

## *Pedobacter nyackensis* sp. nov., *Pedobacter alluvionis* sp. nov. and *Pedobacter borealis* sp. nov., isolated from Montana flood-plain sediment and forest soil

Nathan S. Gordon, Alejandra Valenzuela, Sandra M. Adams, Philip W. Ramsey, Jarrod L. Pollock, William E. Holben and James E. Gannon

### Correspondence

Nathan S. Gordon  
nathan.gordon@umontana.edu

Microbial Ecology Program, Division of Biological Sciences, The University of Montana, Missoula, MT 59812-1006, USA

Three Gram-negative, rod-shaped, non-spore-forming eubacterial strains were isolated in western Montana, USA, and subjected to taxonomic studies. Strains NWG-II14<sup>T</sup> and NWER-II11<sup>T</sup> were isolated from hyporheic sediments of a large alluvial flood plain, whereas strain G-1<sup>T</sup> was isolated from a conifer forest soil. On the basis of 16S rRNA gene sequence similarity, strains NWG-II14<sup>T</sup>, NWER-II11<sup>T</sup> and G-1<sup>T</sup> were shown to belong to the family *Sphingobacteriaceae* and are most closely related to various species of the genus *Pedobacter*. The results of molecular, physiological and biochemical tests allowed genotypic and phenotypic differentiation of these three strains from 23 *Pedobacter* species with validly published names. The three isolates therefore represent novel species, for which the names *Pedobacter nyackensis* sp. nov. (type strain NWG-II14<sup>T</sup> = DSM 19625<sup>T</sup> = LMG 24260<sup>T</sup>), *Pedobacter alluvionis* sp. nov. (type strain NWER-II11<sup>T</sup> = DSM 19624<sup>T</sup> = LMG 24258<sup>T</sup>) and *Pedobacter borealis* sp. nov. (type strain G-1<sup>T</sup> = DSM 19626<sup>T</sup> = LMG 24259<sup>T</sup>) are proposed.

The genus *Pedobacter* was initially described by Steyn *et al.* (1998) and, at the time of writing, included 23 species: *Pedobacter heparinus*, *P. piscium*, *P. africanus* and *P. saltans* (Steyn *et al.*, 1998), *P. cryoconitis* (Margesin *et al.*, 2003), *P. himalayensis* (Shivaji *et al.*, 2005), *P. caeni* (Vanparrys *et al.*, 2005), *P. sandarakinus* (Yoon *et al.*, 2006), *P. roseus* (Hwang *et al.*, 2006), *P. aquatilis* (Gallego *et al.*, 2006), *P. ginsengisoli* (Ten *et al.*, 2006), *P. panaciterrae* (Yoon *et al.*, 2007a), *P. suwonensis* (Kwon *et al.*, 2007), *P. insulae* (Yoon *et al.*, 2007b), *P. lentus* and *P. terricola* (Yoon *et al.*, 2007c), *P. koreensis* (Baik *et al.*, 2007), *P. duraquae*, *P. westerhofensis*, *P. metabolipauper*, *P. hartonius* and *P. steynii* (Muurholm *et al.*, 2007) and *P. terrae* (Yoon *et al.*, 2007d). All of these species are aerophilic or microaerophilic, Gram-negative rods with MK-7 as the predominant isoprenoid quinone and a DNA G+C content of 36–45 mol% ( $T_m$ ). Four species exhibit gliding motility, five exhibit non-gliding motility and four species have been reported to produce heparinase. *Pedobacter* species have been isolated from soil, fish, activated sludge, glacier

cryoconite, glacial water, drinking water, fresh water, a hypertrophic pond and a nitrifying enrichment of surface water. This range of habitats suggests that *Pedobacter* species are generalists, possessing a wide array of enzymes capable of degrading a diverse set of carbon structures for energy. Such metabolic diversity suggests that *Pedobacter* species have the potential to dominate aerobic heterotrophic microbial communities in various terrestrial and aquatic environments. In this report, the taxonomic characterization of three novel strains that group at the species level with other members of the genus *Pedobacter* is reported. One of these strains, NWG-II14<sup>T</sup>, showed seasonal domination of the readily cultured heterotrophic sediment community of an alluvial flood plain in western Montana.

Sediment and interstitial water samples were collected in sterile tubes from the hyporheic zone of the Nyack flood plain along the Middle Fork of the Flathead River, Montana, USA. Soil samples were collected from the rhizosphere of a western Montana conifer forest. During the isolation of organisms extracted from hyporheic sediments and soil, strains NWG-II14<sup>T</sup>, NWER-II11<sup>T</sup> and G-1<sup>T</sup> were recovered on environmental water agar (EWA), a Gelrite (Sigma) layer over dilute one-tenth-strength R2A agar (Difco) and undiluted R2A agar, respectively. EWA was composed of 1% agarose (Sigma) and filter-sterilized

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains NWG-II14<sup>T</sup>, G-1<sup>T</sup> and NWER-II11<sup>T</sup> are EU030686, EU030687 and EU030688, respectively.

Fatty acid profiles of strains NWG-II14<sup>T</sup>, G-1<sup>T</sup> and NWER-II11<sup>T</sup> and strains of related *Pedobacter* species are available as supplementary material with the online version of this paper.

formation water from wells on the flood plain. EWA was used to provide the bacterial community with nutrient levels that most closely represented *in situ* conditions. The Gelrite layer was used to encapsulate bacterial cells in a 3D matrix and solidified using divalent cations contained in the underlying dilute R2A agar. All plates were incubated aerobically for 2–4 weeks at 10 °C in the dark (*in situ* temperature ranges seasonally from 4 to 12 °C). Initial colonies of strain NWG-II14<sup>T</sup> on EWA were 0.2–0.5 mm in diameter, round and transparent, with creamy white centres developing after 6 weeks of incubation. Initial colonies of strains NWER-II11<sup>T</sup> and G-1<sup>T</sup> on Gelrite and R2A agar, respectively, were 0.3–1.0 mm in diameter, round and produced a reddish pigment. All isolates were subsequently cultivated on one-fifth-strength R2A agar at 10 °C for 7 days. On this medium, all strains were able to grow at 4–32 °C, but not at 0 or 37 °C. Growth of all strains was also observed at 10 and 30 °C on tryptic soy agar (TSA; Difco) and nutrient agar (NA; Difco). No growth was observed on MacConkey agar (BBL).

Nearly full-length 16S rRNA gene sequences of strains NWG-II14<sup>T</sup>, NWER-II11<sup>T</sup> and G-1<sup>T</sup> were obtained by direct PCR amplification of 1 µl culture (grown as described above) using the primers 27f, 907r and 1492r (Lane, 1991) and 536f (Holben *et al.*, 2004). PCR products were purified using the QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions and then subjected to direct bidirectional DNA sequence analysis (Murdock Molecular Biology Facility, University of Montana, Missoula). The Sequence Match version 3 component of the Ribosomal Database Project was used to determine  $S_{ab}$  scores (similarity *a* versus *b*) that indicate the closest matching relatives of the three strains.  $S_{ab}$  scores are generated by calculating the number of unique 7 bp oligomers shared between two partial 16S rRNA sequences divided by the smallest number of unique 7-mers in either sequence (Cole *et al.*, 2007). BLAST 2 SEQUENCES was used to generate 16S rRNA gene sequence similarities (Tatusova & Madden, 1999). Similarity scores indicated that strain NWG-II14<sup>T</sup> was most closely related to the type strains of *P. caeni* ( $S_{ab}$ =0.895; 97.2% sequence similarity), *P. steynii* ( $S_{ab}$ =0.890; 97.7%) and *P. heparinus* ( $S_{ab}$ =0.884; 97.4%). Strain NWER-II11<sup>T</sup> was most closely related to the type strains of *P. roseus* ( $S_{ab}$ =0.927; 98.9%), *P. sandarakinus* ( $S_{ab}$ =0.882; 97.3%) and *P. suwonensis* ( $S_{ab}$ =0.870; 97.5%). Strain G-1<sup>T</sup> was most closely related to the type strains of *P. sandarakinus* ( $S_{ab}$ =0.895; 98.1%), *P. suwonensis* ( $S_{ab}$ =0.894; 98.2%) and *P. roseus* ( $S_{ab}$ =0.883; 98.0%).

Multiple alignments were performed with the sequences of these novel strains and the type strains of species with validly published names obtained from the NCBI GenBank database (Wheeler *et al.*, 2000; Benson *et al.*, 2000) for the genera *Sphingobacterium* and *Pedobacter* using CLUSTAL\_X (Thompson *et al.*, 1997). PAUP version 4.0 beta 10 (Swofford, 2003) was used to generate phylogenetic trees using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) algorithms.

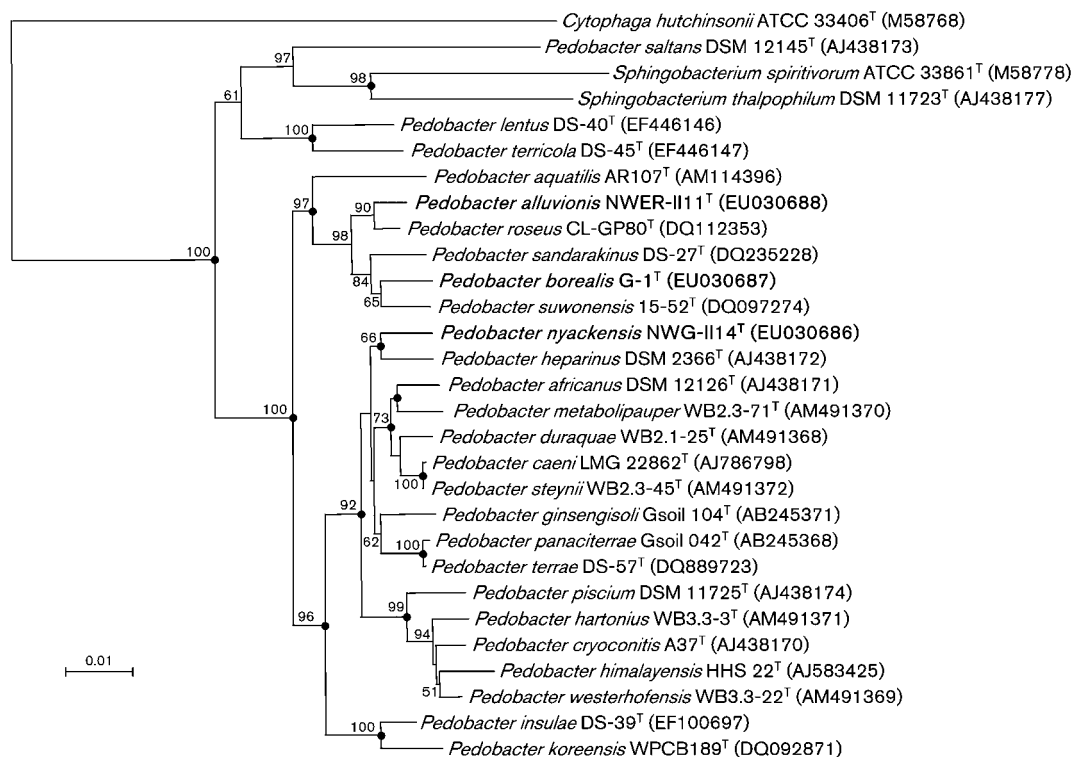
PAUP was also used to generate bootstrap support for all tree topologies using 1000 random replicates from the original sequence data (Felsenstein, 1985). Strains NWG-II14<sup>T</sup>, NWER-II11<sup>T</sup> and G-1<sup>T</sup> clustered with members of the genus *Pedobacter* with a bootstrap resampling value of 100% (Fig. 1). On the basis of the phylogenetic data, it is clear that the three novel strains should be classified in the genus *Pedobacter*.

For chemotaxonomic analyses, cell biomass was obtained from cells cultured in one-fifth-strength R2A broth at 20 °C. Isoprenoid quinones were extracted and purified according to Collins *et al.* (1977) with the TLC modification as noted by Tamaoka *et al.* (1983). Purified isoprenoid quinones were analysed by reversed-phase HPLC using a Phenomenex Synergi 4u Fusion-RP 80A (250 × 4.6 mm) column. Phospholipid fatty acids were extracted from cell pellets and analysed by the method of White & Ringelberg (1998) as described previously by Rillig *et al.* (2006). The G+C content of the genomic DNA of strains NWG-II14<sup>T</sup>, NWER-II11<sup>T</sup> and G-1<sup>T</sup> was determined by HPLC analysis of deoxyribonucleosides according to the method of Mesbah *et al.* (1989), using a reversed-phase column (Synergi 4u Fusion-RP 80A; Phenomenex).

DNA–DNA hybridization experiments were conducted between strain NWER-II11<sup>T</sup> and *P. roseus* JCM 13399<sup>T</sup> (98.9% 16S rRNA gene sequence similarity) and between strain G-1<sup>T</sup> and *P. suwonensis* DSM 18130<sup>T</sup> (98.2% 16S rRNA gene sequence similarity) by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). DNA–DNA relatedness values were determined in duplicate and mean values were calculated. DNA was isolated using a French pressure cell (ThermoSpectronic) and was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization experiments were carried out as described by De Ley *et al.* (1970) with the modifications described by Huß *et al.* (1983) using a Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian).

Cell morphology and motility were determined using a Zeiss Axioskop light microscope at × 1000 with cells grown for 3 days at 10 °C on TSA. Gram-staining reaction and catalase and oxidase activities were tested according to the methods of Gerhardt *et al.* (1994). Heparinase activity was detected by the method of Joubert *et al.* (1984). Carbon assimilation and enzyme production tests were conducted using API 50CH, API 20NE and API ZYM strips (bioMérieux) under aerobic conditions.

Strains NWG-II14<sup>T</sup>, NWER-II11<sup>T</sup> and G-1<sup>T</sup> possessed biochemical characteristics that associated them with members of the genus *Pedobacter* and differentiated them from members of the genus *Sphingobacterium* (Steyn *et al.*, 1998). They lacked urease activity and did not assimilate melezitose. The major isoprenoid quinone of the three strains was MK-7. The DNA G+C contents of strains



**Fig. 1.** Neighbour-joining tree based on 16S rRNA sequences showing the phylogenetic positions of strains NWG-II14<sup>T</sup>, NWER-II11<sup>T</sup> and G-1<sup>T</sup> in relation to the type strains of 23 *Pedobacter* and two *Sphingobacterium* species. *Cytophaga hutchinsonii* ATCC 33406<sup>T</sup> was used as a monophyletic outgroup. Bootstrap support values greater than 50% (percentages of 1000 replicates) are shown at branch points. Filled circles indicate nodes that were also recovered using maximum-parsimony algorithms. Bar, 0.01 substitutions per nucleotide site.

NWG-II14<sup>T</sup>, NWER-II11<sup>T</sup> and G-1<sup>T</sup> were  $41.1 \pm 0.4$ ,  $39.3 \pm 0.6$  and  $39.7 \pm 0.5$  mol%, respectively. These values are consistent with the 36–45 mol% range previously suggested for the genus *Pedobacter* (Steyn *et al.*, 1998). All three strains contained iso-C<sub>15:0</sub>, iso-C<sub>17:0</sub> 3-OH, C<sub>16:1</sub>ω5c and C<sub>16:1</sub>ω7c as major cellular fatty acids, which is also in accordance with the description of the genus *Pedobacter* (Steyn *et al.*, 1998). The fatty acid compositions of strains NWG-II14<sup>T</sup>, NWER-II11<sup>T</sup>, G-1<sup>T</sup> and strains of related *Pedobacter* species are detailed in Supplementary Table S1, available in IJSEM Online. Strains NWER-II11<sup>T</sup> and G-1<sup>T</sup> exhibited low levels of DNA–DNA relatedness to *P. roseus* JCM 13399<sup>T</sup> (6.3%) and *P. suwonensis* DSM 18130<sup>T</sup> (4.4%), respectively, indicating that both strains are unique at the species level according to the recommended threshold of 70% DNA–DNA similarity (Wayne *et al.*, 1987). In contrast to other members of the genus *Pedobacter* (except *P. suwonensis*), strains NWG-II14<sup>T</sup>, NWER-II11<sup>T</sup> and G-1<sup>T</sup> demonstrated α-fucosidase activity.

On the basis of the phylogenetic, phenotypic and chemotaxonomic data presented, strains NWG-II14<sup>T</sup>, NWER-II11<sup>T</sup> and G-1<sup>T</sup> are proposed as the type strains of three novel species in the genus *Pedobacter*, for which the names *Pedobacter nyackensis* sp. nov., *Pedobacter*

*alluvionis* sp. nov. and *Pedobacter borealis* sp. nov., respectively, are proposed.

#### Description of *Pedobacter nyackensis* sp. nov.

*Pedobacter nyackensis* (ny.ack.en'sis. N.L. masc. adj. *nyackensis* from Nyack, a region of north-western Montana, USA).

Colonies grown on TSA for 48 h at 20 °C are 0.5–5.0 mm in diameter, round, convex, opaque and beige, darkening with age. Cells are Gram-negative, oxidase- and catalase-positive, non-motile, non-spore-forming rods (0.2–0.7 × 1.0–4.0 μm), with an oxidative metabolism. Good growth is observed after 96 h at 10 and 30 °C on TSA, NA and R2A. Growth does not occur at 37 °C or on MacConkey agar. Growth occurs at pH 5–10 (optimal pH 7–8). It can be differentiated from other *Pedobacter* species with validly published names by its ability to assimilate L-sorbose; it can be differentiated from its closest phylogenetic relatives, *P. caeni*, *P. heparinus* and *P. africanus*, by its ability to assimilate glycogen and 2-ketogluconate. The fatty acid profile is composed of C<sub>14:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>15:0</sub> 2-OH, C<sub>16:1</sub>ω7c and C<sub>16:1</sub>ω5c. The major isoprenoid quinone is MK-7. Additional phenotypic properties are shown in Table 1.

**Table 1.** Differential characteristics of strains NWGII14<sup>T</sup>, NWERII11<sup>T</sup> and G-1<sup>T</sup> and phylogenetically related species of the genus *Pedobacter*

Taxa: 1, strain NWGII14<sup>T</sup>; 2, strain NWERII11<sup>T</sup>; 3, strain G-1<sup>T</sup>; 4, *P. heparinus*; 5, *P. africanus*; 6, *P. piscium*; 7, *P. cryoconitis*; 8, *P. himalayensis*; 9, *P. caeni*; 10, *P. sandarakinus*; 11, *P. roseus*; 12, *P. aquatilis*; 13, *P. ginsengisoli*; 14, *P. suwonensis*; 15, *P. steynii*. Unless otherwise indicated, data were compiled from Steyn *et al.* (1998), Margesin *et al.* (2003), Shivaji *et al.* (2005), Vanparys *et al.* (2005), Yoon *et al.* (2006), Hwang *et al.* (2006), Gallego *et al.* (2006), Ten *et al.* (2006), Muurholm *et al.* (2007), Kwon *et al.* (2007) and the present study. +, Positive; (+), weak reaction; -, negative; v, variable; NA, data not available. All taxa are positive for the following features: aerobic growth, presence of catalase, oxidase and alkaline phosphatase (NA for *P. ginsengisoli*) and assimilation of D-glucose, D-mannose, D-lactose, cellobiose, N-acetylglucosamine, amygdalin (NA for *P. himalayensis* and *P. roseus*), salicin (v for *P. africanus*), melibiose (v for *P. saltans*; NA for *P. roseus*), sucrose (v for *P. saltans*) and trehalose (v for *P. saltans*). All taxa are negative for the following features: Gram-staining, sporulation, indole production, nitrate reduction, urease activity and assimilation of D-lyxose (NA for *P. himalayensis* and *P. roseus*), D-fucose (NA for *P. himalayensis* and *P. roseus*), erythritol (NA for *P. roseus* and *P. ginsengisoli*), inositol and dulcitol.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Source	Flood-plain sediment	Flood-plain sediment	Soil	Soil	Soil, activated sludge	Fish	Glacier cryoconite	Glacier water	Nitrifying inoculum	Soil	Hypertrophic freshwater	Drinking water	Soil	Cabbage rhi-zosphere	Hard-water creek
Growth temperature range (°C)	<2–32	4–30	4–30	5–30	NA	5–30	1–25	4–25	NA	4–33	5–33	4–30	4–30	1–37	10–30
DNA G+C content (mol%)	41.1	39.3	39.7	42–43	43–45	40–43	43.4	41.0	42.7	39.7	41.3	38	43.6	44.2	NA
Gelatin hydrolysis	–	+	+	–	v	–	+	+	–	–	+	–	–	+	–
Arginine dihydrolase	–	+	+	–	–	NA	–	+	–	–	–	–	–	–	–
Heparinase	+	–	–	+	+	–	–	+	NA	NA	–	NA	NA	–	–
Assimilation of (API 50CH):															
Glycerol	–	–	–	–	–	–	–	+	–	–	+	–	+	–	+
D-Arabinose	–	+	–	–	v	–	–	+	–	–	NA	–	–	–	–
L-Arabinose	+	+	+	–	v	–	+	+	+	–	+	+	–	+	–
D-Ribose	+	+	–	+	v	+	–	+	–	–	–	–	–	–	–
D-Xylose	+	–	+	+	v	–	+	+	–	–	NA	–	+	+	+
L-Xylose	–	–	–	–	–	–	–	+	+	–	+	–	+	–	NA
D-Adonitol	–	+	–	+	–	–	–	+	–	–	NA	–	–	–	–
Methyl β-D-xyloside	–	+	–	–	–	–	–	NA	–	–	NA	–	NA	NA	–
D-Galactose	+	+	+	+	+	v	+	+	–	+	+	+	+	+	–
D-Fructose	+	+	+	+	v	+	+	+	–	–	+	+	+	(+)	–
L-Sorbose	+	+	–	–	–	–	–	–	–	–	NA	–	–	–	NA
L-Rhamnose	+	+	+	+	+	v	–	+	–	–	+	+	–	+	–
D-Mannitol	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–
D-Sorbitol	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–
Methyl α-D-mannoside	+	+	+	+	+	–	–	+	+	(+)	NA	+	NA	+	–
Methyl α-D-glucoside	+	+	+	+	+	+	(+)	+	+	(+)	NA	+	NA	+	+
Arbutin	+	+	+	v	v	+	+	+	+	–	NA	+	NA	+	+
Aesculin	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+
Maltose	+	+	+	+	v	+	+	+	+	+	+	+	+	+	+
Inulin	+	+	–	–	–	–	(+)	+	+	–	+	–	+	–	–
Melezitose	–	–	–	–	–	–	–	–	–	–	NA	+	NA	+	–
Raffinose	+	+	+	–	–	+	+	+	+	–	+	+	+	+	+
Starch	+	+	+	–	v	+	+	+	+	+	NA	+	–	+	+
Glycogen	+	–	–	–	–	–	+	–	–	+	+	–	–	+	+
Xylitol	–	–	–	–	–	–	–	–	+	–	NA	–	–	–	–
β-Gentiobiose	+	+	+	+	v	+	+	NA	+	+	NA	+	NA	+	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Turanose	+	+	+	+	+	+	+	NA	-	-	NA	+	NA	+	+
D-Tagatose	-	-	-	-	-	-	-	NA	-	-	NA	-	NA	NA	-
L-Fucose	-	-	-	+	V	-	-	+	-	-	NA	-	NA	-	-
D-Arabitol	-	-	-	-	-	-	-	NA	-	-	NA	-	NA	NA	-
L-Arabitol	-	-	-	-	-	-	-	NA	-	-	NA	-	NA	NA	-
Gluconate	-	-	-	-	-	-	-	+	-	-	-	-	-	NA	-
2-Ketogluconate	+	-	-	-	-	-	+	NA	-	-	NA	-	NA	NA	-
5-Ketogluconate	+	-	+	-	-	-	-	NA	-	+	NA	-	NA	NA	-
Enzyme activity (API ZYM)															
Esterase	+	+	+	V	-	V	NA	NA	-	+	+	+	NA	+	+
Esterase lipase	+	+	+	+	+	+	-	NA	+	+	+	+	NA	+	+
Lipase	-	-	-	-	-	V	-	-	-	-	-	-	-	-	-
Leucine arylamidase	+	+	+	+	+	+	NA	NA	+	+	+	+	NA	+	+
Valine arylamidase	+	+	+	-	V	+*	NA	NA	-	-	+	+	NA	+	+
Cystine arylamidase	+	+	+	V	-	+*	NA	NA	-	(+)	-	+	NA	-	-
Trypsin	-	+	+	-	-	+*	NA	NA	-	-	+	+	NA	+	NA
Chymotrypsin	-	-	-	V	-	+*	NA	NA	-	-	+	-	NA	-	NA
Acid phosphatase	+	+	+	+	+	+	NA	+	+	+	+	+	NA	+	NA
Naphthol-AS-BI-phosphohydrolyase	+	+	+	+	+	NA	NA	NA	-	+	+	+	NA	+	+
$\alpha$ -Galactosidase	+	+	+	V	-	+*	NA	NA	-	-	+	-	NA	-	NA
$\beta$ -Galactosidase	+	+	+	+	+	+	+	+	+	+	V	+	-	+	+
$\beta$ -Glucuronidase	-	-	+	-	-	V*	NA	NA	-	-	-	-	NA	-	NA
$\alpha$ -Glucosidase	+	+	+	+	+	+	NA	NA	-	+	+	+	NA	+	+
$\beta$ -Glucosidase	+	+	V	V	V	+*	+	+	-	+	-	+	NA	+	+
N-Acetyl- $\beta$ -glucosaminidase	+	+	+	+	+	+	NA	NA	-	+	+	+	NA	+	+
$\alpha$ -Mannosidase	+	-	-	V	V	+*	NA	NA	-	-	-	+	NA	-	-
$\alpha$ -Fucosidase	+	+	+	-	-	-*	NA	NA	-	-	-	-	NA	+	-

\*Data from Takeuchi &amp; Yokota (1992).

The type strain is NWG-III4<sup>T</sup> (=DSM 19625<sup>T</sup> =LMG 24260<sup>T</sup>), isolated from hyporheic sediments of the Nyack flood plain in north-western Montana, USA. The DNA G+C content of the type strain is 36.1 mol%.

### Description of *Pedobacter alluvionis* sp. nov.

*Pedobacter alluvionis* (al.lu.vi.o'nis. L. gen. n. *alluvionis* of alluvial land).

Colonies grown on TSA for 48 h at 20 °C are 0.2–2.0 mm in diameter, round, convex, opaque and have a reddish-pink pigment. Cells are Gram-negative, oxidase- and catalase-positive, non-motile, non-spore-forming rods (0.3–0.6 × 1.0–3.0 µm), with an oxidative metabolism. Good growth is observed after 96 h at 10 and 30 °C on TSA, NA and R2A. Growth does not occur at 37 °C or on MacConkey agar. Growth occurs at pH 5–10 (optimal pH 7–8). It can be differentiated from other *Pedobacter* species with validly published names by its ability to assimilate methyl β-D-xyloside and L-sorbose; it can be differentiated from its closest phylogenetic relatives, *P. roseus*, *P. sandarakinus* and *P. aquatilis*, by the presence of arginine dihydrolase activity and its ability to assimilate D-arabinose, D-ribose and D-adonitol. The fatty acid profile is composed of C<sub>14:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>15:0</sub> 2-OH, C<sub>16:1</sub>ω7c and C<sub>16:1</sub>ω5c. The major isoprenoid quinone is MK-7. Additional phenotypic properties are shown in Table 1.

The type strain is NWER-III1<sup>T</sup> (=DSM 19624<sup>T</sup> =LMG 24258<sup>T</sup>), isolated from hyporheic sediments of the Nyack flood plain in north-western Montana, USA. The DNA G+C content of the type strain is 34.4 ± 0.2 mol%.

### Description of *Pedobacter borealis* sp. nov.

*Pedobacter borealis* (bo.re.a'lis. M.L. masc. adj. *borealis* related to the north, boreal).

Colonies grown on TSA for 48 h at 20 °C are 0.2–2.0 mm in diameter, round, convex, opaque and have a reddish-pink pigment. Cells are Gram-negative, oxidase- and catalase-positive, non-motile, non-spore-forming rods (0.3–0.5 × 0.7–3.0 µm), with an oxidative metabolism. Good growth is observed after 96 h at 10 and 30 °C on TSA, NA and R2A. Growth does not occur at 37 °C or on MacConkey agar. Growth occurs at pH 5–7 (optimal pH 7). It can be differentiated from other *Pedobacter* species with validly published names by the presence of the enzyme β-glucuronidase; it can be differentiated from its closest phylogenetic relatives, *P. sandarakinus*, *P. roseus* and *P. aquatilis*, by the presence of arginine dihydrolase activity and its ability to assimilate D-xylose. The fatty acid profile is composed of C<sub>14:0</sub>, iso-C<sub>15:0</sub>, iso-C<sub>15:0</sub> 2-OH, C<sub>16:1</sub>ω7c and C<sub>16:1</sub>ω5c. The major isoprenoid quinone is MK-7. Additional phenotypic properties are shown in Table 1.

The type strain is G-1<sup>T</sup> (=DSM 19626<sup>T</sup> =LMG 24259<sup>T</sup>), isolated from the rhizosphere of a conifer forest in western

Montana, USA. The DNA G+C content of the type strain is 35.5 ± 0.5 mol%.

### Acknowledgements

Special thanks go to the Dalimata family, Brian Reid and Jack Stanford's team at the Flathead Lake Biological Station for their help in facilitating this research. This work was supported by National Science Foundation Microbial Observatory grant no. MCB-0348773.

### References

- Baik, K. S., Park, Y.-D., Kim, M. S., Park, S. C., Moon, E. Y., Rhee, M. S., Choi, J. H. & Seong, C. N. (2007). *Pedobacter koreensis* sp. nov., isolated from fresh water. *Int J Syst Evol Microbiol* 57, 2079–2083.
- Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., Rapp, B. A. & Wheeler, D. L. (2000). GenBank. *Nucleic Acids Res* 28, 15–18.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for base ratio determination of bacterial DNA. *Anal Biochem* 81, 461–466.
- Cole, J. R., Chai, B., Farris, R. J., Wang, Q., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Bandela, A. M., Cardenas, E., Garrity, G. M. & Tiedje, J. M. (2007). The ribosomal database project (RDP-II): introducing *myrDP* space and quality controlled public data. *Nucleic Acids Res* 35, D169–D172.
- Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 100, 221–230.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* 12, 133–142.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Gallego, V., Garcia, M. T. & Ventosa, A. (2006). *Pedobacter aquatilis* sp. nov., isolated from drinking water, and emended description of the genus *Pedobacter*. *Int J Syst Evol Microbiol* 56, 1853–1858.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Holben, W. E., Feris, K. P., Kettunen, A. & Apajalahti, J. H. A. (2004). GC fractionation enhances microbial community diversity assessment and detection of minority populations of bacteria by denaturing gradient gel electrophoresis. *Appl Environ Microbiol* 70, 2263–2270.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* 4, 184–192.
- Hwang, C. Y., Choi, D. H. & Cho, B. C. (2006). *Pedobacter roseus* sp. nov., isolated from a hypertrophic pond, and emended description of the genus *Pedobacter*. *Int J Syst Evol Microbiol* 56, 1831–1836.
- Joubert, J. J., van Rensburg, E. J. & Pitout, M. J. (1984). A plate method for demonstrating the breakdown of heparin and chondroitin sulfate by bacteria. *J Microbiol Methods* 2, 197–202.
- Kluge, A. G. & Farris, J. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* 18, 1–32.
- Kwon, S.-W., Kim, B.-Y., Lee, K.-H., Jang, K.-Y., Seok, S.-J., Kwon, J.-S., Kim, W.-G. & Weon, H.-Y. (2007). *Pedobacter suwonensis* sp. nov., isolated from the rhizosphere of Chinese cabbage (*Brassica campestris*). *Int J Syst Evol Microbiol* 57, 480–484.

- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Margesin, R., Spröer, C., Schumann, P. & Schinner, F. (2003). *Pedobacter cryoconitis* sp. nov., a facultative psychrophile from alpine glacier cryoconite. *Int J Syst Evol Microbiol* **53**, 1291–1296.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Muurholm, S., Cousin, S., Päuker, O., Brambilla, E. & Stackebrandt, E. (2007). *Pedobacter duraquae* sp. nov., *Pedobacter westerhofensis* sp. nov., *Pedobacter metabolipauper* sp. nov., *Pedobacter hartonius* sp. nov. and *Pedobacter steynii* sp. nov., isolated from a hard-water rivulet. *Int J Syst Evol Microbiol* **57**, 2221–2227.
- Rillig, M. C., Mummey, D. L., Ramsey, P. W., Klironomos, J. N. & Gannon, J. E. (2006). Phylogeny of arbuscular mycorrhizal fungi predicts community composition of symbiosis-associated bacteria. *FEMS Microbiol Ecol* **57**, 389–395.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shivaji, S., Chaturvedi, P., Reddy, G. S. N. & Suresh, K. (2005). *Pedobacter himalayensis* sp. nov., from the Hamta glacier located in the Himalayan mountain ranges of India. *Int J Syst Evol Microbiol* **55**, 1083–1088.
- Steyn, P. L., Segers, P., Vancanneyt, M., Sandra, P., Kersters, K. & Joubert, J. J. (1998). Classification of heparinolytic bacteria into a new genus, *Pedobacter*, comprising four species: *Pedobacter heparinus* comb. nov., *Pedobacter piscium* comb. nov., *Pedobacter africanus* sp. nov. and *Pedobacter saltans* sp. nov. Proposal of the family *Sphingobacteriaceae* fam. nov. *Int J Syst Bacteriol* **48**, 165–177.
- Swofford, D. L. (2003). PAUP\*: Phylogenetic analysis using parsimony (\*and other methods), version 4.0B10. Sunderland, MA: Sinauer Associates.
- Takeuchi, M. & Yokota, A. (1992). Proposals of *Sphingobacterium faecium* sp. nov., *Sphingobacterium piscium* sp. nov., *Sphingobacterium heparinum* comb. nov., *Sphingobacterium thalophilum* comb. nov., and two genospecies of the genus *Sphingobacterium* and synonymy of *Flavobacterium yabuuchiae* and *Sphingobacterium spiritivorum*. *J Gen Appl Microbiol* **38**, 465–482.
- Tamaoka, J., Katayama-Fujimura, Y. & Kuraishi, H. (1983). Analysis of bacterial menaquinone mixtures by high performance liquid chromatography. *J Appl Bacteriol* **54**, 31–36.
- Tatusova, T. A. & Madden, T. L. (1999). BLAST 2 Sequences, a new tool for comparing protein and nucleotide sequences. *FEMS Microbiol Lett* **174**, 247–250.
- Ten, L. N., Liu, Q.-M., Im, W.-T., Lee, M., Yang, D.-C. & Lee, S.-T. (2006). *Pedobacter ginsengisoli* sp. nov., a DNase-producing bacterium isolated from soil of a ginseng field in South Korea. *Int J Syst Evol Microbiol* **56**, 2565–2570.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Vanparys, B., Heylen, K., Lebbe, L. & De Vos, P. (2005). *Pedobacter caeni* sp. nov., a novel species isolated from a nitrifying inoculum. *Int J Syst Evol Microbiol* **55**, 1315–1318.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Wheeler, D. L., Chappey, C., Lash, A. E., Leipe, D. D., Madden, T. L., Schuler, G. D., Tatusova, T. A. & Rapp, B. A. (2000). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* **28**, 10–14.
- White, D. C. & Ringelberg, D. B. (1998). Signature lipid biomarker analysis. In *Techniques in Microbial Ecology*, pp. 255–272. Edited by R. S. Burlage, R. Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors. Oxford University Press.
- Yoon, J.-H., Lee, M.-H., Kang, S.-J., Park, S.-Y. & Oh, T.-K. (2006). *Pedobacter sandarakinus* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **56**, 1273–1277.
- Yoon, M.-H., Ten, L. N., Im, W.-T. & Lee, S.-T. (2007a). *Pedobacter panaciterrae* sp. nov., isolated from soil in South Korea. *Int J Syst Evol Microbiol* **57**, 381–386.
- Yoon, J.-H., Kang, S.-J., Oh, H. W. & Oh, T.-K. (2007b). *Pedobacter insulae* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **57**, 1999–2003.
- Yoon, J.-H., Kang, S.-J., Park, S. & Oh, T.-K. (2007c). *Pedobacter lentus* sp. nov. and *Pedobacter terricola* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **57**, 2089–2095.
- Yoon, J.-H., Kang, S.-J. & Oh, T.-K. (2007d). *Pedobacter terrae* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **57**, 2462–2466.