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

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Five isolates that were related phylogenetically to members of the genus *Pedobacter* were isolated from freshwater of the hard-water creek Westerhöfer Bach, North Germany. The five strains (WB 2.1-25^T, WB 2.3-71^T, WB 3.3-3^T, WB 3.3-22^T and WB 2.3-45^T) were Gram-negative and chemoheterotrophic, with rod-shaped cells. Most of their metabolic properties matched those given in the description of the genus *Pedobacter*. Consistent with the genus description, their fatty acids included mainly iso-C_{15:0} and summed feature 3 (C_{16:1}ω7c, iso-C_{15:0} 2-OH or both); C_{16:1}ω5c, C_{16:0}, iso-C_{15:0} 3-OH, C_{16:0} 3-OH and iso-C_{17:0} 3-OH were present in smaller amounts. The major isoprenoid quinone was menaquinone 7. With one exception, binary similarity values of the almost complete 16S rRNA gene sequences determined among the isolates as well as between the isolates and type strains of *Pedobacter* species were lower than 98.5%. The only exception was the close relationship between *Pedobacter caeni* DSM 16990^T and strain WB 2.3-45^T (99.2% similarity). DNA–DNA reassociation values determined for this pair of strains was 29.8%, indicating that strain WB 2.3-45^T represents a unique genospecies. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strains WB 2.1-25^T and WB 2.3-71^T form a group that is moderately related to *P. caeni* and strain WB 2.3-45^T (98.5% similarity). Strains WB 3.3-3^T and WB 3.3-22^T (98.5% similarity) branched separately from these four organisms. The five phylogenetically isolated strains differed from each other as well as from the type strain of the type species (*Pedobacter heparinus* DSM 2366^T) and some related representatives of the genus in several metabolic reactions and cultural parameters. On the basis of phenotypic and phylogenetic distinctiveness, five novel species are proposed: *Pedobacter duraquae* sp. nov., with WB 2.1-25^T (=DSM 19034^T=CIP 109481^T) as the type strain; *Pedobacter westerhofensis* sp. nov., with WB 3.3-22^T (=DSM 19036^T=CIP 109479^T) as the type strain; *Pedobacter metabolipauper* sp. nov., with WB 2.3-71^T (=DSM 19035^T=CIP 109480^T) as the type strain; *Pedobacter hartonius* sp. nov., with WB 3.3-3^T (=DSM 19033^T=CIP 109468^T) as the type strain; and *Pedobacter steynii* sp. nov., with WB 2.3-45^T (=DSM 19110^T=CIP 109507^T) as the type strain.

The genus *Pedobacter* (Steyn *et al.*, 1998) was established to accommodate *Sphingobacterium heparinum* (Takeuchi & Yokota, 1992) and some other heparinolytic bacteria that, on the basis of DNA–DNA hybridization (Steyn *et al.*,

1992, 1993), were shown to fall outside the realm of the genus *Sphingobacterium*. Several novel species have been added to the genus *Pedobacter* during the past few years, isolated mainly from soil (Ten *et al.*, 2006; Yoon *et al.*, 2007), freshwater (Gallego *et al.*, 2006; Hwang *et al.*, 2006; Vanparys *et al.*, 2005) and glaciers (Margesin *et al.*, 2003; Shivaji *et al.*, 2005). During a recent survey on the microbial composition of a hard-water creek, almost 1000 strains were isolated on R2A agar, about 50% of which had swarming (40%) or slime-producing (10%)

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains WB 2.1-25^T, WB 3.3-22^T, WB 2.3-71^T, WB 3.3-3^T and WB 2.3-45^T are AM491368–AM491372, respectively.

A table showing antibiotic sensitivities of the *Pedobacter* WB isolates is available as supplementary material with the online version of this paper.

capacities. Whereas almost all of the swarming bacteria and many slime-formers could be affiliated to the genus *Flavobacterium* (Brambilla *et al.*, 2007; Cousin *et al.*, 2007), other slime-producing organisms were members of different proteobacterial taxa (Stackebrandt *et al.*, 2007; unpublished data) and of the phylum *Bacteroidetes* (unpublished data). Among the latter organisms, five strains, identified by partial 16S rRNA gene sequences as members of the genus *Pedobacter*, were subjected to a polyphasic taxonomic study; subsequently, all were described as representatives of novel species.

The isolation of strains from the Westerhöfer Bach on medium R2A (Difco) has been described by Brambilla *et al.* (2007). Strains WB 2.1-25^T, WB 2.3-71^T and WB 2.3-45^T were isolated about 180 m downstream of the spring (station 2; station 1 being the source of Westerhöfer Bach), whereas strains WB 3.3-3^T and WB 3.3-22^T were isolated about 250 m downstream of the spring (station 3). Following isolation, strains were transferred and maintained on medium 67 (M67; DSMZ, 2001) at 25 °C for several days. The same medium was also used to cultivate the reference strains *Pedobacter heparinus* DSM 2366^T, *Pedobacter africanus* DSM 12126^T, *Pedobacter caeni* DSM 16990^T and *Pedobacter cryoconitis* DSM 14825^T. Maintenance in glycerol served as a medium-term preservation method. Cultures were preserved in liquid N₂ and freeze-dried.

DNA extraction and PCR amplification of the 16S rRNA genes were carried out as described by Rainey *et al.* (1996). The PCR amplicons were purified by using the QIAquick PCR Purification kit (QIAGEN) according to the instructions of the manufacturer. Sequencing of the PCR products, manual alignment of the sequences with those of *Pedobacter* type strains and determination of similarity coefficients were done as described by Rainey *et al.* (1996). The algorithm of De Soete (1983) and the neighbour-joining algorithm (Felsenstein, 1993) were used to generate tree topologies.

Binary similarity values of the almost complete sequences of the 16S rRNA gene, determined for the five WB strains,

were lower than 98.5 %. Two strain clusters emerged from the analysis, one containing strains WB 2.1-25^T, WB 2.3-71^T and WB 2.3-45^T (97.7–98.5 % similarity) and the other containing strains WB 3.3-3^T and WB 3.3-22^T (98.5 % similarity). The similarity values between the two groups were 95.8–97.0 %. With the exception of *P. caeni* DSM 16990^T, which shared a 16S rRNA gene sequence similarity value of 99.2 % with strain WB 2.3-45^T, the type strains of other *Pedobacter* species were more distantly related to the isolates (91.5–98.2 % similarity). *P. caeni* DSM 16990^T, *P. africanus* DSM 12126^T and *P. cryoconitis* DSM 14825^T were included in the following studies because they showed the highest similarity values with the novel WB strains. *P. heparinus* DSM 2366^T was included as the type strain of the type species of the genus. Phylogenetic relationships of the novel strains to other *Pedobacter* species type strains are shown in a phylogenetic tree (Fig. 1) generated based on the algorithm of De Soete (1983). Topologies of the neighbour-joining and maximum-likelihood trees differed slightly in the branching points of the deeply branching clades (not shown), but the affiliation of phylogenetic neighbours are in accord with those published previously (Gallego *et al.*, 2006; Margesin *et al.*, 2003; Shivaji *et al.*, 2005; Ten *et al.*, 2006; Yoon *et al.* 2007).

Literature data compiled from hundreds of species descriptions in 2005 indicated that strains sharing less than 98.8 % sequence similarity belong to different genospecies (Stackebrandt & Ebers, 2006); however, values above this threshold value are not necessarily indicative of novel genospecies. Therefore, the genomic relatedness between *P. caeni* 16990^T and WB 2.3-45^T was determined by using the spectrophotometric DNA–DNA reassociation method. DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by hydroxyapatite chromatography as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970), with the modifications of Huß *et al.* (1983) and Escara & Hutton (1980), using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a

Fig. 1. Relationship of the WB isolates to type strains of *Pedobacter* species. The dendrogram is based on 16S rRNA gene sequence comparisons using the algorithm of De Soete (1983). Bar, 2 % sequence divergence, as determined by measuring the length of the horizontal lines connecting any two species.

Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian). Two reassociation experiments resulted in 35.7 and 23.9 % reassociation (mean of 29.8 %). These low values indicate that strain WB 2.3-45^T represents a novel genospecies.

The novel strains and some *Pedobacter* reference strains were subjected to fatty acid methyl ester analysis to confirm membership to the genus. Cultures of all strains were grown on trypticase soy agar (TSA; Difco) for 24 h. Fatty acids were extracted and analysed (Miller, 1982) according to the standard protocol of the Microbial Identification System (MIS; MIDI Microbial ID). Extracts were analysed using a Hewlett Packard model HP6890A GC equipped with an FID as described by Kämpfer & Kroppenstedt (1996). The major fatty acids were summed feature 3 (C_{16:1}ω7c, iso-C_{15:0} 2-OH or both) (35–48 % of total) and iso-C_{15:0} (15–26.5 %); C_{16:1}ω5c, C_{16:0}, iso-C_{15:0} 3-OH, C_{16:0} 3-OH and iso-C_{17:0} 3-OH were present in smaller amounts. The amounts of fatty acids determined in the analysed reference strains agreed by and large with the values indicated in the literature (e.g. Steyn *et al.*, 1998; Gallego *et al.*, 2006). A dendrogram of fatty acid relationships is shown in Fig. 2. Although most strains differed in the quantities of their fatty acids only, the separation of *P. heparinus* DSM 2366^T and *P. africanus* DSM 12126^T from *P. cryoconitis* DSM 14825^T, *P. caeni* DSM 16990^T and the WB strains is in accord with the 16S rRNA gene tree (Fig. 1), as is the position of strain WB 2.3-45^T adjacent to the type strain of *P. caeni*. Menaquinones were determined according to Minnikin *et al.* (1984). MK-7 was the major peak (97–100 %) in all strains tested.

To test whether the novel genospecies could also be defined in terms of distinguishing phenotypic properties, strains were subjected to cultural, biochemical and morphological analyses. Results are indicated in Table 1 and the species description. All strains were grown on R2A medium. Strains WB 2.1-25^T, WB 2.3-45^T, WB 2.3-71^T, WB 3.3-22^T, *P. africanus* DSM 12126^T, *P. caeni* DSM 16990^T and *P. heparinus* DSM 2366^T were grown at 25 °C, whereas strain WB 3.3-3^T and *P. cryoconitis* DSM 14825^T were grown at 20 °C. Growth was measured by reading the OD at 600 nm. The temperature optimum was tested over a

period of 3–6 days using a temperature gradient incubator model TN-3 (Toyo Kagaku Sangyo) at 1–36 °C in steps of 2 °C. The optimal pH for growth was tested in buffered M67 at 25 °C at pH 4.0–9.5 in steps of 0.2 pH units using three different buffer solutions (Na₂HPO₄/NaH₂PO₄, succinic acid/NaOH and 2-amino-2-methyl-1,3-propanediol/HCl). Analysis was done at 1 day intervals over a period of 6 days. Salt tolerance was tested on R2A medium supplemented with 1–10 % NaCl in steps of 2 %.

Morphology of cells grown on R2A media for 6 h, 30 h and 8 days was investigated by light microscopy at 2400 × magnification. Colony morphology was determined after 24 h, 5 days and 14 days at optimal growth temperature on media R2A, nutrient agar (NA; Difco) and TSA. Growth was also determined on MacConkey agar (Difco). Micro-aerophilic growth was tested on R2A plates in a candle jar for 3 days at 23 °C (Gerhardt *et al.*, 1981). Cells were tested for gliding movement by the hanging drop method as described by Bernardet *et al.* (2002); motility was tested with cells from 2-day-old liquid cultures on soft agar incubated for 3 days at 23 °C. The presence of flexirubin pigments (Bernardet *et al.*, 2002), Gram-stain using the aminopeptidase and KOH reactions, catalase activity (H₂O₂ test) and heparin hydrolysis (Zimmermann *et al.*, 1990) were also tested. Cytochrome *c* oxidase was determined by adding a few drops of tetramethyl-*p*-phenylenediamine solution to a 3-day-old slant of each strain. All WB isolates were aerophilic and microaerophilic under the conditions provided in a candle jar, non-gliding and motile.

Antimicrobial susceptibilities were determined with the disc diffusion method using a ST6090 Disc Dispenser (Oxoid). Isolates were classified into three categories (sensitive, resistant and intermediate) based on the quantitative interpretation criteria recommended by the NCCLS (2000). All strains studied were resistant to cefalotin (30 µg), cefotaxime (30 µg), oxacillin (5 µg), mezlocillin (30 µg), aztreonam (30 µg), gentamicin (10 µg), colistin (10 µg), vancomycin (30 µg), amikacin (30 µg), polymyxin B (300 IU), kanamycin (30 µg) and neomycin (30 µg); all were sensitive to doxycycline (30 µg), imipenem (10 µg) and tetracycline (30 µg). Results in which the WB strains differ from each other

Fig. 2. Dendrogram showing the fatty acid relationships among the WB isolates and phylogenetically neighbouring *Pedobacter* reference strains. The dendrogram was generated by treating the Euclidian distances of the fatty acids with the unweighted pair group method with arithmetic means algorithm. Numerical analyses were done using standard MIS software (Microbial ID).

Table 1. Differential biochemical properties of the *Pedobacter* WB isolates and phylogenetically related *Pedobacter* type strains

Strains: 1, WB 2.1-25^T; 2, WB 2.3-71^T; 3, WB 3.3-3^T; 4, WB 3.3-22^T; 5, *P. heparinus* DSM 2366^T; 6, *P. caeni* DSM 16990^T; 7, WB 2.3-45^T; 8, *P. cryoconitis* DSM 14825^T; 9, *P. africanus* DSM 12126^T. All strains are positive for the following features: aminopeptidase, KOH test, catalase, oxidase, *N*-acetyl- β -glucosaminidase, α -D-lactose, D-melibiose, trehalose, alkaline phosphatase, leucine arylamidase, β -glucosidase, esterase lipase (C8), naphthol-AS-BI-phosphohydrolase and β -galactosidase. All strains tolerate up to 2 % NaCl, hydrolyse Tween 80, aesculin and starch, and assimilate the following substrates: D-mannose, D-glucose, L-arabinose, methyl α -D-glucopyranoside, arbutin, aesculin, ferric citrate, amidon, D-cellobiose, maltose and D-turanose. All strains are negative for the following features: nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, lipase (C14), cystine arylamidase, α -mannosidase, α -fucosidase, flexirubin reaction with 20 % KOH, gelatinase and urease. The following substrates are not assimilated: L-proline, malate, citrate, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, DL- α -glycerol phosphate, Tween 40, adonitol, D-arabitol, i-erythritol, *myo*-inositol, L-rhamnose, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D-serine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glucose 6-phosphate, methyl β -D-xylopyranoside, L-dulcitol, D-melezitose, xylitol, D-tagatose, D-fucose, L-fucose, L-arabitol, 2-ketogluconate, 5-ketogluconate, gluconate, capric acid, adipic acid and phenylacetic acid. +, Positive; –, negative; w, weak reaction; v, variable.

Utilization of:	1	2	3	4	5	6	7	8	9
API 50 CH and API 20NE									
Amygdalin	+	–	+	–	– (+)*	+	–	–	– (+)*
D-Arabitol	–	–	+	–	–	–	–	–	–
D-Ribose	+	–	–	–	–	–	–	–	–
D-Xylose	+	–	+	+	+	– (+)*	+	+	+
D-Adonitol	+	–	–	–	– (+)*	–	–	–	–
Gentiobiose	+	+	+	+	– (+)*	+	+	+	– (+/6)*
Inulin	+	–	–	–	–	–	–	w	–
L-Rhamnose	+	–	+	+	+	–	–	–	+
Salicin	+	–	+	–	– (+)*	+	+	– (+)*	– (+/6)*
Methyl α -D-mannopyranoside	–	+	–	+	– (+)*	+	–	–	+
<i>N</i> -Acetyl-D-glucosamine	–	–	+	w	+	+	+	–	+
D-Fructose	+	–	+	– (+)†	+	–	–	+	– (2/6)*
D-Mannitol	–	–	– (+)†	–	– (+)*†	– (+)†	–	–	–
D-Galactose	+	–	+	+	– (+)*†	–	+	+	–
Sucrose	+	– (+)†	–	+	– (+)*	+	–	+	+
D-Sorbitol	–	–	–	–	+	–	–	–	–
D-Raffinose	– (+)†	–	–	– (+)†	– (+)*	+	– (+)†	– (+)*	–
Glycerol	–	–	–	+	–	– (+)*	+	– (+)*	–
Glycogen	–	–	–	–	–	–	+	+	–
Biolog									
D-Arabinose	+	–	–	–	–	–	–	–	–
α -Cyclodextrin	+	–	–	w	–	+	+	+	–
Dextrin	+	–	w	+	–	+	+	+	–
<i>N</i> -Acetyl-D-galactosamine	–	–	–	–	–	+	–	–	–
L-Arabinose	–	–	w	w	– (+)*	+	–	– (+)*	–
Lactulose	–	–	w	+	w	+	+	–	–
Methyl β -D-glucoside	–	–	+	+	– (+)*	+	+	– (w)*	–
Psicose	–	–	–	w	–	–	–	–	–
D-Glucuronic acid	–	–	–	–	–	w	–	–	–
Succinic acid	–	–	–	–	–	w	–	–	–
L-Glutamic acid	–	–	–	–	–	+	+	+	–
Glycyl L-aspartic acid	–	–	–	–	–	+	w	–	+
Glycyl L-glutamic acid	–	–	–	–	–	+	w	–	+
L-Serine	–	–	–	+	– (v)*	w	+	w	–

Table 1. cont.

Utilization of:	1	2	3	4	5	6	7	8	9
L-Threonine	—	—	—	w	— (v)*	+	+	—	—
Glucose 1-phosphate	—	—	—	—	—	—	—	—	+

*Reactions in parentheses are as given in the original description. For *P. africanus* (Steyn *et al.*, 1998), the type strain has not been recorded individually so the number of positive scoring strains is indicated in parentheses.

†Reactions in parentheses according to Biolog.

are presented in Supplementary Table S1 available in IJSEM Online.

Enzymic activities and carbon assimilation tests were determined using the commercial API ZYM, API 50 CH and API 20NE systems (bioMérieux) and the utilization or oxidization of carbon sources was determined using the GN MicroPlate system (Biolog) according to the instructions of the manufacturers. A modified AUX media (Somvanchi *et al.*, 2006) with 0.6 g MgSO₄·7H₂O instead of 6 g was used for strain WB 2.1-25^T. API ZYM tests were read after 6 h incubation, whereas API 50 CH, API 20NE and GN MicroPlate tests were read after 48 h. Incubation was at 25 °C except for *P. cryoconitis* DSM 14825^T and strain WB 3.3-3^T, which were inoculated at 20 °C. Results are included in the species descriptions and Table 1.

Many of the reactions indicated as positive for all strains of the genus *Pedobacter* also scored positive in our tests (Table 1). Some other substrates were used by the majority of strains, i.e. D-xylose, sucrose, gentiobiose and salicin. The occurrence of reactions scored as 'weak', however, somehow hinders a clear-cut interpretation of inter-strain similarities at the level of physiological reactions. In several tests recorded, *P. heparinus* DSM 2366^T showed an unexpectedly significant number of reactions that deviated from those indicated in the original description (Steyn *et al.*, 1998). In its negative reaction towards the API and Biolog substrates, strain DSM 2366^T was similar to strain WB 2.3-71^T. Of the reactions listed in Table 1, these two strains do not have a single positive score in common, although both of them share between two and four positive scores with other representatives. Except for *P. heparinus* DSM 2366^T and *P. africanus* DSM 12126^T, none of the strains investigated were heparinase-positive. The presence of phenotypic differences observed among the isolates confirms their distinct phylogenetic position determined by 16S rRNA gene sequence analysis and, where done, by DNA–DNA reassociation experiments. It is concluded that the five WB isolates each represents a novel species in the genus *Pedobacter*; descriptions are given below.

Description of *Pedobacter duraquae* sp. nov.

Pedobacter duraquae (dur.a'quae. L. adj. *durus*, -a, -um hard; L. fem. n. *aqua* water; N.L. gen. fem. n. *duraquae* from/of hard water).

Gram-negative rods, 1.9–2.5 × 0.63–0.83 µm, motile and non-spore-forming. Grows at 9–32 °C; optimum growth at 25–27 °C. Grows at pH 5.70–8.15. Weak growth in 4 % NaCl. Colonies on R2A, TSA and NA media are circular, butyrous, opaque, smooth, entire and non-pigmented. Colonies are ivory white and convex (0.1 mm in diameter) on R2A, sand yellow (0.4 mm in diameter) and raised on TSA, and translucent and flat (0.2 mm in diameter) on NA. Microaerophilic. Hydrolyses DNA. Positive for valine arylamidase, β-glucuronidase, α-glucosidase and β-glucosidase and weak for esterase (C4) and chymotrypsin (API ZYM). Positive for amygdalin, D-arabinose, D-ribose, D-xylose, D-adonitol, gentiobiose, inulin, L-rhamnose, salicin, D-fructose, D-galactose, sucrose and D-raffinose (API 50 CH and API 20NE). Positive for α-cyclodextrin and dextrin and negative for D-raffinose (Biolog). Additional reactions are listed in Table 1 and Supplementary Table S1.

The type strain, WB 2.1-25^T (=DSM 19034^T=CIP 109481^T), was isolated from a site about 180 m downstream of the spring of the Westerhöfer Bach, Westerhof, 40 km north of Göttingen, Germany (51° 45' 49" N 10° 05' 31.7" E).

Description of *Pedobacter hartonius* sp. nov.

Pedobacter hartonius (har.to'ni.us. M.L. masc. n. *Harto* the Harz, a mountain range in northern Germany; N.L. masc. adj. *hartonius* pertaining to the Harz).

Gram-negative rods, 1.5–2.5 × 0.63–0.83 µm, motile and non-spore-forming. Grows at 6–26 °C; optimum growth at 12–16 °C. Grows at pH 5.7–7.8 and in 4 % NaCl. Colonies on R2A, TSA and NA media are circular, butyrous, convex, opaque, smooth, entire and non-pigmented. Colonies are pearl white (0.1 mm in diameter) on R2A and sand yellow (0.4 mm in diameter) and ivory white (0.1 mm in diameter) on TSA and NA, respectively. Microaerophilic. Hydrolyses gelatin. Positive for α-glucosidase and weak for esterase (C4) and β-glucosidase (API ZYM). Positive for amygdalin, D-arabitol, D-xylose, gentiobiose, L-rhamnose, salicin, N-acetyl-D-glucosamine, D-fructose, D-mannitol and D-galactose (API 50 CH and API 20NE). Positive for methyl β-D-glucoside and α-D-lactose, weak reactions with dextrin, L-arabinose and lactulose, and negative for D-mannitol (Biolog). Additional reactions are listed in Table 1 and Supplementary Table S1.

The type strain, WB 3.3-3^T (=DSM 19033^T=CIP 109468^T), was isolated from a site about 250 m downstream of the spring of the Westerhöfer Bach, Westerhof, 40 km north of Göttingen, Germany (51° 45' 49" N 10° 05' 31.7" E).

Description of *Pedobacter metabolipauper* sp. nov.

Pedobacter metabolipauper [me.ta'bo.li.pau'per. N.L. n. *metabolismus* metabolism (word stem *metabol-*); L. masc. adj. *pauper* poor; N.L. masc. adj. *metabolipauper* metabolically poor].

Gram-negative rods, 1.7–2.7 × 0.83 µm, motile and non-spore-forming. Grows at 8–31 °C; optimum growth at 24–26 °C. Grows at pH 5.9–8.0. No growth in 4% NaCl. No growth on TSA. Colonies on R2A and NA media are 0.1 mm in diameter, ivory white, circular, butyrous, raised, opaque, smooth and entire. Colonies are non-pigmented. Microaerophilic. Hydrolyses DNA weakly. Positive for trypsin and α-glucosidase and weak for esterase (C4) and chymotrypsin (API ZYM). Positive for gentiobiose, methyl α-D-mannopyranoside and sucrose (API 50 CH and API 20NE). Negative for sucrose (Biolog). Additional reactions are listed in Table 1 and Supplementary Table S1.

The type strain, WB 2.3-71^T (=DSM 19035^T=CIP 109480^T), was isolated from a site about 180 m downstream of the spring of the Westerhöfer Bach, Westerhof, 40 km north of Göttingen, Germany (51° 45' 49" N 10° 05' 31.7" E).

Description of *Pedobacter westerhofensis* sp. nov.

Pedobacter westerhofensis (wes.ter.ho.fen'sis. N.L. masc. adj. *westerhofensis* pertaining to Westerhof, a village in the Harz Mountains).

Gram-negative rods, 1.7–2.5 × 0.83–1.0 µm, motile and non-spore-forming. Grows at 9–28 °C; optimum growth at 22–24 °C. Grows at pH 5.3–7.8. No growth in 4% NaCl. Colonies on R2A, TSA and NA media are 0.1–0.2 mm in diameter, pearl white, circular, butyrous, convex, opaque, smooth and entire. Colonies are non-pigmented. Microaerophilic. Hydrolyses gelatin. Hydrolyses DNA weakly. Positive for α-glucosidase and β-glucosidase (API ZYM). Positive for D-xylose, gentiobiose, L-rhamnose, methyl α-D-mannopyranoside, D-galactose, sucrose and D-raffinose and weak reactions with N-acetyl-D-glucosamine (API 50 CH and API 20NE). Positive for dextrin, α-D-lactose, lactulose, methyl β-D-glucoside and L-serine, weak reactions with α-cyclodextrin, L-arabinose, psicose and L-threonine, and negative for D-raffinose (Biolog). Additional reactions are listed in Table 1 and Supplementary Table S1.

The type strain, WB 3.3-22^T (=DSM 19036^T=CIP 109479^T), was isolated from a site about 250 m downstream of the spring of the Westerhöfer Bach, Westerhof,

40 km north of Göttingen, Germany (51° 45' 49" N 10° 05' 31.7" E).

Description of *Pedobacter steynii* sp. nov.

Pedobacter steynii (stey'ni.i. N.L. gen. masc. n. *steynii* of/ from Steyn, named after P. L. Steyn, the microbiologist who described the genus *Pedobacter*).

Gram-negative rods, 1.70–3.75 × 0.83 µm, motile and non-spore-forming. Grows at 10–30 °C; optimum growth at 25–27 °C. Grows at pH 5.70–8.45. Weak growth in 4% NaCl. Colonies on R2A, TSA and NA media are circular, butyrous, convex (raised on NA), opaque, smooth, entire, non-pigmented and slimy. Colonies are 0.4 mm in diameter and pearl white on R2A, and 0.2 mm in diameter and ivory white on TSA and NA media. Microaerophilic. Hydrolyses casein, DNA and gelatin. Positive for esterase (C4), valine arylamidase and α-glucosidase (API ZYM). Positive for protease gelatin, D-xylose, gentiobiose, salicin, D-galactose, N-acetyl-D-glucosamine, D-raffinose, glycerol and glycogen (API 50 CH and API 20NE). Positive for α-cyclodextrin, dextrin, α-D-lactose, methyl β-D-glucoside, L-glutamic acid, L-serine and L-threonine, weak reactions with D-glucuronic acid and succinic acid, and negative for D-raffinose (Biolog). Additional reactions are listed in Table 1 and Supplementary Table S1.

The type strain, WB 2.3-45^T (=DSM 19110^T=CIP 109507^T), was isolated from a site about 180 m downstream of the spring of the Westerhöfer Bach, Westerhof, 40 km north of Göttingen, Germany (51° 45' 49" N 10° 05' 31.7" E).

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