Palleronia soli sp. nov., isolated from a soil sample on reclaimed tidal land, and emended description of the genus *Palleronia*

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A Gram-stain-negative, strictly aerobic, non-motile, non-spore-forming, short rod-shaped and moderately halophilic bacterial strain, CAU 1105^T, was isolated from soil on reclaimed tidal land in Modo, Republic of Korea, and its taxonomic position was investigated using a polyphasic approach. Strain CAU 1105^T grows optimally at a temperature of 37 °C at pH 7 in the presence of 3 % (w/v) sea salt. Based on 16S rRNA gene sequence similarity analyses, the novel isolate was assigned to the genus *Palleronia* within the class *Alphaproteobacteria* and showed the highest 16S rRNA gene sequence similarity with *Palleronia marisminoris* B33^T (95.4 %). Strain CAU 1105^T contained ubiquinone-10 as the only respiratory quinone and $C_{18:1}\omega7c$ as the major cellular fatty acid. The DNA G+C content of strain CAU 1105^T was 64.3 mol%. On the basis of phenotypic differentiation, phylogenetic and chemotaxonomic data, strain CAU 1105^T represents a novel species of the genus *Palleronia*, for which the name *Palleronia soli* sp. nov. is proposed. The type strain is CAU 1105^T (=KCTC 42298^T=NBRC 110740^T). An emended description of the genus *Palleronia* is also provided.

Since Gram-negative bacteria belonging to the *Roseobacter* clade within the family *Rhodobacteraceae* (class *Alphaproteobacteria*) were first described by Shiba (1991), 17 different genera have been described. The *Roseobacter* clade is marine or hypersaline, with characterized isolates demonstrating either a salt tolerance or requirement (Buchan *et al.*, 2005; Zan *et al.*, 2014). Members of the *Roseobacter* clade are known to be numerically abundant and ecologically important in marine environments, and they have been isolated from many marine environments such as seawater, sediment, tidal flats, marine algae, hypersaline microbial mats and coastal biofilms (Choi *et al.*, 2007; Lee *et al.*, 2007; Kim *et al.*, 2008; Yoon *et al.*, 2009; Vandecandelaere *et al.*, 2009; Wang *et al.*, 2009; Zheng *et al.*, 2010). The genus *Palleronia* was created by Martínez-Checa *et al.* (2005) as a member of the *Roseobacter* clade. The genus *Palleronia* consists of Gram-negative, rod-shaped, moderately halophilic bacteria. Ubiquinone Q-10 is the dominating respiratory quinone. At the time of writing, this genus consists of only one recognized species, *Palleronia marisminoris*, which was isolated from a hypersaline soil bordering a saline saltern on the Mediterranean seaboard near Murcia, South-eastern Spain (Martínez-Checa *et al.*, 2005). During the past 10 years, no new species of this genus with validly published names have been described (Parte, 2014). The Yellow Sea coast of the Republic of Korea comprises huge tidal flats, which are rich in valuable biological resources. A novel Gram-stain-negative, aerobic, moderately halophilic bacterium, designated CAU 1105^T, was isolated from a soil sample on reclaimed tidal land, collected in Modo (37° 32′ 12.28″ N 126° 24′ 51.47″ E), Republic of Korea. The objective of this study was to evaluate the taxonomic position of this bacterial strain using a polyphasic approach.

A marine environmental sample was serially diluted and plated on marine agar 2216 (MA; Difco) at 30 °C for 7 days under aerobic conditions as described by Gordon & Mihm (1962). Colonies were randomly selected and strain CAU 1105^T was purified from a single colony on MA. The strain was grown aerobically on MA at 37 °C for 3 days. The strain was preserved at −80 °C in marine broth 2216 (MB; Difco) containing 25 % glycerol. Strain CAU 1105^T has been deposited in the Korean Collection for Type Cultures (KCTC; Taejon, Korea) and the Biological Resource Center, NITE (NBRC; Chiba, Japan) as KCTC 42298^T and NBRC 110740^T, respectively.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAU 1105^T is KP064190.

A supplementary figure is available with the online Supplementary Material.

Genomic DNA was extracted from strain CAU 1105^T using the method described by Marmur (1961). PCR amplification of 16S rRNA genes from the isolate was performed following the procedures described in Lane (1991). Sequencing of the amplified 16S rRNA gene was performed using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 3730; Applied Biosystems). The 16S rRNA gene sequence of strain CAU 1105^T was determined and compared with available reference sequences in the GenBank database (Benson *et al.*, 2013) accessed December 2014. Sequence similarity levels between strain CAU 1105^T and other related strains were calculated using the EzTaxon-e server (Kim *et al.*, 2012; http://www.ezbiocloud.net/eztaxon) and multiple alignments with reference sequences were carried out using CLUSTAL X 2.1 (Larkin *et al.*, 2007). The type strains of closely related taxa, including *Palleronia marisminoris* B33^T, *Hwanghaeicola aestuarii* Y26^T, *Maribius pelagius* B5-6^T, *Maribius salinus* CL-SP27^T and *Maritimibacter alkaliphilus* HTCC2654^T, were used as reference strains for the phylogenetic analysis. Evolutionary distance matrices were generated through the neighbour-joining method (Jukes & Cantor, 1969). Phylogenetic trees were reconstructed by using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and least-squares (Fitch & Margoliash, 1967) algorithms in the PHYLIP package (Felsenstein, 1989). The tree topology reconstructed by the neighbour-joining algorithm was evaluated using a bootstrap analysis based on 1000 replicates (Felsenstein, 1985) using the SEQBOOT and CONSENSE programs from the PHYLIP package. The G+C content of the DNA was performed using HPLC according to Tamaoka & Komagata (1984) and analysed by reversed-phase HPLC.

The temperature range for growth of strain CAU 1105^T was determined by growing the isolate at different temperatures (4, 10, 20, 30, 37, 40 and 45 °C) on MA. The pH range for growth was examined at 37 °C in MB adjusted to pH 4.5–11.5 at 0.5 pH unit intervals. The pH values of <6, 6–9 and >9 were obtained by using sodium acetate/acetic acid, Tris/HCl and sodium carbonate buffers, respectively. Salt requirement was determined in MY medium (Moraine & Rogovin, 1966), and the salt concentrations examined ranged from 0–15 % (w/v) and consisted of a mixture of sea salts as described by Rodríguez-Valera *et al.* (1981). The Gram reaction was performed using a Gram staining kit (bioMérieux). Catalase and oxidase tests were carried out according to Cappuccino & Sherman (2002). Hydrolysis of gelatin, casein, starch, aesculin and citrate were examined as described by Lányí (1987) and Smibert & Krieg (1994). Cell morphology was observed using light microscopy (DM 1000; Leica) and transmission electron microscopy (JEM 1010; JEOL). The presence or absence of flagella and their arrangement were determined using transmission electron microscopy of an exponentially growing culture after negatively staining of cells with 1 % (w/v) phosphotungstic acid. Biochemical characterizations were examined by using the API 20E and API 20NE systems (bioMérieux). Additionally, enzyme activities were determined using the API ZYM kit (bioMérieux) according to the manufacturer's instructions. API 20E and API ZYM strips were read after 24 h and API 20NE strips after 48 h. Antibiotic susceptibility was determined on MA at 37 °C by the disc diffusion method using Sensi-Disc susceptibility test discs (BBL). The following antibiotics were examined (μg per disc unless otherwise stated): amoxicillin (20), ampicillin (10), carbenicillin (100), cefoxitin (30), cephalothin (30), chloramphenicol (30), erythromycin (15), kanamycin (30), penicillin (10 U), polymyxin B (300 U), rifampicin (5), streptomycin (10), tobramycin (10), nalidixic acid (30) and trimethoprim/sulfamethoxazole (1.25/23.75). An inhibition zone >10 mm diameter indicated susceptibility and the absence of an inhibition zone represented resistance.

Isoprenoid quinones were extracted according to Komagata & Suzuki (1987) and analysed by HPLC. The isoprenoid quinones were eluted by an isocratic solvent system [methanol/isopropyl ether (3 : 1, v/v)] using a flow rate of 1 ml min^{-1}. The cell mass of strain CAU 1105^T for fatty acid analysis was harvested from tryptic soy agar (TSA; Difco) in late-exponential growth phase after cultivation for 3 days at 37 °C. Fatty acids were methylated, saponified, and extracted according to the standard MIDI protocol (Sherlock Microbial Identification System version 6.1). Fatty acid methyl esters were obtained by the method of Minnikin *et al.* (1980), and separated using an automated gas chromatography system (model 6890N and 7683 autosampler; Agilent). The peaks were confirmed by using the Microbial Identification System software package (MOORE library version 5.0; MIDI database TSBA6).

The 16S rRNA gene sequence of strain CAU 1105^T (1447 bp) was determined and compared with the available reference sequences in the GenBank database (accessed December 2014). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain CAU 1105^T formed a distinct lineage within the genus *Palleronia* and the most closely related strain was *P. marisminoris* B33^T (GenBank accession no. AY926462, similarity 95.4 %) (Fig. 1). Other closely related phylogenetic neighbours based on 16S rRNA gene sequences were *H. aestuarii* Y26^T (FJ230842, similarity 94.4 %), *Maribius pelagius* B5-6^T (DQ514326, similarity 94.3 %), *Maribius salinus* CL-SP27^T (AY906863, similarity 94.2 %) and *Maritimibacter alkaliphilus* HTCC2654^T (DQ915443, similarity 92.9 %). The DNA G+C content of strain CAU 1105^T was 64.3 mol %.

Strain CAU 1105^T grew at temperatures between 20 and 40 °C (optimum 37 °C) and sea salt concentrations from 1–11 % sea salt (optimum 3 %). Visible growth occurred at pH 4.5–11 and strain CAU 1105^T showed the highest visible growth at pH 7. Recently, in the course of screening micro-organisms from an alkaline soil in Korea, many bacterial strains were isolated and characterized as capable of growth at these high pH values (Yoon *et al.*, 2005). Cells of strain CAU 1105^T were Gram-stain-negative, aerobic,

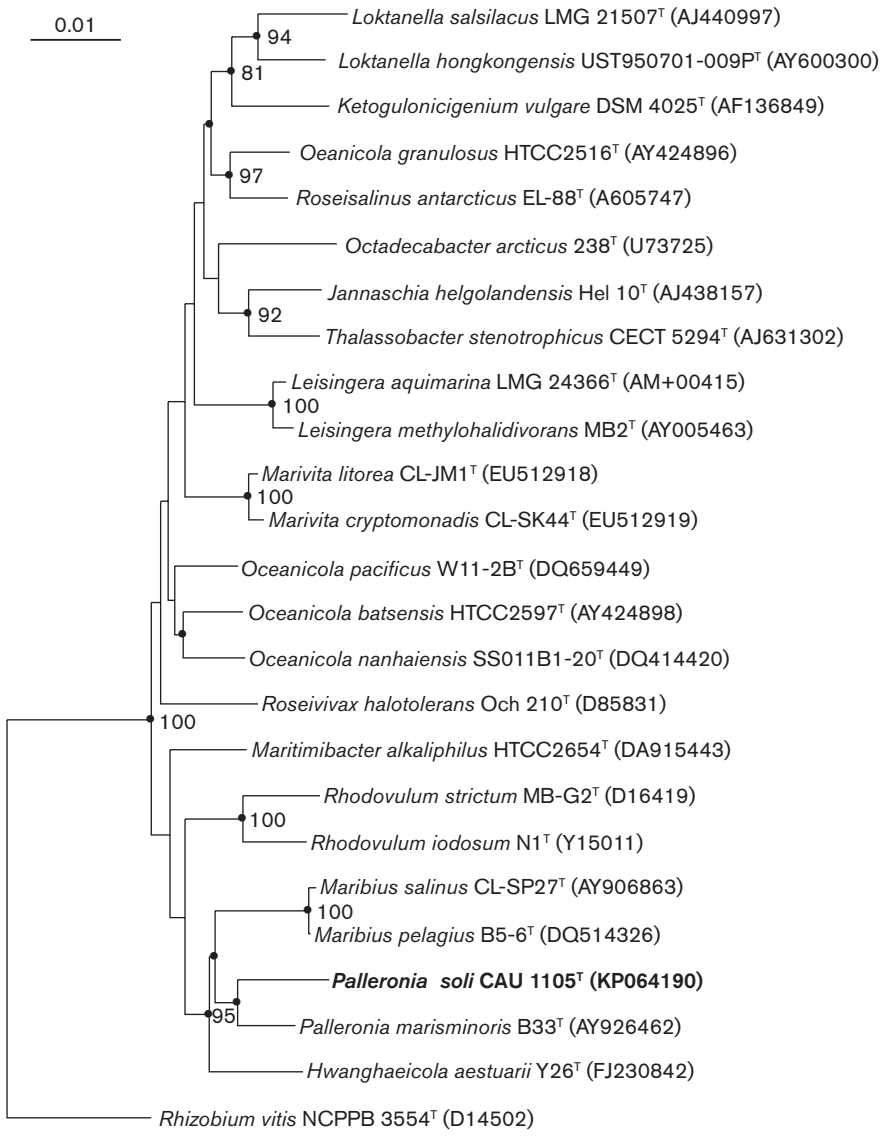

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain CAU 1105^T and related taxa. Filled circles indicate that corresponding nodes were recovered in trees generated with the maximum-likelihood, maximum-parsimony and least-squares algorithms. Numbers at the nodes are bootstrap values based on a neighbour-joining analysis (1000 replicates); only values >70 % are shown. *Rhizobium vitis* NCPPB 3554^T (GenBank accession no. D14502) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

non-motile, short rod-shaped (0.5–0.8 μm in diameter and 1.2–2.6 μm in length), and did not have flagella (Fig. S1, available in the online Supplementary Material). The catalase test was positive, but the oxidase test was negative. Detailed phenotypic features of strain CAU 1105^T are presented in the species description and compared with those of closely related taxa in Table 1.

The only respiratory quinone detected in strain CAU 1105^T was Q-10. This character is in agreement with *P. marisminoris*, the type species of the genus *Palleronia* (Martínez-Checa *et al.*, 2005). The cellular fatty acids of strain CAU 1105^T (present at >1 %) were $C_{18:1}\omega7c$, $C_{18:0}$, $C_{17:0}$, 11-methyl $C_{18:1}\omega7c$, $C_{16:0}$, $C_{10:0}$ 3-OH, $C_{17:1}\omega8c$ and $C_{12:0}$ (Table 2). This fatty acid profile was very similar to that of the closely related *P. marisminoris* LMG 22959^T in that the unsaturated fatty acid $C_{18:1}\omega7c$ was predominant, although there were differences in the percentages of some fatty acids. However, strain CAU 1105^T could be distinguished from *P. marisminoris* LMG 22959^T by the absence of the unsaturated fatty acid $C_{19:0}$ cyclo $\omega8c$ in strain CAU 1105^T.

Table 1. Phenotypic properties that differentiate strain CAU 1105^T from the type species of the genus *Palleronia*

Strains: 1, CAU 1105^T (data from this study); 2, *Palleronia marisminoris* B33^T (Martínez-Checa *et al.*, 2005). Both strains are negative for the following: flagella; oxidase; indole production; Voges–Proskauer; hydrolysis of gelatin, aesculin and urea; sole carbon source of D-glucose, D-mannose, glycerol, inositol, mannitol, L-alanine and L-lysine. Both strains are sensitive to amoxicillin, ampicillin, carbenicillin, cefoxitin, cephalothin, chloramphenicol, erythromycin, kanamycin, rifampicin, streptomycin and tobramycin. +, Positive; −, negative; W, weakly positive; R, resistant; S, sensitive; ND, no data available.

Characteristic	1	2
Source	Reclaimed land, Yellow sea	Hypersaline soil
Pigment colour	Beige	Pink
Temperature range (°C)	20–40	20–37
pH range	4.5–11	5–10
Salt concentration for growth (%, w/v)		
Range	1–11	0.5–15
Optimum	3	5
Hydrolysis of casein	+	−
Citrate utilized as sole carbon source	+	−
β-Galactosidase (API 20E)	−	+
Antibiotic susceptibility		
Penicillin	S	ND
Polymyxin B	S	R
DNA G+C content (mol%)	64.3	64.2

Therefore, these data provide appropriate evidence to support the identification of strain CAU 1105^T by phylogenetic, phenotypic, biochemical and chemotaxonomic analyses as representative of a novel species of the genus *Palleronia*, for which the name *Palleronia soli* sp. nov. is proposed. An emended description of the genus *Palleronia* is also provided.

Table 2. Cellular fatty acid contents (%) of strain CAU 1105^T and the type species of the genus *Palleronia*

Strains: 1, CAU 1105^T (data from this study); 2, *Palleronia marisminoris* B33^T (Martínez-Checa *et al.*, 2005). Fatty acids that represented <1 % in both strains are not shown. −, Not detected.

Fatty acid	1	2
Saturated		
$C_{12:0}$	1.1	−
$C_{16:0}$	5.1	4.3
$C_{17:0}$	7.5	−
$C_{18:0}$	9.7	3.4
Unsaturated		
$C_{17:1}\omega8c$	1.5	−
$C_{18:1}\omega7c$	62.9	68.9
11-methyl $C_{18:1}\omega7c$	5.3	−
$C_{19:0}$ cyclo $\omega8c$	−	12.8
$C_{20:1}\omega7c$	−	−
Hydroxy		
$C_{10:0}$ 3-OH	2.2	5.0
Unknown	−	2.3

Emended description of the genus *Palleronia* Martinez-Checa *et al.* 2005

The description is as given by Martínez-Checa *et al.* (2005) with the following amendments. Colonies contain pink or beige pigment. Cells contain or do not contain poly-β-hydroxyalkanoate (PHA) granules. Moderately or strictly halophilic. The principal cellular fatty acids are $C_{18:1}\omega7c$ or $C_{18:1}\omega7c$ and $C_{19:0}$ cyclo $\omega8c$.

Description of *Palleronia soli* sp. nov

Palleronia soli (so′li. L. n. *solum* soil; L. gen. n. *soli* of the soil).

Moderately halophilic, non-spore-forming, Gram-stain-negative, aerobic, non-motile and short rod-shaped, 0.5–0.8 μm in diameter and 1.2–2.6 μm in length. Colonies on MA are beige, circular and convex with entire margins after 3 days incubation at 37 °C. Optimal growth occurs at 37 °C (range 20–40 °C), pH 7 (range pH 4.5–11) and with 3% (w/v) sea salt (range 1–11%). Catalase-positive, but oxidase-negative. ONPG test is positive, but indole, Voges–Proskauer and the tryptophan test are negative. Casein is hydrolysed but urea, gelatin, starch and aesculin are not. Nitrate is not reduced. Citrate is utilized. H_2S is not produced. Arginine, lysine and ornithine decarboxylase tests are negative. Esterase (C4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase activities are positive, but, alkaline phosphate, lipase (C14), leucine arylamidase, cystine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphate, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-

glucosaminidase, α-mannosidase and α-fucosidase activities are not. Acid production from D-mannitol, D-glucose, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose and L-arabinose is negative. The following carbohydrates are not assimilated: D-mannose, *N*-acetylglucosamine, maltose, potassium gluconate (API 20NE), glycerol, D-ribose, D-galactose, methyl α-D-glucopyranoside, amygdalin, arbutin, lactose, melibiose, trehalose, inulin, raffinose, gentiobiose, erythritol, D-arabinose, L-arabinose, D-xylose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-fructose, L-sorbose, dulcitol, methyl α-D-mannopyranoside, salicin, cellobiose, sucrose, melezitose, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol and potassium 2-ketogluconate (API 50CH). Potassium 5-ketogluconate is utilized. Susceptible to amoxicillin (20 μg), ampicillin (10 μg), carbenicillin (100 μg), cefoxitin (30 μg), cephalothin (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), kanamycin (30 μg), penicillin (10 U), polymyxin B (300 U), rifampicin (5 μg), streptomycin (10 μg) and tobramycin (10 μg), but resistant to nalidixic acid (30 μg) and trimethoprim/sulfamethoxazole (1.25/23.75 μg). The only respiratory quinone is ubiquinone Q-10. The major cellular fatty acid is $C_{18:1}\omega7c$.

The type strain is CAU 1105^T (=KCTC 42298^T=NBRC 110740^T), isolated from reclaimed land on Modo in the Republic of Korea. The DNA G+C content of the type strain is 64.3 mol%.

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