Niastella populi sp. nov., isolated from soil of Euphrates poplar (*Populus euphratica*) forest, and emended description of the genus *Niastella*

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A novel bacterial strain, designated THYL-44^T, was isolated from the soil of a Euphrates poplar (*Populus euphratica*) forest in Xinjiang, China. The cells were strictly aerobic, Gram-staining-negative, non-flagellated, non-motile and filamentous. Growth occurred at 17–37 °C (optimum 30 °C), at pH 5.0–8.0 (optimum pH 7.0) and with 0–1 % NaCl (w/v; optimum 0 %). Flexirubin pigments were not produced. A phylogenetic analysis based on 16S rRNA gene sequences revealed that strain THYL-44^T was closely related to *Niastella koreensis* KACC 11465^T (95.5 % sequence similarity). The major respiratory quinone was MK-7 and the predominant cellular fatty acids were iso-C_{15:0} (28.6 %), iso-C_{17:0} 3-OH (23.9 %) and iso-C_{15:1} G (17.4 %). The DNA G + C content was 45.2 mol%. Therefore, the phylogenetic, physiological and chemotaxonomic data demonstrated that strain THYL-44^T represents a novel species of the genus *Niastella*, for which the name *Niastella populi* sp. nov. is proposed. The type strain is THYL-44^T (=CCTCC AB 208238^T=KCTC 22560^T). On the basis of new data, an emended description of the genus *Niastella* is also proposed.

The genus *Niastella*, containing *Niastella koreensis* (type species) and *Niastella yeongjuensis*, was proposed by Weon *et al.* (2006) for bacterial strains isolated from soil cultivated with Korean ginseng. These organisms are Gram-staining-negative, aerobic, filamentous bacteria that are motile by gliding, that do not produce flexirubin pigments and do not reduce nitrate. Their cellular fatty acids include large amounts of iso-C_{15:0}, iso-C_{15:1} G and iso-C_{17:0} 3-OH and their major respiratory quinone is MK-7. Phylogenetically, the genus *Niastella* is a member of the family *Chitinophagaceae*, phylum *Bacteroidetes* (<http://www.bacterio.net>).

In the present study, a novel member of the genus *Niastella* was characterized. Strain THYL-44^T was isolated from the soil of a Euphrates poplar (*Populus euphratica*) forest (84° 15'–84° 30' E, 40° 0'–40° 55' N) in Xinjiang, China. The soil sample was diluted with sterile water and the dilutions were plated onto 0.1 × TSA (tryptic soy agar; Difco) plates. The strain was isolated after incubation at 30 °C for one week. However, it was later noticed that the isolate grew better on R2A agar (Difco).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain THYL-44^T is EU877262.

A maximum-parsimony phylogenetic tree and a transmission electron micrograph of cells of strain THYL-44^T are available as supplementary figures in the online version of this paper.

Cell morphology was examined by phase-contrast microscopy (BX51; Olympus) and transmission electron microscopy (8100; Hitachi), using cells negatively stained with 2 % (w/v) phosphotungstic acid after air drying. Gram staining was carried out according to the classical Gram procedure described by Doetsch (1981). Motility was examined according to the method of Bernardet *et al.* (2002). Growth at 4, 17, 22, 25, 30, 37, 42 and 50 °C and at pH 5.0–10.0 (at 1.0 pH unit intervals) was tested after 5 days of incubation in R2A broth. The pH was adjusted using 1 M HCl or 1 M NaOH and the broth was then filter-sterilized at 0.2 µm. Salt tolerance was tested on R2A agar supplemented with 0–10 % (w/v) NaCl (at 1 % intervals). 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. Growth under anaerobic conditions was tested on R2A agar in a GasPak (BBL) jar at 30 °C for 10 days. The following tests were performed on strain THYL-44^T and on the type strains of the recognized species of the genus *Niastella*. Hydrolysis of carboxymethylcellulose, casein, chitin, DNA, agar, tyrosine and starch was performed using the methods of Smibert & Krieg (1994). Flexirubin pigments were detected by means of a colour shift after exposure to a 20 % (w/v) KOH solution (Reichenbach, 1992). Congo red absorption was tested by directly flooding colonies on agar plates with 0.01 % aqueous

Congo red solution. Other biochemical tests were carried out by using API 20NE, API ID 32GN and API ZYM test kits according to the manufacturer's instructions (bioMérieux). Growth was also evaluated on nutrient agar (NA; Difco), TSA (Difco) and MacConkey agar (Difco) at 30 °C.

Genomic DNA was extracted with a Bacteria Genomic DNA isolation kit (Shanghai Chaoshi Bio Technologies Co. Ltd). The 16S rRNA gene was amplified by PCR with bacterial universal primers 27F and 1527R (Rainey *et al.*, 1996) and the PCR products were sequenced by Invitrogen Biotechnology Co. Ltd. Sequence similarity was calculated by pairwise alignment obtained from the EzTaxon database (Chun *et al.*, 2007). Analysis of the 16S rRNA gene sequence data was performed by using the MEGA version 4.0 software package (Tamura *et al.*, 2007), after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). A distance matrix method (distance options according to the Kimura two-parameter model) using clustering with the neighbour-joining (Saitou & Nei, 1987) method (Fig. 1) as well as a discrete character-based maximum-parsimony (Fitch, 1971) method (see Supplementary Fig. S1 available in IJSEM Online) were used. In each case, bootstrap values were calculated based on 1000 replications (Felsenstein, 1985). The 16S rRNA gene sequence of strain THYL-44^T was a continuous stretch of 1445 bp. Sequence similarity calculations indicated that strain THYL-44^T showed the highest degree of similarity to *Niastella koreensis* KACC 11465^T (95.5 %) and *N. yeongjuensis* KACC 11466^T (95.4 %). Lower sequence similarities (<93.6 %) were found with all other organisms in Fig. 1. The 16S rRNA gene sequence of strain THYL-44^T is therefore distinctly different from those of members of the family *Chitinophagaceae* (Stackebrandt & Goebel, 1994).

To determine the whole-cell fatty acid profiles, strain THYL-44^T, *N. koreensis* DSM 17620^T and *N. yeongjuensis* DSM 17621^T were cultured on R2A agar at 30 °C for 3 days. Fatty

acid methyl esters were obtained by saponification, methylation and extraction as described previously (Kämpfer & Kroppenstedt, 1996) and separated by GC (6890; Agilent). Peaks were integrated automatically and fatty acid names and percentages were determined using the Sherlock Microbial Identification System (MIDI). The fatty acid profile of strain THYL-44^T revealed iso-C_{15:0} (28.6 %), iso-C_{17:0} 3-OH (23.9 %) and iso-C_{15:1}G (17.4 %) as the major fatty acids. This composition was essentially in agreement with the fatty acid patterns of species of the genus *Niastella* obtained using different (Weon *et al.*, 2006) and identical (this study; Table 1) growth conditions. *Segetibacter koreensis* KCTC 12655^T showed large amounts of fatty acid C_{16:1}ω5c (An *et al.*, 2007) and thus could be clearly differentiated from *N. koreensis* DSM 17620^T, *N. yeongjuensis* DSM 17621^T and strain THYL-44^T.

Biomass for molecular systematic and chemotaxonomic studies was obtained from R2A broth cultures in shaken flasks at 30 °C for 3 days. The genomic DNA of strain THYL-44^T was prepared according to a modification of the procedure of Wilson (1987). The determination of the DNA G + C content was performed according to the method of Mesbah *et al.* (1989) using reversed-phase HPLC (UltiMate 3000; Dionex). The DNA G + C content of strain THYL-44^T was 45.2 mol% which was in line with the values reported previously for species of the genus *Niastella* (44.3–45.8 mol%). Respiratory quinones were isolated from lyophilized cells as described by Collins *et al.* (1977) and were analysed by HPLC as described by Xie & Yokota (2003). The presence of MK-7 supported the affiliation of strain THYL-44^T to the family *Chitinophagaceae*, in which all species investigated to date have MK-7 as the major quinone.

In terms of physiological and biochemical properties, strain THYL-44^T could be distinguished from members of the genera *Terrimonas* (Xie & Yokota, 2006), *Flavisolibacter* and *Segetibacter* (Yoon & Im, 2007) by the ability to hydrolyse casein, the absence of oxidase activity and the enzyme

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship of strain THYL-44^T and related taxa. Bootstrap values (>50 %) are shown at nodes as percentages of 1000 replicates. *Flavobacterium aquatile* ATCC 11947^T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Table 1. Fatty acid contents (%) of strain THYL-44^T and the type strains of the recognized species of the genus *Niastella*

Strains: 1, THYL-44^T; 2, *N. koreensis* DSM 17620^T; 3, *N. yeongjuensis* DSM 17621^T. All data are from this study. —, Not detected; tr, traces (<1 %). Fatty acids amounting to <1 % of total fatty acids in all three strains are omitted. The major fatty acids are indicated in bold type.

Fatty acid	1	2	3
Saturated fatty acids			
C _{16:0}	4.0	4.8	2.9
C _{18:0}	2.0	2.4	tr
Unsaturated fatty acids			
C _{16:1ω5c}	—	—	1.0
Branched-chain fatty acids			
anteiso-C _{15:0}	4.9	4.5	5.5
iso-C _{15:0}	28.6	23.3	29.1
iso-C _{15:0} 3-OH	1.6	1.3	2.1
iso-C _{15:1} G	17.4	15.8	14.8
iso-C _{17:0}	tr	tr	1.0
iso-C _{17:0} 3-OH	23.9	25.9	23.4
Hydroxy fatty acids			
C _{16:0} 3-OH	1.6	1.4	1.7
C _{17:0} 2-OH	1.8	2.9	2.3
C _{17:0} 3-OH	tr	1.5	tr
Summed feature 3*	6.2	6.8	5.7

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contained C_{16:1ω5c} and/or iso-C_{15:0} 2-OH.

reaction pattern in the API ZYM test kit. Furthermore, strain THYL-44^T could be differentiated from the two recognized members of the genus *Niastella* on the basis of its ability to assimilate a number of carbohydrates, inability to hydrolyse chitin and absence of gliding motility. Other differentiating characteristics are shown in Table 2.

On the basis of these results, strain THYL-44^T represents a novel species of the genus *Niastella*, for which the name *Niastella populi* sp. nov. is proposed. As a consequence of newly reported characteristics, the description of the genus *Niastella* Weon *et al.* 2006 needs to be emended.

Emended description of the genus *Niastella* Weon *et al.* 2006

The main characteristics are as those given for the genus description by Weon *et al.* (2006). In addition, cells are motile by gliding or are non-motile. Growth on TSA and hydrolysis of chitin, carboxymethylcellulose and tyrosine are species-dependent.

Description of *Niastella populi* sp. nov.

Niastella populi (po'pu.li. L. gen. n. *populi* of a poplar, pertaining to *Populus*, the Latin name of the poplars that grow in the forest from which the strain was isolated).

Table 2. Phenotypic characteristics that differentiate strain THYL-44^T from the type strains of the recognized species of the genus *Niastella*

Strains: 1, THYL-44^T; 2, *Niastella koreensis* DSM 17620^T; 3, *Niastella yeongjuensis* DSM 17621^T. All data are from this study except the DNA G + C contents of taxa 2 and 3 (Weon *et al.*, 2006). +, Positive; —, negative.

Characteristic	1	2	3
Oxidase activity	—	—	+
Gliding motility	—	+	+
Colony colour	Yellow	Light yellow	Milky
Hydrolysis of chitin	—	+	+
Enzyme activities (API ZYM):			
α-Chymotrypsin	—	—	+
α-Glucosidase	+	+	+*
β-Glucosidase	+	+	+*
Assimilation of (API 20NE and API ID 32GN):			
N-Acetyl-β-glucosamine	+	—	—
L-Arabinose	+	—	—
D-Glucose	+	—	—
L-Histidine	+	—	—
Maltose	+	—	—
D-Mannose	+	—	—
Melibiose	+	—	—
L-Rhamnose	+	—	—
Salicin	+	—	—
L-Serine	+	—	—
Sucrose	+	—	—
DNA G + C content (mol%)	45.2	45.8	44.3

*Weak positive reaction according to Weon *et al.* (2006).

Cells are strictly aerobic, Gram-staining-negative, filamentous rods approximately 0.2–0.5 µm wide and 6–30 µm long (see Supplementary Fig. S2 available in IJSEM Online) and are devoid of flagella and exhibit no gliding motility. Colonies on R2A agar are rough, convex, irregular and yellow-coloured. Growth occurs at 17–37 °C, at pH 5.0–8.0 and in the presence of 0–1 % NaCl (w/v); growth is optimal at 30 °C, at pH 7.0 and in the absence of NaCl. Grows on R2A, NA and TSA, but not on MacConkey agar. Oxidase- and catalase-negative. Flexirubin pigments are not produced and Congo red is not absorbed. Casein is hydrolysed. Chitin, carboxymethylcellulose, starch, tyrosine, agar and DNA are not hydrolysed. In API 20NE and API ID 32GN strips, D-glucose, L-arabinose, D-mannose, L-rhamnose, N-acetyl-β-glucosamine, L-serine, salicin, melibiose, maltose, sucrose and L-histidine are assimilated. D-Mannitol, potassium glucosamine, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, D-ribose, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-fucose, D-sorbitol, propionic acid, capric acid, valeric acid, trisodium citrate, potassium 2-ketogluconate, 3-hydroxy-

butyric acid, 4-hydroxybenzoic acid and L-proline are not assimilated. In API 20NE and API ZYM strips, reactions are positive for aesculin and gelatin hydrolysis and for alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase activities; weakly positive for esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase and α -galactosidase activities, and negative for nitrate reduction, indole production, glucose fermentation and arginine dihydrolase, urease, trypsin, α -chymotrypsin and β -glucuronidase activities. The predominant isoprenoid quinone is MK-7. The major cellular fatty acids (>17%) are iso-C_{15:0}, iso-C_{17:0} 3-OH and iso-C_{15:1} G.

The type strain, THYL-44^T (=CCTCC AB 208238^T =KCTC 22560^T), was isolated from the soil of a Euphrates poplar forest in Xinjiang, China. The DNA G+C content of the type strain is 45.2 mol%.

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