.2	46.2

*GY, Golden yellow; Y, yellow; YO, yellow-orange.

†Data from this study.

‡Data for KCTC 2943^T.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the positions of strains JS13-10^T and JS16-4^T. Numbers at nodes indicate bootstrap percentages (based on 1000 resamplings); only values >50% are given. Filled circles indicate that the corresponding branches were also recovered in the maximum-parsimony phylogenetic tree. Bar, 0.01 substitutions per nucleotide position.

Description of *Chitinophaga niastensis* sp. nov.

Chitinophaga niastensis [ni.as.ten'sis. N.L. fem. adj. *niastensis* pertaining to NIAST, the National Institute of Agricultural Science and Technology (Suwon, Republic of Korea), where taxonomic studies were conducted].

Cells are Gram-negative, aerobic, non-motile, rod-shaped, 0.6–0.7 µm wide and 1–13 µm long. Oxidase- and catalase-positive. Colonies are yellowish and smeared. Grows at temperatures between 5 and 30 °C, with optimum growth occurring at 28 °C. The pH range for growth is 5.0–9.0,

Table 2. Fatty acid compositions of strains JS13-10^T, JS16-4^T and other strains of the genus *Chitinophaga*

Strains: 1, JS13-10^T; 2, JS16-4^T; 3, *C. arvensicola* KCTC 2943^T; 4, *C. filiformis* DSM 527^T; 5, *C. ginsengisegetis* KACC 12723^T; 6, *C. ginsengisoli* KACC 12724^T; 7, *C. japonensis* DSM 13484^T; 8, *C. pinensis* DSM 2588^T; 9, *C. sancti* DSM 784^T; 10, *C. skermanii* CIP 109140^T; 11, *C. terrae* KACC 12755^T; 12, '*C. soli*' KACC 12833; 13, KACC 12832. All strains were grown at 30 °C for 2 days. –, <1.0% or not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
C _{12:1} AT 11-12	–	–	–	–	–	–	–	–	4.0	–	–	–	–
C _{14:0}	1.2	1.8	1.6	–	1.8	–	–	–	1.0	2.0	1.6	1.4	1.2
anteiso-C _{15:0}	–	1.2	–	–	–	–	–	–	–	–	–	–	1.1
iso-C _{15:0}	38.2	36.6	35.6	41.0	38.3	39.0	39.1	33.2	38.8	45.8	49.0	51.0	41.3
iso-C _{15:0} 3-OH	7.7	4.7	4.2	3.2	4.2	3.3	2.6	3.1	4.3	5.7	5.0	4.6	4.4
C _{16:0}	2.8	6.5	4.3	4.0	3.4	1.6	4.4	4.1	4.6	3.8	3.4	3.1	6.8
C _{16:0} 2-OH	–	–	–	–	–	–	1.8	–	–	–	–	–	–
C _{16:0} 3-OH	2.0	1.4	1.9	–	–	1.2	–	1.0	–	2.3	1.0	1.5	1.4
iso-C _{16:0}	–	–	–	–	–	–	–	–	–	–	–	–	1.2
C _{16:1} ω5c	19.6	32.5	36.1	27.9	34.2	31.7	26.0	34.7	23.9	19.0	21.3	19.1	21.6
C _{16:1} ω11c	–	–	2.0	2.7	–	1.1	2.1	3.5	3.4	2.6	–	–	–
C _{17:0} 2-OH	–	–	–	–	–	–	–	–	–	–	–	–	1.2
C _{17:0} 3-OH	1.6	–	–	–	–	–	–	–	–	–	–	1.2	–
iso-C _{17:0}	–	–	–	–	–	–	1.4	–	–	–	–	–	–
iso-C _{17:0} 3-OH	17.2	9.2	8.6	7.8	8.3	9.6	11.4	11.2	7.7	9.2	6.5	10.1	11.7
Unknown fatty acids*													
ECL 13.565	–	1.7	2.0	1.3	4.5	1.6	2.1	–	2.4	1.7	1.3	2.4	–
ECL 14.959	–	–	–	–	–	–	–	–	–	–	1.7	–	–
Summed features†													
3	4.4	4.4	3.7	8.0	5.3	9.1	2.1	5.5	9.9	5.6	4.2	3.5	4.7
4	–	–	–	–	–	–	1.4	–	–	–	–	–	–

*ECL, Equivalent chain length.

†Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 comprises iso-C_{15:0} 2-OH and/or C_{16:1} ω7c. Summed feature 4 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B.

with an optimum between pH 6.0 and 7.0. Grows in the absence of NaCl and in the presence of 2.0 % (w/v) NaCl, but not with 3.0 % (w/v) NaCl. Grows on R2A, nutrient agar and trypticase soy agar, but not on MacConkey agar. Hydrolyses Tween 80, but not casein, chitin, carboxymethylcellulose, DNA, hypoxanthine, pectin, starch, Tween 80, tyrosine or xanthine. Other physiological properties are shown in Table 1. The major isoprenoid quinone is MK-7. The major fatty acids (>10 %) are iso-C_{15:0} and C_{16:1}ω5c.

The type strain, JS16-4^T (=KACC 12954^T=JCM 15441^T), was isolated from soil from Jeju Island, Republic of Korea. The DNA G + C content of the type strain is 43 mol% (by HPLC).

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