Parasegetibacter luojiensis gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from a forest soil

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A novel golden-yellow-coloured strain, designated RHYL-37^T, was isolated from a soil sample from a forest of Euphrates poplar (*Populus euphratica*) in Xinjiang, China. The cells were strictly aerobic, Gram-staining-negative, heterotrophic, non-spore-forming rods that were motile by gliding. Growth occurred at 17–37 °C (optimum, 30 °C), at pH 5.0–9.0 (optimum, pH 7.0) and with 0–1 % NaCl (w/v; optimum, 0 %). Phylogenetic analysis based on 16S rRNA gene sequences indicated that the novel isolate formed a cluster with members of the genera *Segetibacter*, *Terrimonas*, *Niastella*, *Niabella* and *Chitinophaga* in the family 'Chitinophagaceae' in the phylum *Bacteroidetes*. Sequence similarities with the type strains of recognized species ranged from 90.6 to 94.0 %. The predominant isoprenoid quinone was MK-7 and the major cellular fatty acids were iso-C_{15:0}, iso-C_{15:1}, C_{16:0} and iso-C_{17:0} 3-OH. The DNA G + C content was 39.7 mol%. On the basis of the phylogenetic, physiological and chemotaxonomic data, strain RHYL-37^T represents a novel genus and species in the family 'Chitinophagaceae', for which the name *Parasegetibacter luojiensis* gen. nov., sp. nov. is proposed. The type strain of *Parasegetibacter luojiensis* is RHYL-37^T (=CCTCC AB 208240^T=KCTC 22561^T).

Bacteria constituting the phylum *Bacteroidetes* (Garrity & 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 forest of Euphrates poplar (*Populus euphratica*) in Xinjiang, China (40° 0'–40° 55' N 84° 15'–84° 30' E), a large number of novel bacterial strains were isolated, most of them belonging to the phyla *Proteobacteria* and *Bacteroidetes*. During preliminary identification, one of these isolates, designated RHYL-37^T, was found to be a member of the phylum *Bacteroidetes* and was subjected to further taxonomic investigations. On the basis of the results obtained in this study, we propose that strain RHYL-37^T represents a new genus and species in the phylum *Bacteroidetes*.

Strain RHYL-37^T was isolated from a soil sample that was diluted with sterile water; the dilutions were plated onto 0.5 × R2A agar (Difco). After 1 week of incubation at

30 °C, single colonies were transferred onto new plates of 0.5 × R2A agar and on 0.3 × marine agar (MA) [11.22 g marine broth 2216 (MB; Difco) and 15 g agar per litre distilled water]. Routine cultivation was performed on 0.3 × MA or in 0.3 × MB at 30 °C for 24 h to 1 week.

Cell morphology was examined by phase-contrast (Olympus BX51) and transmission electron (Hitachi 8100) microscopy, using cells negatively stained with 1 % (w/v) phosphotungstic acid after air drying. Gram staining was carried out according to the classical procedure described by Doetsch (1981). Gliding motility was assessed as described by Bowman (2000). Catalase and oxidase activities were revealed by bubble production in 3 % (v/v) H₂O₂ and using 1 % (w/v) tetramethyl-*p*-phenylenediamine, respectively. Growth under anaerobic conditions was tested in a GasPak (BBL) jar at 30 °C for 10 days on 0.3 × MA. Carbon-source utilization and enzyme activities were tested by using the API 20NE, API 20E, API ID 32GN and API ZYM test kits (bioMérieux) at 30 °C for 10 days. Hydrolysis of cellulose, casein, chitin, DNA, tyrosine and starch was tested according to the methods of Smibert & Krieg (1994). The presence of flexirubin pigments was assessed from a colour shift after exposure to a 20 % (w/v) KOH solution (Reichenbach, 1992). Growth at 4, 10, 17, 22, 30, 37, 42 and 50 °C and at pH 5.0–10.0 (at 1.0 pH unit intervals) was assessed after 5 days of incubation in 0.3 ×

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RHYL-37^T is EU877263.

Detailed fatty acid compositions of strain RHYL-37^T and its closest phylogenetic neighbours are available as supplementary material with the online version of this paper.

MB; for pH experiments, the pH was adjusted using 1 M HCl or NaOH and the medium was then filter-sterilized (0.2 µm pore size). Salt tolerance was tested on 0.5 × R2A agar supplemented with 1–10 % (w/v) NaCl (at 1 % intervals) after 5 days of incubation. Growth was also evaluated on nutrient agar (NA; Difco), tryptic soy agar (TSA; Difco), 0.1 × TSA and MacConkey agar (Difco) at 30 °C.

Cells of strain RHYL-37^T were strictly aerobic, Gram-staining-negative, heterotrophic, non-spore-forming rods that were motile by gliding. Other characteristics are

summarized in the species description, and those that differentiate strain RHYL-37^T from closely related type strains are shown in Table 1.

The 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 using bacterial universal primers (Rainey *et al.*, 1996), and the PCR products were sequenced by Invitrogen Biotechnology Co. Ltd. An almost-complete 16S rRNA gene sequence (1442 bp) of strain RHYL-37^T was determined and subjected to comparative analysis. Sequence similarity

Table 1. Differentiating characteristics of strain RHYL-37^T and related taxa

Strains: 1, RHYL-37^T (data from this study); 2, *Segetibacter koreensis* KCTC 12655^T; 3, *Niastella koreensis* KACC 11465^T; 4, *Niastella yeongjuensis* KACC 11466^T; 5, *Terrimonas ferruginea* ATCC 13524^T; 6, *Terrimonas lutea* DY^T; 7, *Flavisolibacter ginsengiterrae* KCTC 12656^T; 8, *Flavisolibacter ginsengisoli* KCTC 12657^T; 9, *Chitinophaga pinensis* DSM 2588^T; 10, *Niabella aurantiaca* KACC 11698^T. Data for reference strains are from An *et al.* (2007), Weon *et al.* (2006), Yoon & Im (2007), Xie & Yokota (2006), Sangkhobol & Skerman (1981) and Kim *et al.* (2007) unless indicated. All strains are negative for assimilation of inositol and nitrate reduction. All strains have MK-7 as the major respiratory quinone. +, Positive; –, negative; w, weak reaction; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10
Production of flexirubin pigments	–	ND	–*	–	–*	ND	–	–	+	+
Gliding motility	+	–	+	+	–	–	–	–	+	–
Oxidase	+	+	–	+	+	+	+	–	+	–
Catalase	+	+	–	–	+	w	–	+	–	+
Major fatty acids (>10 %)†	i-C _{15:0} , i-C _{15:1} , C _{16:0} , i-C _{17:0} 3-OH	i-C _{15:0} , C _{16:1ω5c} , i-C _{15:1} , i-C _{15:1} , i-C _{17:0} 3-OH	i-C _{15:0} , i-C _{15:1} , i-C _{17:0} 3-OH*	i-C _{15:0} , i-C _{15:1} , i-C _{17:0} 3-OH	i-C _{15:0} , i-C _{15:1} , i-C _{17:0} 3-OH*	i-C _{15:0} , i-C _{15:1} , i-C _{17:0} 3-OH, SF3	i-C _{15:0} , i-C _{17:0} 3-OH, SF4	i-C _{15:0} , i-C _{17:0} 3-OH	i-C _{15:0} , C _{16:1ω5c} , i-C _{17:0} 3-OH, SF3*	i-C _{15:0} , i-C _{15:1} , i-C _{17:0} 3-OH, SF3*
Hydrolysis of:										
Casein	–	–	+	+	–	ND	–	–	+	+
Chitin	–	–	+	+	–	–	–	–	+	–
Gelatin	+	–	+	+	–	+	+	+	+	–
Starch	+	–	–	–	+	ND	–	–	–	–
Urea	–	+	–	–	–	–	–	–	+	–
Growth at 37 °C	+	–	+	–	+	+	–	+	+	–
Maximum NaCl concentration (% w/v)	1.0	3.0	ND	ND	1.0	1.0	3.0	1.0	ND	3.0
Assimilation of:										
N-Acetyl-D-glucosamine	+	+	–	–	–	+	+	–	+	+
L-Arabinose	+	–	–	–	–	+	+	–	+	+
Gluconate	+	–	–	–	–	–	+	–	–	–
D-Mannose	–	+	–	–	+	+	+	–	+	+
Maltose	+	+	–	–	+	+	+	–	+	+
α-Melibiose	–	w	–	–	–	+	+	–	–	+
L-Rhamnose	–	+	–	–	+	–	+	–	+	+
D-Ribose	+	–	–	–	–	–	–	–	–	–
Sucrose	+	–	–	–	–	+	+	+	+	+
Salicin	–	+	–	–	–	–	+	–	+	+
DNA G + C content (mol%)	39.7	40.4	45.8	44.3	48.9	47.2	43.0	42.7	44.6	45.0

*Data from this study.

†i, Iso-branched; SF3, summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1ω7c}); SF4, summed feature 4 (iso-C_{17:1} I and/or anteiso-C_{17:1} B).

was searched by NCBI BLAST and calculated by pairwise alignment obtained from the EzTaxon database (Chun *et al.*, 2007). Analysis of the 16S rRNA gene sequence was performed by using the software package MEGA version 4.0 (Tamura *et al.*, 2007), after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic trees were inferred using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. Bootstrap analysis based on 1000 replications was undertaken to test the robustness of the phylogenetic trees (Felsenstein, 1985). All branches of the neighbour-joining tree (Fig. 1) were also present in the maximum-parsimony tree (not shown).

Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate formed a cluster with members of the genera *Segetibacter*, *Terrimonas*, *Niastella*, *Flavisolibacter*, *Niabella* and *Chitinophaga* (Fig. 1). The family 'Chitinophagaceae' has recently been proposed to group these genera (<http://www.bacterio.net>). However, the levels of 16S rRNA gene sequence similarity with the type strains of recognized species of these genera ranged from 90.6 (*Chitinophaga* species) to 94.0% (*Terrimonas* species), demonstrating that strain RHYL-37^T is a distinct member of the family 'Chitinophagaceae', phylum *Bacteroidetes*.

Biomass for molecular systematic and chemotaxonomic studies was obtained after incubation at 30 °C for 3 days in shaken flasks containing R2A. Isoprenoid quinones were isolated from lyophilized cells as described by Collins *et al.* (1977), and were analysed by HPLC (UltiMate 3000; Dionex) as described by Xie & Yokota (2003). Genomic DNA of strain RHYL-37^T was prepared according to a modification of the procedure of Wilson (1987). The DNA G+C content was determined by HPLC according to Mesbah *et al.* (1989), using reversed-phase HPLC. To determine its whole-cell fatty acid profile, strain RHYL-37^T was cultured at 30 °C for 4 days on R2A agar. The type strains of some of the closest phylogenetic neighbours of strain RHYL-37^T (*Niastella koreensis* KACC 11465^T, *Terrimonas ferruginea* ATCC 13524^T, *Chitinophaga pinensis* DSM 2588^T and *Niabella aurantiaca* KACC 11698^T) were cultured under the same conditions and analysed in parallel. Analysis of fatty acid methyl esters was carried out according to the standard protocol of the Sherlock Microbial Identification System (MIDI).

The major respiratory quinone of strain RHYL-37^T was MK-7. The G+C content of the genomic DNA was 39.7 mol%, a value much lower than those of the most closely related organisms (47.2–48.9 mol% for members of the genus *Terrimonas* and 44.3–45.8 mol% for members of the genus *Niastella*). As shown in Table 1, the major fatty acids of strain RHYL-37^T were iso-C_{15:0} (19.5%), iso-C_{15:1} (14.7%), C_{16:0} (10.1%) and iso-C_{17:0} 3-OH (16.3%). The complete fatty acid compositions of strain RHYL-37^T and its phylogenetic neighbours are given in Supplementary Table S1 (available in IJSEM Online). The presence of 5.2% iso-C_{13:0} 3-OH and 3.7% anteiso-C_{15:1}, the absence of C_{16:1}ω5c and differences in the proportions of other fatty acids distinguished strain RHYL-37^T from related organisms.

In terms of physiological and biochemical properties, strain RHYL-37^T differed from most members of related genera in being able to glide and to hydrolyse starch. Furthermore, it could be distinguished from *Segetibacter koreensis* KCTC 12655^T on the basis of growth at 37 °C, the ability to hydrolyse gelatin and absence of urease. In addition, strain RHYL-37^T could assimilate several carbohydrates, whereas the two *Niastella* species could not assimilate any of the substrates included in the API 20NE and API ID 32 GN test kits. The characteristics that differentiate strain RHYL-37^T from closely related species are listed in Table 1.

On the basis of a variety of taxonomic criteria, strain RHYL-37^T formed a distinct and coherent taxonomic entity. Thus, it represents a novel genus and species in the phylum *Bacteroidetes*, for which the name *Parasegetibacter luojiensis* gen. nov., sp. nov. is proposed.

Description of *Parasegetibacter* gen. nov.

Parasegetibacter (Pa.ra.se.ge.ti.bac'ter. Gr. prep. *para* beside; N.L. masc. n. *Segetibacter* the name of a bacterial genus; N.L. masc. n. *Parasegetibacter* resembling the genus *Segetibacter*).

Cells are Gram-staining-negative, aerobic, non-spore-forming rods devoid of flagella but motile by gliding. Oxidase- and catalase-positive. Flexirubin pigments are not produced. Nitrate is not reduced to nitrite. The major respiratory quinone is MK-7. The DNA G+C content of the type strain of the type species is 39.7 mol%.

Fig. 1. Neighbour-joining tree, showing the phylogenetic positions of strain RHYL-37^T and related taxa based on 16S rRNA gene sequences. Bootstrap values (>50%) are shown at nodes as percentages of 1000 replicates. The sequence of *Balneola vulgaris* 13IX/A01/164^T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Phylogenetically, the genus *Parasegetibacter* is a member of the family 'Chitinophagaceae', phylum Bacteroidetes. The type species is *Parasegetibacter luojiensis*.

Description of *Parasegetibacter luojiensis* sp. nov.

Parasegetibacter luojiensis (lu.o.ji.en'sis. N.L. masc. adj. *luojiensis* pertaining to Luojia hill, where the campus of Wuhan University is located, where studies of the type strain were conducted).

The characteristics are the same as those given in the genus description. In addition, cells are 0.3–0.8 µm in diameter and 2–20 µm long. Colonies on 0.3 × MA are golden yellow, smooth, convex and circular with regular edges, 2–3 mm in diameter after 4 days of incubation. Growth occurs at 17–37 °C, at pH 5.0–9.0 and in the presence of 0–1 % NaCl (w/v); growth is optimal at 30 °C, pH 7.0 and in the absence of NaCl. Hydrolyses starch and tyrosine. Does not hydrolyse casein, chitin, cellulose or DNA. In API 20NE and API ID 32 GN strips, assimilates D-glucose, L-arabinose, D-mannose, N-acetyl-D-glucosamine, maltose, sucrose, D-ribose, potassium 2-ketogluconate and potassium 5-ketogluconate. Does not assimilate L-rhamnose, potassium glucosamine, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, glycogen, 3-hydroxybenzoic acid, L-serine, D-mannitol, salicin, melibiose, L-fucose, D-sorbitol, propionic acid, capric acid, valeric acid, trisodium citrate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid or L-proline. In API 20NE and API ZYM strips, tests positive for aesculin and gelatin hydrolysis and alkaline phosphatase, esterase (C4), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities; weakly positive for esterase lipase (C8) and lipase (C14) activities; negative for nitrate reduction, indole production, glucose fermentation and arginine dihydrolase, urease, β-glucuronidase and α-glucosidase activities. The major cellular fatty acids are iso-C_{15:0}, iso-C_{15:1}, C_{16:0} and iso-C_{17:0} 3-OH.

The type strain, RHYL-37^T (=CCTCC AB 208240^T =KCTC 22561^T), was isolated from soil from a forest of Euphrates poplar in Xinjiang, China.

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