Pseudomonas pohangensis sp. nov., isolated from seashore sand in Korea

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A novel bacterium, designated strain H3-R18^T, was isolated from seashore sand collected from Homi cape, Pohang city, Korea. Cells were Gram-negative, aerobic, non-motile, cream-coloured, mesophilic and slightly halotolerant. 16S rRNA gene sequence analysis indicated that the organism was a member of the genus *Pseudomonas*, but the sequence showed $\leq 96.3\%$ sequence similarity to that of the type strains of all recognized *Pseudomonas* species. Highest sequence similarities were to *Pseudomonas brenneri* CFML 97-391^T (96.3%) and *Pseudomonas migulae* CIP 105470^T (96.3%). The major fatty acids were summed feature 3 and C_{16:0}, with lesser amounts of C_{12:0}, C_{12:0} 3-OH, C_{18:1} ω7c and C_{14:0}. The major isoprenoid quinone was Q-9. The DNA G + C content was 64.0 mol%. The phylogenetic, phenotypic and genetic properties of strain H3-R18^T suggest that it represents a novel species, for which the name *Pseudomonas pohangensis* sp. nov. is proposed. The type strain is H3-R18^T (= KACC 11517^T = DSM 17875^T).

Members of the genus *Pseudomonas* are widely distributed in a variety of environments such as soil, plants, water, air and clinical specimens. According to the original definition given by Migula (1894), the genus *Pseudomonas* comprises 'the polarly flagellated strictly aerobic rods with a respiratory type of metabolism in which oxygen is used'. Following several revisions to this description, the genus *Pseudomonas* includes about 100 species with validly published names (<http://www.bacterio.cict.fr/>). *Pseudomonas* species isolated from seawater or marine sediments are *Pseudomonas alcaligenes*, *Pseudomonas pseudoalcaligenes* (Palleroni, 1984), *Pseudomonas stutzeri* (junior synonym *Pseudomonas perfectomarina*) (Baumann *et al.*, 1983; Rosselló-Mora *et al.*, 1993) and *Pseudomonas alcaliphila* (Yumoto *et al.*, 2001), and some strains of *Pseudomonas balearica* (Bennasar *et al.*, 1996).

During characterization of the bacterial community of seashore sand collected from Homi cape, Pohang city, Korea, strain H3-R18^T was isolated on marine agar 2216 (MA; Difco). After 3 days incubation at 28 °C on MA, colonies

were milky, convex and circular (< 1 mm in diameter) with a clear margin. The isolate grew poorly on nutrient agar (NA; Difco) and trypticase soy agar (TSA; Difco), and did not grow on MacConkey agar (Difco).

16S rRNA gene sequences of the type strains (about 100) of recognized *Pseudomonas* species were obtained from the GenBank library, and a preliminary phylogenetic tree was constructed. Strain H3-R18^T was clustered with *Pseudomonas pachastrellae* CCUG 46540^T but with low bootstrap value (data not shown). The 16S rRNA gene sequence of strain H3-R18^T (1443 nt) was aligned together with those of representative members from the preliminary tree by using the CLUSTAL W program (Thompson *et al.*, 1994). A phylogenetic tree was produced using the software package MEGA version 3.1 (Kumar *et al.*, 2004). Distances (using distance options according to the Kimura two-parameter model) and clustering using the neighbour-joining and maximum-parsimony methods were determined by using bootstrap values based on 1000 replicates. According to the neighbour-joining tree (Fig. 1), strain H3-R18^T was clearly affiliated with the genus *Pseudomonas*, and formed a single group with *Pseudomonas luteola* IAM 13000^T. Based on a BLAST search against the GenBank library, strain H3-R18^T showed high 16S rRNA gene sequence similarity (99.6%) to uncultured bacterial clone 20up (GenBank accession no.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain H3-R18^T is DQ339144.

A transmission electron micrograph showing a cell of strain H3-R18^T is available as supplementary material in IJSEM Online.

Fig. 1. Neighbour-joining phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of strain H3-R18^T with some members of the genus *Pseudomonas*. Bootstrap values (expressed as percentages of 1000 replications) greater than 40% are shown at the branch points. Bar, 0.005 substitutions per nucleotide position.

AY212656), which was isolated from equine faecal contamination (Simpson *et al.*, 2004). However, the 16S rRNA gene sequence of strain H3-R18^T showed $\leq 96.3\%$ similarity to that of the type strains of all recognized *Pseudomonas* species, suggesting that it represented a novel genomic species, without the need for DNA–DNA hybridization characterization (Stackebrandt & Goebel, 1994). Highest sequence similarities were shown to *Pseudomonas brenneri* CFML 97-391^T (96.3%) and *Pseudomonas migulae* CIP 105470^T (96.3%).

The DNA G+C content of strain H3-R18^T, determined using a HPLC method (Mesbah *et al.*, 1989), was 64.0 mol%. Fatty acid methyl esters were extracted and prepared by the standard protocol of the Microbial Identification System (MIDI; Microbial ID) after cells were grown on MA for 48 h at 28 °C. The dominant cellular fatty acids of H3-R18^T were summed feature 3 (39.3%; comprising iso-C_{15:0} 2-OH and/or C_{16:1} ω7c), C_{16:0} (30.7%), C_{12:0} (8.2%), C_{12:0} 3-OH (7.1%), C_{18:1} ω7c (6.5%), C_{14:0} (3.7%), C_{10:0} 3-OH (1.6%) and C_{17:0} cyclo (1.0%). Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). The major isoprenoid quinone was ubiquinone 9 (Q-9).

To investigate morphological and some physiological characteristics, strain H3-R18^T was routinely cultivated on MA at 28 °C. Cell morphology was examined by light microscopy (AXIO; Zeiss) and transmission electron microscopy. For the latter, cells were grown for 48 h on MA, negatively stained with 0.5% (w/v) uranyl acetate and examined

with a LEO model 912AB electron microscope (see Supplementary Fig. S1 available in IJSEM Online). The test for motility was performed on motility medium (0.1% yeast extract, 0.01% K₂HPO₄, 0.2% agar) and one-tenth strength marine broth 2216 (Difco) supplemented with 0.2% agar. Anaerobic growth was checked using BBL anaerobic agar (Becton Dickinson). The temperature range for growth was determined using MA in the range 4–45 °C. The requirement for NaCl was tested in marine broth supplemented with 0, 1, 3, 5, 7 and 9% (w/v) NaCl, and the pH range (pH 4.0–10.0 at intervals of 1.0 pH unit) for growth was determined in marine broth that was buffered with citrate-phosphate buffer or Tris/HCl buffer (Breznak & Costilow, 1994). Gram staining, catalase and oxidase activities, poly-β-hydroxybutyrate accumulation, and hydrolysis of aesculin, casein, DNA, gelatin and starch were determined as described by Smibert & Krieg (1994). King A medium and King B medium were used to test production of pyocyanin and formation of fluorescent pigments, respectively (King *et al.*, 1954). Hydrolysis of alginate (0.5%, w/v), carboxymethylcellulose (Sigma; 0.1%, w/v), chitin from crab shells (1%, w/v), pectin (0.5%, w/v) and tyrosine (0.5%, w/v) was also tested. For the various physiological tests, API 20NE and API ZYM test strips (bioMérieux) were used according to the manufacturer's instructions. To test the utilization of carbon substrates, Biolog GN 2 plates were used with a half-strength solution of sea salts for cell suspension.

In API 20NE test strips, strain H3-R18^T showed positive reactions for nitrate reduction, and arginine dihydrolase

and aesculin hydrolysis, but negative reactions for indole production, glucose fermentation, urease, gelatin hydrolysis and β -galactosidase. The strain assimilated capric acid, but not D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, D-maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate or phenylacetic acid. Enzyme activity was observed for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but not for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase or α -fucosidase. In Biolog GN 2 plates, strain H3-R18^T utilized Tweens 40 and 80, pyruvic acid methyl ester, β -hydroxybutyric acid, DL-lactic acid, L-alanine, L-aspartic acid, L-glutamic acid and L-proline. Phenotypic characteristics that distinguish strain H3-R18^T from some phenotypically similar and

phylogenetically related *Pseudomonas* species are shown in Table 1.

On the basis of the data presented, H3-R18^T is proposed as the type strain of a novel species in the genus *Pseudomonas*, *Pseudomonas pohangensis* sp. nov.

Description of *Pseudomonas pohangensis* sp. nov.

Pseudomonas pohangensis (po.hang.en'sis. N.L. adj. *pohangensis* referring to Pohang city in Korea, where the type strain was found).

Cells are Gram-negative, non-motile, non-spore-forming rods approximately $0.6\text{--}0.8 \times 1.5\text{--}3.0$ μm in size. Colonies are irregular and cream-coloured after 48 h on MA. Cells are non-fluorescent on King A and B media. Catalase- and oxidase-positive. Growth occurs at temperatures of $4\text{--}35$ °C

Table 1. Differential phenotypic characteristics of *Pseudomonas pohangensis* sp. nov. and other *Pseudomonas* species

Taxa: 1, strain H3-R18^T; 2, *Pseudomonas aeruginosa* (Palleroni, 1984); 3, *Pseudomonas alcaligenes* (Palleroni, 1984); 4, *Pseudomonas alcaliphila* (Yumoto *et al.*, 2001); 5, *Pseudomonas balearica* (Bennasar *et al.*, 1996); 6, *Pseudomonas brenneri* (Baïda *et al.*, 2001); 7, *Pseudomonas fluorescens* biovar 1 (Palleroni, 1984); 8, *Pseudomonas luteola* (Kodama *et al.*, 1985); 9, *Pseudomonas migulae* (Verhille *et al.*, 1999); 10, *Pseudomonas pachastrellae* (Romanenko *et al.*, 2005); 11, *Pseudomonas pseudoalcaligenes* (Palleroni, 1984); 12, *Pseudomonas putida* biovars (Palleroni, 1984); 13, *Pseudomonas stutzeri* (Palleroni, 1984). +, At least 90 % of strains positive; –, at least 90 % of strains negative; d, 11–89 % of strains positive; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Yellow or orange cellular pigment	–	–	d	–	–	–	–	+	ND	–	–	–	d
Fluorescent pigments	–	d	–	–	–	ND	+	–	–	–	–	+	–
Pyocyanin	–	+	–	–	–	–	–	–	–	–	–	–	–
Number of flagella	0	1	1	1	1	1	>1	1	1	1	1	>1	1
Poly- β -hydroxybutyrate accumulation	–	–	–	ND	ND	–	–	+	ND	ND	d	–	–
Denitrification	+	+	+	–	+	+	–	–	+	–	d	–	+
Arginine dihydrolase	+	+	+	+	–	+	+	+	+	–	d	+	–
Growth at:													
4 °C	+	–	–	+	ND	+	+	–	+	–	–	d	–
41 °C	–	+	+	–	+	–	–	+	–	+	+	–	+
Tolerance of 8 % NaCl	–	–	–	–	+	–	–	–	–	+	–	–	–
Hydrolysis of:													
Gelatin	–	+	d	+	–	ND	+	+	–	–	d	–	–
Starch	–	–	–	–	+	ND	–	–	–	–	–	–	+
Tween 80	+	d	d	+	ND	ND	d	+	–	+	–	d	+
Assimilation of:													
D-Glucose	–	+	–	+	ND	+	+	+	+	–	–	+	+
Maltose	–	–	–	–	+	–	–	+	–	–	–	–	+
Fructose	–	+	–	+	ND	+	+	+	+	–	+	+	d
D-Mannose	–	–	–	–	ND	+	+	+	+	–	–	d	–
Sucrose	–	–	–	–	ND	–	+	–	–	–	–	d	–
Trehalose	–	–	–	–	ND	+	+	+	+	–	–	–	–
Glycerol	–	+	–	+	ND	+	+	+	+	–	d	+	+
D-Mannitol	–	+	–	+	–	+	+	+	+	–	–	d	d
Gluconate	–	+	–	ND	ND	+	+	+	+	–	d	+	d
Malate	–	+	+	+	+	–	+	+	+	–	+	+	+
DNA G + C content (mol%)	64.0	67.2	64–68	62.3–63.2	64.1–64.4	58	60.5	54.8	58–59	61.1–61.5	62–64	60.7–62.5	60.6–66.3

(optimum 25–30 °C), pH 5–9 (optimum pH 7–8) and at NaCl concentrations of up to 3.0% (optimum 1.0%). Does not hydrolyse alginic acid, casein, chitin, carboxymethylcellulose, DNA, gelatin, starch, tyrosine or urea. Major fatty acids are summed feature 3 and C_{16:0}, with lesser amounts of C_{12:0}, C_{12:0} 3-OH, C_{18:1ω7c} and C_{14:0}. The major isoprenoid quinone is Q-9. The DNA G+C content is 64.0 mol%.

The type strain, H3-R18^T (=KACC 11517^T=DSM 17875^T), was isolated from seashore sand collected from Homi cape, Pohang city, Korea.

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