Pseudomonas hussainii sp. nov., isolated from droppings of a seashore bird, and emended descriptions of Pseudomonas pohangensis, Pseudomonas benzenivorans and Pseudomonas segetis

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Two Gram-staining-negative, aerobic, rod-shaped, non-spore-forming bacterial strains that are motile by a monopolar flagellum, designated CC-AMH-11T and CC-AMHZ-5, were isolated from droppings of a seashore bird off the coast of Hualien, Taiwan. The strains showed 99.7 % mutual pairwise 16S rRNA gene sequence similarity, while exhibiting <96.2 % sequence similarity to strains of other species of the genus Pseudomonas (95.7–95.9 % similarity with type species, Pseudomonas aeruginosa LMG 1242T), and formed a distinct co-phyletic lineage in the phylogenetic trees. The common major fatty acids (>5 % of the total) were C18:1ω7c and/or C18:1ω6c (summed feature 8), C16:1ω6c and/or C16:1ω7c (summed feature 3), C16:0 and C12:0. Phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylserine, an unidentified lipid and an unidentified phospholipid were detected as common polar lipids. The DNA G+C contents of strains CC-AMH-11T and CC-AMHZ-5 were 61.1 and 61.6 mol%, respectively. The common major respiratory quinone was ubiquinone 9 (Q-9), and the predominant polyamine was putrescine. The DNA–DNA hybridization obtained between the two strains was 79.0 % (reciprocal value 89.4 % using CC-AMHZ-5 DNA as the probe). The very high 16S rRNA gene sequence similarity and DNA–DNA relatedness and the poorly distinguishable phenotypic features witnessed between CC-AMH-11T and CC-AMHZ-5 suggested unambiguously that they are two distinct strains of a single genomic species. However, the strains also showed several genotypic and phenotypic characteristics that distinguished them from other closely related species of Pseudomonas. Thus, the strains are proposed to represent a novel species of Pseudomonas, for which the name Pseudomonas hussainii sp. nov. is proposed. The type strain is CC-AMH-11T (=JCM 19513T=BCRC 80696T); a second strain of the same species is CC-AMHZ-5 (=JCM 19512=BCRC 80697). In addition, emended descriptions of the species Pseudomonas pohangensis, Pseudomonas benzenivorans and Pseudomonas segetis are also proposed.

The genus Pseudomonas, affiliated to the family Pseudomonadaceae, class Gammaproteobacteria, was described by Migula (1894) and includes 212 species and 18 subspecies with validly published names at the time of writing (http://www.bacterio.net/pseudomonas.html). Members of the genus Pseudomonas are ubiquitous in distribution, and have been isolated from various sources such as water (Liu et al., 2013; Pascual et al., 2012; Lang et al., 2010; Hauser et al., 2004), soil (Lin et al., 2013a; Feng et al., 2012; Park et al., 2006), seashore sand (Weon et al., 2006) and food-waste compost (Lin et al., 2013b). The nutritional studies of Stanier et al. (1966) on Pseudomonas provided baseline assistance to the phenotypic characterization of representative isolates. Species of the genus Pseudomonas differ considerably in their nutritional versatility and can metabolize xenobiotic compounds (Lang et al., 2010; Busse et al., 1989). The members of...
the genus *Pseudomonas* have undergone multiple taxonomic reassessment on the basis of physiological, molecular and phenotypic features (Sneath *et al.*, 1981), DNA–DNA hybridization (Palleroni, 1984), 16S rRNA gene sequence similarity (Anzai *et al.*, 2000) and chemotaxonomic data (Oyaizu & Komagata, 1983). Here, we describe the polyphasic taxonomic characterization of two strains of the genus *Pseudomonas*, designated CC-AMH-1^T^ and CC-AMHZ-5.

Strains CC-AMH-1^T^ and CC-AMHZ-5 were isolated from the droppings of a seashore bird (unidentified) off the coast of Hualien, Taiwan. The sample was subjected to standard dilution-to-extinction plating using marine agar 2216 (MA; BD Difco) (30 °C, 48–96 h). Pale-yellow colonies of strains CC-AMH-1^T^ and CC-AMHZ-5 were isolated, purified and preserved in nutrient broth (NB; Himedia) supplemented with 20% (v/v) glycerol at −80 °C. Taxonomic investigations were carried out according to previously published guidelines (Tindall *et al.*, 2010). Three type strains of the genus *Pseudomonas* [*Pseudomonas pohangensis* DSM 17875^T^ (Weon *et al.*, 2006), *P. benzenovorans* DSM 8628^T^ (Lang *et al.*, 2010) and *P. segetis* KCTC 12331^T^ (Park *et al.*, 2006)] were used for direct comparative analysis. All five strains were cultured by using nutrient agar (NA; Himedia) or NB for 48 h at 30 °C, unless specified otherwise.

Genomic DNA of the novel strains was isolated by using the UltraClean Microbial Genomic DNA isolation kit (Mo Bio) by following the manufacturer’s instructions. The partial 16S rRNA gene was amplified by PCR as given by Shahina *et al.* (2013). Gene sequencing was performed by using the Big Dye terminator kit (Heiner *et al.*, 1998) and an automatic DNA sequencer (ABI PRISM 310; Applied Biosystems) (Watts & MacBeath, 2001). Sequence fragments were then assembled using the Fragment Assembly System program from the Wisconsin Package (GGC, 1995). Sequence similarity values were computed using the *BLAST* (Altschul *et al.*, 1990) search program of NCBI and EzTaxon-e server (Kim *et al.*, 2012). Sequence data were analysed by using *MEGA* 5, version 5.0 (Tamura *et al.*, 2011), after multiple alignment by *CLUSTAL_X* (Thompson *et al.*, 1997). A distance matrix method (distance options according to Kimura’s two-parameter model; Kimura, 1980) including clustering by neighbour-joining (NJ; Saitou & Nei, 1987), a discrete character-based maximum-parsimony (MP; Fitch, 1971) method and the maximum-likelihood (ML; Felsenstein, 1981) method were used. The topologies of the trees were evaluated by using bootstrap resampling method based on 1000 replications (Felsenstein, 1985).

The amplified 16S rRNA genes of strains CC-AMH-1^T^ and CC-AMHZ-5 were each 1500 bp long. The strains shared 99.7% mutual pairwise 16S rRNA gene sequence similarity, while exhibiting <96.2% sequence similarity to strains of other *Pseudomonas* species. Strain CC-AMH-1^T^ shared the highest pairwise 16S rRNA gene sequence similarity with *Pseudomonas guariconensis* PCAVU11^T^ (96.2%), followed by *P. segetis* FR1439^T^ (96.1%) and *Pseudomonas aeruginosa* LMG 1242^T^ (95.9%; type species). In contrast, strain CC-AMHZ-5 shared a maximum of 96.2% 16S rRNA gene similarity with *P. benzenovorans* DSM 8628^T^ and *P. guariconensis* PCAVU11^T^, followed by *P. segetis* FR1439^T^ (95.9%). In the NJ (Fig. 1) and ML (not shown) phylogenetic trees, the novel strains formed distinct co-phyletic lineages associated with *Pseudomonas psychrotolerans* C36^T^, having 39 and 38% bootstrap confidence of the node, respectively. However, in the MP tree, the novel strains were associated with *P. pohangensis* H3-R18^T^ (42% bootstrap support). The low bootstrap values clearly indicated an unstable taxonomic position of these two strains with regard to related species of the genus *Pseudomonas*. On the other hand, the strains that shared high pairwise 16S rRNA gene sequence similarity with the novel strains also occupied a distant phylogenetic neighbourhood in the NJ (Fig. 1), MP and ML phylogenetic trees (not shown). Thus, *P. pohangensis* H3-R18^T^, *P. benzenovorans* DSM 8628^T^ and *P. segetis* FR1439^T^ were chosen randomly for direct comparative taxonomic analysis.

Because of the highly conserved nature of the 16S rRNA gene, nearly identical sequences do not guarantee species identity (Fox *et al.*, 1992). Therefore, we investigated the genetic relatedness between strains CC-AMH-1^T^ and CC-AMHZ-5 further through DNA–DNA hybridization, which was performed by using the DIG DNA labelling and detection kit (cat. no. 11 093 657 910; Roche Diagnostics) according to the manufacturer’s protocol. The DNA–DNA hybridization obtained between strains CC-AMH-1^T^ and CC-AMHZ-5 was 79.0% (reciprocal value 89.4% using CC-AMHZ-5 DNA as probe). These values are well above the 70% threshold that has been recommended for species distinction (Tindall *et al.*, 2010), suggesting that these two strains are possibly affiliated to the same genomic species.

The following phenotypic tests were carried out exclusively on the novel strains. Colonies were examined for morphological features such as appearance, size, shape, texture and pigmentation. Presence of endospores was assessed by phase-contrast microscopy (model A3000; Zeiss) after malachite-green staining (Smibert & Krieg, 1994) of cells grown on NA for 7 days. Cell morphology including the presence of flagella was determined by placing the cells (1–2 days old) on a carbon-coated copper grid followed by staining with 0.2% uranyl acetate for 5–10 s, brief air-drying and observation under a transmission electron microscope (JEOL JEM-1400). Gram staining was performed according to Murray *et al.* (1994). The requirement for NaCl was tested on R2A agar (BD Difco) supplemented with 0–10% (w/v) NaCl (at 1% intervals). The pH range for growth was determined in NB that was adjusted before incubation to pH 4.0–10.0 (at 1.0 pH unit intervals) using appropriate buffers (Hameed *et al.*, 2013). Growth at 10, 20, 25, 30, 37, 40, 45, 50 and 55 °C was tested in NB after 1 week of incubation. Strains were inoculated in the Biolog GN2 MicroPlate and API 50 CH strip according to the manufacturers’ instructions. Results were recorded after 48 h of incubation at 30 °C.

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*Pseudomonas hussainii* sp. nov.
The following phenotypic tests were carried out on all five strains. Activities of catalase, oxidase and DNase and hydrolysis of starch, egg yolk, Tween 20, colloidal chitin, CM-cellulose, xylan and L-tyrosine were tested as described previously (Hameed et al., 2014); NA was used as the basal medium for hydrolytic studies. Bacterial cell suspensions were inoculated separately in API 20 NE and API ZYM strips (bioMérieux) and the results were recorded after 48 h of incubation at 30 °C.

The phenotypic characteristics of the isolates are shown in Fig. S1 (available in the online Supplementary Material) and are given in the species description. Both strains were rod-shaped, varying in cell size, and motile by means of a flagellum. They were identified as follows:

- **Pseudomonas aeruginosa** LMG 1242T (Z76651)
- **Pseudomonas otitidis** MCC10330T (AY953147)
- **Pseudomonas resinovorans** LMG 2274T (Z76668)
- **Pseudomonas alcaligenes** LMG 1224T (Z76653)
- **Pseudomonas guguanensis** CC-G9AT (JQ864237)
- **Pseudomonas sagittaria** CC-OPY-1T (JQ277453)
- **Pseudomonas tuomuerensis** 78-123T (DQ868767)
- **Pseudomonas oleovorans** DSM 1045T (Z76664)
- **Pseudomonas mendocina** LMG 1223T (Z76664)
- **Pseudomonas alciphila** AL15-21T (AB030583)
- **Pseudomonas toytomomiesis** HT-3T (AB061701)
- **Pseudomonas stutzeri** CCUG 11256T (U26262)
- **Pseudomonas xanthomarina** KMM 1447T (AB176954)
- **Pseudomonas guanciconesis** PCAVU11T (HF674459)
- **Pseudomonas cremonicolata** IAM 1541T (AB060137)
- **Pseudomonas oryzihabitans** IAM 1568T (D84004)
- **Pseudomonas putida** IAM 1236T (D84020)
- **Pseudomonas fulva** IAM1529T (D84015)
- **Pseudomonas jessenii** CIP 105274T (AF068259)
- **Pseudomonas asplenii** ATCC 23835T (AB021397)
- **Pseudomonas fuscovaginae** ICMP 5940T (FJ483519)
- **Pseudomonas japonica** IAM 15071T (AB126621)
- **Pseudomonas entomophila** LA8T (AY907566)
- **Pseudomonas mosselli** CIP 105259T (AF072668)
- **Pseudomonas taiwanensis** BCRC 17751T (EU103629)
- **Pseudomonas plecglossicidica** FPC951T (AB009457)
- **Pseudomonas monteilii** CIP 104883T (AF064458)
- **Pseudomonas argentinensis** CH01T (AY691188)
- **Pseudomonas straminea** IAM 1598T (D84023)
- **Pseudomonas Flavescens** B62T (U01916)
- **Pseudomonas segetis** FR1439T (AY770691)
- **Pseudomonas marincola** KMM 3042T (AB301071)
- **Pseudomonas bortori** R-20821T (AM114527)
- **Pseudomonas anguilliseptica** NCIMB 1949T (X99540)
- **Pseudomonas punonensis** LMT03T (JQ344321)
- **Pseudomonas seleniipraecipitatus** CA5T (FJ422810)
- **Pseudomonas plecoglossicida** FPC951T (AB009457)
- **Pseudomonas psychrotolerans** C36T (AJ575816)
- **Pseudomonas benzenivorans** DSM 8628T (FM208263)
- **Pseudomonas psychrotolerans** C36T (AJ575816)
- **Pseudomonas luteola** IAM 13000T (D84002)
- **Pseudomonas psychrotolerans** C36T (AJ575816)
- **Pseudomonas formosensis** CC-CY503T (JF432053)
- **Cellvibrio ostraviensis** LMG 19434T (JF493853)

Fig. 1. NJ phylogenetic tree based on 16S rRNA gene sequences showing the taxonomic positions of strains CC-AMH-11T and CC-AMHZ-5 with respect to some closely related members of the genus *Pseudomonas*. Bootstrap values (>70%) based on 1000 replications are shown at nodes. *Cellvibrio ostraviensis* LMG 19434T was used as an outgroup. Filled circles indicate that the corresponding nodes were recovered in trees generated with the MP and ML algorithms as well. Bar, 0.01 substitutions per nucleotide position.
monopolar flagellum. Strains CC-AMHZ-5 and CC-AMH-11\textsuperscript{T} shared similar phenotypic characteristics. However, strain CC-AMHZ-5 differed slightly from strain CC-AMH-11\textsuperscript{T} as the former exhibited strong oxidation of \(\alpha\)-ketoglutaric acid, which was oxidized weakly by the latter; weak oxidation of \(\alpha\)-hydroxybutyric acid, which was oxidized strongly by the latter; weak oxidation of D-mannitol, citric acid and sebacic acid, which were not oxidized by the latter; no oxidation of \(L\)-leucine and putrescine, which were oxidized by the latter; and no oxidation of \(L\)-arabinose, gentiobiose, lactulose, \(\gamma\)-hydroxybutyric acid, itaconic acid, \(\alpha\)-ketovaleric acid, quinic acid, D-saccharic acid, L-aspartic acid, glycyl L-aspartic acid, glycyl -L-glutamic acid, urocanic acid, 2-aminoethanol and glycerol, which were oxidized weakly by the latter. In addition, strain CC-AMHZ-5 failed to produce acid from D-glucose, unlike strain CC-AMH-11\textsuperscript{T}. Other features that distinguished the isolates from each other as well as from the reference strains are given in Table 1.

For cellular fatty acid analysis, fatty acid methyl esters of the novel isolates and the three reference strains were extracted from cells cultivated on NA at 30 °C for 2–3 days. Cell samples were harvested during the exponential growth phase and subjected to saponification, methylation and extraction as described previously (Kämpfer & Kroppenstedt, 1996) followed by gas chromatography (model 7890A; Agilent). Peaks were automatically integrated and fatty acid names and percentages were determined using the Microbial Identification Standard software package MIDI (version 6) (Sasser, 1990) by adopting the database RTSBA6.

### Table 1. Differential phenotypic characteristics of representatives of the genus Pseudomonas

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<th>4</th>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(L)-Tyrosine*</td>
<td>– (DP-)</td>
<td>– (DP-)</td>
<td>– (DP-)</td>
<td>+ (DP-)</td>
<td>+ (DP+)</td>
</tr>
<tr>
<td>Egg yolk</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Chitin, CM-cellulose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Xylan</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Aesculin (API 20 NE)</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin (API 20 NE)</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Enzyme activity (API 20 NE)</td>
<td></td>
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<tr>
<td>Arginine dihydrolase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Assimilation of (API 20 NE):</td>
<td></td>
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<tr>
<td>(D)-Glucose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(D)-Mannitol</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Potassium gluconate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Capric acid</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Malate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trisodium citrate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>+</td>
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<tr>
<td>Phenylacetic acid</td>
<td>–</td>
<td>–</td>
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<td>Enzyme activity (API ZYM)</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>W</td>
<td>W</td>
<td>+</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>(\alpha)-Glucosidase</td>
<td>W</td>
<td>W</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(N)-Acetyl-(\beta)-glucosaminidase</td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>W</td>
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</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>61.1</td>
<td>61.6</td>
<td>64\textsuperscript{a}</td>
<td>NA</td>
<td>56.3\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\*DP, Diffusible pigments.

\textsuperscript{a}Data from: \(a\), Weon et al. (2006); \(b\), Park et al. (2006).
In both strains, the common major fatty acids (>5% of the total) were C18:1ω7c and/or C18:1ω6c (summed feature 8; 29.2–29.6%), C16:1ω7c and/or C16:1ω6c (summed feature 3; 23.1–23.7%), C16:0 (15.3–17.9%) and C12:0 (8.0–6.8%) (Table 2). The fatty acid profiles of the two strains were similar, and the predominance of the above-mentioned fatty acids was in line with those of the three reference strains as well as information available in the literature (Liu et al., 2013; Lin et al., 2013a, b; Feng et al., 2012; Pascual et al., 2012; Weon et al., 2006; Hauser et al., 2004).

Respiratory quinones of the novel strains were extracted and analysed by two-dimensional TLC (Embley & Wait, 1994). Authentic phosphatidylcholine and phosphatidylserine standards were purchased from Sigma-Aldrich. The spot of phosphatidylcholine was confirmed to lack phosphatidylcholine (Pascual et al., 2013, 2013a, b; Lang et al., 2012; Pascual et al., 2013; Lin et al., 2013a, b; Feng et al., 2012; Pascual et al., 2012; Weon et al., 2006; Hauser et al., 2004).

Polar lipids of all five strains were extracted and analysed by reversed-phase HPLC according to Collins (1985) with minor modifications (Shahina et al., 2013). Both strains contained ubiquinone 9 (Q-9) as the major respiratory quinone, similar to quinone data reported previously from species of the genus Pseudomonas (Liu et al., 2013; Lin et al., 2013a, b; Feng et al., 2012; Pascual et al., 2012; Weon et al., 2006; Hauser et al., 2004).

Table 2. Whole-cell fatty acid profiles of representatives of the genus Pseudomonas

<table>
<thead>
<tr>
<th>Fatty acid</th>
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<tr>
<td>Saturated</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C10:0</td>
<td>3.1</td>
<td>2.6</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>C12:0</td>
<td>8.0</td>
<td>6.8</td>
<td>8.0</td>
<td>7.2</td>
<td>12.8</td>
</tr>
<tr>
<td>C14:0</td>
<td>2.7</td>
<td>2.9</td>
<td>TR</td>
<td>TR</td>
<td>1.2</td>
</tr>
<tr>
<td>C16:0</td>
<td>15.3</td>
<td>17.9</td>
<td>25.4</td>
<td>23.4</td>
<td>24.1</td>
</tr>
<tr>
<td>C17:0</td>
<td>TR</td>
<td>TR</td>
<td>1.7</td>
<td>1.7</td>
<td>TR</td>
</tr>
<tr>
<td>C18:0</td>
<td>TR</td>
<td>1.0</td>
<td>1.3</td>
<td>1.0</td>
<td>TR</td>
</tr>
<tr>
<td>Unsaturated</td>
<td></td>
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</tr>
<tr>
<td>C17:1ω8c</td>
<td>TR</td>
<td>TR</td>
<td>1.5</td>
<td>1.2</td>
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<tr>
<td>Branched monounsaturated</td>
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<tr>
<td>iso-C17:1ω10c</td>
<td>1.2</td>
<td>TR</td>
<td>1.2</td>
<td>1.1</td>
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</tr>
<tr>
<td>Hydroxy</td>
<td></td>
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<td></td>
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<tr>
<td>C10:0 3-OH</td>
<td>4.9</td>
<td>4.7</td>
<td>TR</td>
<td>4.5</td>
<td>3.4</td>
</tr>
<tr>
<td>C12:0 3-OH</td>
<td>5.0</td>
<td>5.0</td>
<td>7.3</td>
<td>4.9</td>
<td>4.8</td>
</tr>
<tr>
<td>iso-C15:0 3-OH</td>
<td>4.1</td>
<td>3.3</td>
<td>2.8</td>
<td>1.4</td>
<td>3.1</td>
</tr>
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<tr>
<td>C17:0 cyclo</td>
<td>–</td>
<td>–</td>
<td>4.5</td>
<td>13.3</td>
<td>4.2</td>
</tr>
<tr>
<td>C19:0 cyclo ω8c</td>
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<td>–</td>
<td>2.3</td>
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<tr>
<td>Summed features*</td>
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<tr>
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<td>23.7</td>
<td>33.7</td>
<td>15.9</td>
<td>14.5</td>
</tr>
<tr>
<td>Summed feature 8</td>
<td>29.2</td>
<td>29.6</td>
<td>7.4</td>
<td>16.2</td>
<td>25.4</td>
</tr>
</tbody>
</table>

*As indicated by Montero-Calasanz et al. (2013), summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete equivalent chain-lengths (ECL) as well as those where the ECL are not reported separately. Summed feature 3 was listed as C16:1ω6c and/or C16:1ω7c; summed feature 8 was listed as C18:1ω7c and/or C18:1ω6c.

Fig. 2. Polar lipid profiles of representatives of the genus Pseudomonas as determined by two-dimensional TLC. (a) Strain CC-AMH-11T; (b) strain CC-AMHZ-5; (c) P. pohangensis DSM 17875T; (d) P. benzenivorans DSM 8628T; (e) P. segetis KCTC 12331T. Total polar lipids were visualized by spraying the TLC plates with 10% ethanolic molybdophosphoric acid. DPG, Diphasphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; PL1–5, unidentified phospholipids; L1–3, unidentified lipids.
Genomic data for *P. aeruginosa* PAO1 (Stover et al., 2000) and *P. psychrotolerans* L19 (Espírito Santo et al., 2012) revealed a gene that encodes phosphatidylserine decarboxylase, an enzyme that catalyses the reversible decarboxylation of phosphatidyl-L-serine into phosphatidylethanolamine, and we have confirmed the presence of phosphatidylserine in all five strains in this study. Additionally, we speculate that a spot labelled APL, localized at a similar position in *P. psychrotolerans* (Hauser et al., 2004) as well as in many other species of the genus *Pseudomonas* (Liu et al., 2013; Feng et al., 2012), possibly represents phosphatidylserine. However, it should be noted that some species of *Pseudomonas* clearly lack a similar APL spot (Lin et al., 2013b), indicating that the distribution of phosphatidylserine is heterogeneous, similar to phosphatidylcholine. Nevertheless, the detection of diphasphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine in all five strains was in line with data reported previously (Liu et al., 2013; Lin et al., 2013a, b; Pascual et al., 2012; Feng et al., 2012; Hauser et al., 2004).

The polyamines of all five strains were extracted and analysed by reversed-phase HPLC according to Scherer & Kneifel (1983) with minor modifications (see online Supplementary Material). Strains CC-AMH-11T and CC-AMHZ-5 contained putrescine as the predominant polyamine (≥70%), as well as accommodating moderate amounts of spermidine (18.2–21.4%), minor amounts of cadaverine (3.1–5.9%) and trace amounts of triamine-sym-homospermidine (0.1–0.02%) (Table S1). The dominance of putrescine followed by spermidine was evident in the reference strains, except *P. pohangensis* DSM 17875T, which, in contrast, accumulated very large amounts of putrescine (94.3%) and minor amounts of spermidine (3.1%). *P. segetis* KCTC 12331T showed significant amounts of cadaverine (18.4%). The predominance of putrescine in all five strains is in excellent agreement with the polyamine data reported previously (Hauser et al., 2004; Busse et al., 1989), irrespective of some of the above-mentioned differences with regard to other polyamines.

For determination of the genomic DNA G+C content, DNA of the novel strains was subjected to thermal denaturation followed by enzymic digestion into nucleosides as described previously (Mesbah et al., 1989). The resultant nucleoside mixture was separated and quantified by reversed-phase HPLC according to Mesbah et al. (1989) with minor modifications, as indicated by Shahina et al. (2013). The DNA G+C contents of strains CC-AMH-11T and CC-AMHZ-5 were 61.1 and 61.6 mol%, respectively, which were within the range (55.3–64.3 mol%) reported from some species of the genus *Pseudomonas* (Liu et al., 2013; Lin et al., 2013a, b; Feng et al., 2012; Weon et al., 2006; Park et al., 2006).

The high 16S rRNA gene sequence similarity and DNA–DNA hybridization accompanied by poorly distinguishable phenotypic features witnessed in the novel isolates suggested that they represent two distinct strains of the same genomic species. Based on their phylogenetic distinctiveness and differentiating phenotypic characteristics, a novel species of the genus *Pseudomonas* named *Pseudomonas hussainii* sp. nov. is proposed. On the basis of new data obtained in this study, emended descriptions of the species *P. pohangensis*, *P. benzenivorans* and *P. segetis* are also proposed.

**Emended description of *Pseudomonas pohangensis* Weon et al. 2006**

The description is as given by Weon et al. (2006) with the following amendments. Phosphatidylethanolamine is the predominant polar lipid. In addition, diphasphatidylglycerol, phosphatidylglycerol and phosphatidylserine are also detected in significant amounts. Putrescine is the predominant polyamine. In addition, spermidine, cadaverine and triamine-sym-homospermidine are present in minor to trace amounts.

**Emended description of *Pseudomonas benzenivorans* Lang et al. 2012**

The description is as given by Lang et al. (2010) with the following amendments. Phosphatidylethanolamine is the predominant polar lipid followed by phosphatidylglycerol. In addition, diphasphatidylglycerol, phosphatidylcholine, phosphatidylserine, an unidentified lipid and an unidentified phospholipid are detected in moderate to trace amounts. Putrescine is the predominant polyamine, followed by spermidine. In addition, cadaverine and triamine-sym-homospermidine are present in trace amounts.

**Emended description of *Pseudomonas segetis* Park et al. 2006**

The description is as given by Park et al. (2006) with the following amendments. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylserine are the predominant polar lipids. In addition, four unidentified phospholipids and three unidentified lipids are also detected in moderate to trace amounts. Putrescine is the predominant polyamine, followed by cadaverine and spermidine. In addition, triamine-sym-homospermidine is present in trace amounts.

**Description of *Pseudomonas hussainii* sp. nov.**

*Pseudomonas hussainii* (hus.sai’ni.i. N.L. masc. gen. n. hussainii named after S. A. Hussain, an Indian ornithologist and avian gut biologist).

Cells are Gram-staining-negative, aerobic, motile by means of a monopolar flagellum, non-spore-forming, chemoheterotrophic, mesophilic, 1.3–1.9 μm long and 0.6–0.7 μm in diameter. On NA, after 1–2 days of incubation at 30 °C, colonies are 1–3 mm in diameter, round to ovoid with irregular margins, flat and pale yellow. Growth occurs at 15–40 °C (optimum, 30–37 °C), at pH 6.0–8.0 (optimum, pH 7.0) and on R2A agar supplemented with 0–5% NaCl (optimum, 1%). Positive for activities of catalase and
oxidase and hydrolysis of starch, egg yolk and Tween 20. Negative for hydrolysis of L-tyrosine, DNA, colloidal chitin, xylan and CM-cellulose. In the API 20 NE strip, positive for nitrate reduction and assimilation of D-glucose, maltose, capric acid and malate and negative for indole production, fermentation of glucose, activities of arginine dihydrolase and urease, hydrolysis of aesculin and p-nitrophenyl β-D-galactopyranoside and assimilation of L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, potassium gluconate, adipic acid, trisodium citrate and phenylacetic acid; variable for hydrolysis of gelatin. In the API ZYM strip, positive for activities of esterase (C4), esterase lipase (C8) and leucine arylamidase, weakly positive for activities of valine arylamidase, naphthol-AS-BI-phosphohydrolase and α-glucosidase and negative for activities of alkaline and acid phosphatases, lipase (C14), cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. In the Biolog GN2 MicroPlate, positive for the oxidation of dextrin, glycogen, Tweens 40 and 80, L-glucose, maltose, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, ω-hydroxybutyric acid, ω-ketobutyric acid, DL-lactic acid, propionic acid, succinic acid, bromosuccinic acid, L-alaninamide, D- and L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, L-ornithine, L-proline and L-serine; weakly positive for the oxidation of cellobiose, α-ketoglutaric acid, malonic acid and γ-aminobutyric acid; variable for oxidation of L-arabinose, gentiobiose, lactulose, D-mannitol, citric acid, γ-hydroxybutyric acid, itaconic acid, α-ketovaleric acid, quinic acid, D-saccharic acid, sebacic acid, L-aspartic acid, glycoll L-aspartic acid, glycoll L-glutamic acid, L-leucine, urocanic acid, putrescine, 2-aminoethanol and L-aspartic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, D-mannose, D-mannitol, D-galactopyranoside and assimilation of L-arabinose, D-glucose, maltose, pyruvic acid methyl ester, succinic acid, bromosuccinic acid, L-alaninamide, D- and L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, L-ornithine, L-proline and L-serine; weakly positive for the oxidation of dextrin, glycogen, Tweens 40 and 80, L-glucose, maltose, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, ω-hydroxybutyric acid, ω-ketobutyric acid, DL-lactic acid, propionic acid, succinic acid, bromosuccinic acid, L-alaninamide, D- and L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, L-ornithine, L-proline and L-serine; weakly positive for the oxidation of cellobiose, α-ketoglutaric acid, malonic acid and γ-aminobutyric acid; variable for oxidation of L-arabinose, gentiobiose, lactulose, D-mannitol, citric acid, γ-hydroxybutyric acid, itaconic acid, α-ketovaleric acid, quinic acid, D-saccharic acid, sebacic acid, L-aspartic acid, glycoll L-aspartic acid, glycoll L-glutamic acid, L-leucine, urocanic acid, putrescine, 2-aminoethanol and glycocol; and negative for oxidation of the other substrates. In the API 50 CH strip, ac is produced from maltose, starch, glycogen and potassium 5-ketogluconate; acid is produced from maltose, glycogen and potassium 5-ketogluconate; and negative for oxidation of the other substrates. In the API 50 CH strip, acid is produced from maltose, starch, glycogen and potassium 5-ketogluconate; acid is produced from maltose, glycogen and potassium 5-ketogluconate; and negative for oxidation of the other substrates. In the API 50 CH strip, acid is produced from maltose, starch, glycogen and potassium 5-ketogluconate; acid is produced from maltose, glycogen and potassium 5-ketogluconate; and negative for oxidation of the other substrates. In the API 50 CH strip, acid is produced from maltose, starch, glycogen and potassium 5-ketogluconate; acid is produced from maltose, glycogen and potassium 5-ketogluconate; and negative for oxidation of the other substrates.

The type strain, CC-AMH-11^T (= JCM 19513^T = BCRC 80696^T), and a reference strain, CC-AMHZ-5 (=JCM 19512 = BCRC 80697), were isolated from droppings of a seashore bird off coastal Hualien, Taiwan. The DNA G+C contents of strains CC-AMH-11^T and CC-AMHZ-5 are 61.1 and 61.6 mol%.

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