Pseudopedobacter beijingensis gen. nov., sp. nov., isolated from coking wastewater activated sludge, and reclassification of Pedobacter saltans as Pseudopedobacter saltans comb. nov.

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A taxonomic study was carried out on strain GCS-AE-31T, which was isolated from a phenol-degrading consortium, enriched from coking wastewater of the Beijing Shougang Company Limited during the screening of phenol-degrading bacteria. Cells of strain GCS-AE-31T were Gram-stain-negative, short rods, motile by gliding, oxidase- and catalase-positive. Growth was observed at salinities of 0–3 % and at temperatures of 10–37 °C. On the basis of 16S rRNA gene sequence similarity, strain GCS-AE-31T was most closely related to Pedobacter saltans LMG 10337T (96.17 %), but it showed low similarity to all other species of the genus Pedobacter (89.28–92.45 %). It also showed low 16S rRNA gene similarity to all other species of the family Sphingobacteriaceae (87.25–92.45 %) examined. The dominant fatty acids were iso-C15 : 0, summed feature 3 (C16 : 1v7c/C16 : 1v6c), anteiso-C15 : 0 and iso-C17 : 0 3-OH. The menaquinones were MK-7 (95.5 %) and MK-6 (4.5 %). The polar lipids were phosphatidylethanolamine, three aminolipids and three unknown phospholipids. Sphingolipid was present. The G+C content of the chromosomal DNA was 36.2 mol%. According to its phylogenetic position and phenotypic traits, the novel strain could not be assigned to the genus Pedobacter; it should be classified as representing a novel species of a novel genus in the family Sphingobacteriaceae, for which the name Pseudopedobacter beijingensis gen. nov., sp. nov. is proposed (type strain GCS-AE-31T = MCCC 1A01299T = CGMCC 1.12329T = LMG 27180T). The misclassified species Pedobacter saltans is transferred to the novel genus as Pseudopedobacter saltans comb. nov. (type strain LMG 10337T = MCCC 1A06472T = DSM 12145T = CCUG 39354T = CIP 105500T = JCM 21818T = NBRC 100064T).

The genus Pedobacter, belonging to the family Sphingobacteriaceae, was proposed by Steyn et al. (1998) and it comprises 41 species with validly published names (http://www.bacterio.net/p/pedobacter.html) at the time of writing. In this study, we isolated a novel strain GCS-AE-31T from a phenol-degrading consortium, enriched from coking wastewater of the Beijing Shougang Company Limited (Cao et al., 2011). It was phylogenetically related to a member of the genus Pedobacter but formed a separate clade in the family Sphingobacteriaceae. Characterization and classification of strain GCS-AE-31T were done using polyphasic methods. The routine cultivation of the strain and most phenotypic tests were carried out on Luria–Bertani (LB) medium (Sambrook et al., 1989) unless noted otherwise.

Genomic DNA was prepared according to the method of Ausubel et al. (1995) and the 16S rRNA gene was amplified by PCR using primers that have been described previously (Liu & Shao, 2005). Sequences from related taxa were obtained from the GenBank database. The phylogenetic analysis was performed using MEGA version 5 (Tamura et al., 2011). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method of Saitou & Nei (1987), the maximum-likelihood method (Felsenstein, 1981) and the minimum-evolution method of Rzhetsky & Nei (1992, 1993) were determined by using bootstrap values based on 1000 replications.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of Pseudopedobacter beijingensis GCS-AE-31T is KC755039.

Four supplementary figures and a supplementary table are available with the online version of this paper.

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A nearly full-length 16S rRNA gene sequence (1493 nt) of strain GCS-AE-31<sup>T</sup> was obtained. Sequence similarity was determined using the EzTaxon-e server (Kim et al., 2012). Phylogenetic analysis of strain GCS-AE-31<sup>T</sup> indicated that it represented a member of the family Sphingobacteriaceae, forming a separate clade with Pedobacter saltans LMG 10337<sup>T</sup> within the family Sphingobacteriaceae (shown in Fig. 1). All 41 species of the genus Pedobacter and representative species from all the eight genera of the family Sphingobacteriaceae were included in the phylogenetic tree. Strain GCS-AE-31<sup>T</sup> was most closely related to Pedobacter saltans LMG 10337<sup>T</sup> (96.17%), but it showed low 16S rRNA gene similarity to all other species of the genus Pedobacter (89.28–92.45%). It also showed low 16S rRNA gene similarity to all other genera of the family Sphingobacteriaceae (87.25–92.45%). In addition, all type strains of species of the genus Pedobacter formed six clades separated by other genera within the family Sphingobacteriaceae (Shown in Fig. 1 and Figs S1 and S2 available in the online Supplementary Material). Clade 1 contained the type species and main strains. The strains of the other five clades showed a low 16S similarity (<94%) with the type species of the genus Pedobacter; they should represent five novel genera and need further classification. Strain GCS-AE-31<sup>T</sup> showed low similarity to the type species of the genus Pedobacter (Pedobacter heparinus LMG 4024<sup>T</sup>) (91.42%), which indicated that it could not be assigned to the genus Pedobacter. Strain GCS-AE-31<sup>T</sup> and Pedobacter saltans LMG 10337<sup>T</sup> should represent a novel genus in the family Sphingobacteriaceae. Strain GCS-AE-31<sup>T</sup> also showed a low 16S similarity (<85%) with its neighbouring clade (Pedobacter bauzanensis BZ42<sup>T</sup>, 91.82%).

Gram-staining, catalase, oxidase and lipase (Tween 80) activities, hydrolysis of aesculin and starch, the optimal growth temperature and pH, tolerance to NaCl, general cell morphology and electron microscopy were studied as previously described (Lai et al., 2009). Phenol degradation was tested in mineral salt medium (MSM) according to the method of Essam et al. (2010). Other biochemical tests were carried out using API 20NE and API ZYM strips (bioMérieux) and Biolog GN2 according to the manufacturers’ instructions. Heparinase activity was detected as previously described (Zimmermann et al., 1990). Gliding motility was tested as described by Bernardet et al. (2002). Pedobacter saltans LMG 10337<sup>T</sup> and Pedobacter heparinus LMG 4024<sup>T</sup> were obtained from LMG and tested at the same time in this study. The results for the three strains are shown in Table S1. The predominant fatty acids of strain GCS-AE-31<sup>T</sup> were iso-C<sub>15</sub>:0 (34.5%), summed feature 3 (C<sub>16</sub>:1ω7c/C<sub>16</sub>:1ω6c, 18.6%), anteiso-C<sub>15</sub>:0 (7.1%) and iso-C<sub>17</sub>:0 3-OH (6.4%), which accounted for 66.6% of the total fatty acids. A distinguishing feature was that strain GCS-AE-31<sup>T</sup> contained considerable amounts of anteiso-C<sub>15</sub>:0 (7.1%), of which there is only 1.2% in Pedobacter saltans LMG 10337<sup>T</sup> and 3.3% in Pedobacter heparinus LMG 4024<sup>T</sup>.

Antibiotic susceptibility tests were performed by the disc diffusion method according to the protocol of Lai et al. (2009). Strain GCS-AE-31<sup>T</sup> and two type strains of species of the genus Pedobacter were tested at the same time in this study. They were all sensitive to chloromycetin (30 μg per disc; Oxoide), carbencillin (100), cephradin (30), cefobid (30), clindamycin (2), vibramycin (30), erythromycin (15), pipercillin (100), rifampicin (5), streptomycin (10), cotrimoxazole (25) and tetracycline (30); and resistant to cefalexin (30), gentamicin (10), kanamycin (30), metronidazole (5), lincomycin (2), oxacillin (1) and streptomycin (10). The differences in susceptibility to nine other kinds of antibiotics of the three strains are shown in Table 1.

Analysis of the respiratory quinones was carried out by the Identification Service of the DSMZ (Braunschweig, Germany). Like other members of the genus Pedobacter, strain GCS-AE-31<sup>T</sup> contained MK-7 (95.5%) as the predominant menaquinone, and it contained minor amounts of MK-6 (4.5%). Polar lipids were extracted using a chloroform/methanol system and analysed using one- and two-dimensional TLC, as described previously (Kates, 1986). Merck silica gel 60 F<sub>254</sub> aluminium-backed thin-layer plates were used in TLC analysis. The plate dotted with sample was subjected to two-dimensional development, with the first solvent of chloroform/methanol/water (65:25:4, by vol.) followed by the second solvent of chloroform/methanol/acetic acid/water (85:12:15:4, by vol.). The profile for strain GCS-AE-31<sup>T</sup> comprised phosphatidylethanolamine, three aminolipids and three unidentified phospholipids, as shown in Fig. S3. Sphingolipid was detected in strain GCS-AE-31<sup>T</sup>, which is a typical feature of the family Sphingobacteriaceae.

The G + C content of the chromosomal DNA was determined according to the methods described by Mesbah & Whitman (1989) using reverse-phase HPLC. The DNA G+C content of the novel isolate GCS-AE-31<sup>T</sup> was 36.2 mol%, which was close to that of Pedobacter saltans LMG 10337<sup>T</sup>, but lower than that of the type species Pedobacter heparinus DSM 2366<sup>T</sup> (shown in Table 1).

Strain GCS-AE-31<sup>T</sup> was Gram-stain-negative, rod-shaped (Fig. S4) and motile by gliding. GCS-AE-31<sup>T</sup> cannot use phenol. The high 16S rRNA similarity between strain GCS-AE-31<sup>T</sup> and Pedobacter saltans LMG 10337<sup>T</sup> (96.17%) and low similarity to all other species of the genus Pedobacter (89.28–92.45%) strongly indicates that these two strains represent a novel genus. The differences in physiological, biochemical and chemotaxonomic characteristics between
strain GCS-AE-31<sup>T</sup> and Pedobacter saltans LMG 10337<sup>T</sup> and the type species of the genus Pedobacter are given in Table 1. On the basis of phylogenetic position and phenotypic traits, strain GCS-AE-31<sup>T</sup> represents a novel species of a novel genus in the family Sphingobacteriaceae, for which the name Pseudopedobacter beijingsensis gen. nov., sp. nov.
sp. nov. is proposed. The misclassified species Pedobacter saltans is transferred to the novel genus as Pseudopedobacter saltans comb. nov.

**Description of Pseudopedobacter gen. nov.**

Pseudopedobacter (Pseu.do.pe.do.bac.ter. Gr. adj. pseudēs false; N.L. masc. n. Pedobacter a bacterial genus; N.L. masc. n. Pseudopedobacter like Pedobacter, referring to the close relationship to the genus).

Cells are Gram-stain-negative, rod-shaped, with no flagellum and motile by gliding, positive for indole production, D-glucose fermentation, gelatin hydrolysis, denitrification, arginine dihydrolase and urease; and positive for β-glucosidase (aesculin hydrolysis), β-galactosidase and assimilation of D-glucose, D-mannose and N-acetylglucosamine; negative for indole production, D-glucose fermentation, gelatin hydrolysis, denitrification, arginine dihydrolase and urease. Principal fatty acids are iso-C₁₅ : 0, summed feature 3 (C₁₆ : 1ω7c/C₁₆ : 1ω6c), anteiso-C₁₅ : 0 and iso-C₁₇ : 0 3-OH. The major menaquinone is MK-7. Sphingolipid is present. G+C content of the DNA is 36–38 mol%.

The type species is Pseudopedobacter beijingensis.

**Description of Pseudopedobacter beijingensis sp. nov.**

Pseudopedobacter beijingensis (bei.jing.en’sis. N.L. masc. adj. beijingensis of Beijing, the capital of PR China, where the type strain was first isolated).

Cells are Gram-stain-negative, rod-shaped, about 1.4 μm long and 0.3 μm wide, with no flagellum and motile by gliding, positive for indole production, D-glucose fermentation, gelatin hydrolysis, denitrification, arginine dihydrolase and urease; and positive for β-glucosidase (aesculin hydrolysis), β-galactosidase and assimilation of D-glucose, D-mannose and N-acetylglucosamine; negative for indole production, nitrate reduction, D-glucose fermentation, arginine dihydrolase, urease, lipase (Tween 80), gelatinase and denitrification. On LB agar medium, produces smooth milk-white colonies with regular edges that are 1–2 mm in diameter after 2 days of incubation at 28 °C, non-pigmented and slightly raised in the centre. Grows in 0–3 % NaCl (optimum 1 %) at 10–37 °C (optimum 28 °C), but not at 41 °C within a week. Sensitive to chloramphenicol, carbenicillin, cephradin, cefobid, clindamycin, vibramycin, erythromycin, pipercillin, rifampicin, minomycin, co-trimoxazole, ampicillin, ciprofloxacin, norfloxacin.
Pseudopedobacter beijingensis gen. nov., sp. nov.

The type strain, MCCC 1A06472T (=CIP 105500T =JCM 21818T =DSM 12143T) was isolated from coking wastewater and their degradation gene [32].

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References


