The genus *Pseudomonas* was originally created by Migula (1984) and represents a group of Gram-stain-negative bacteria that are aerobic, non-spore-forming, motile and rod-shaped (Timmis, 2002; Zhang et al., 2011). This genus is recognized as a large and widely distributed bacterial group (Nishimori et al., 2000). To date, the names of more than 200 species of the genus *Pseudomonas* have been validly published (http://bacterio.net), which were isolated from various environments, such as plants, animals, soil, water and air (Romanenko et al., 2005a; Vela et al., 2006; Amoozegar et al., 2014). About a dozen species of the genus *Pseudomonas* have been isolated from marine environments, including *Pseudomonas pachastrellae* (Romanenko et al., 2005a), *Pseudomonas xanthomarina* (Romanenko et al., 2005b) and *Pseudomonas marincola* (Romanenko et al., 2008) from marine animals; *Pseudomonas pseudoalkaligenes* (Palleroni, 1984), *Pseudomonas alcaliphila* (Yumoto et al., 2001) and *Pseudomonas litoralis* (Pascual et al., 2012) from seawater; *Pseudomonas balearica* (Bennasar et al., 1996) and *Pseudomonas deceptienss* (Carrión et al., 2011) from marine sediment; and *Pseudomonas aestsusigni* (Sánchez et al., 2014), *Pseudomonas sabulinigri* (Kim et al., 2009) and *Pseudomonas taeanensis* (Lee et al., 2010) from seashore. The presence of the hydroxylated acids C<sub>10</sub>:0 3-OH and C<sub>12</sub>:0 3-OH is presumed to be indicative for the genus *Pseudomonas* (Lang et al., 2010). The genomic G+C content of members of the genus is 58–69 mol% (Palleroni et al., 2005). Based on concatenating the 16S rDNA, gyrB, rpoD and rpoB gene sequences, the genus *Pseudomonas* can be divided into two main lineages, the *Pseudomonas aeruginosa* lineage and the *Pseudomonas fluorescens* lineage. However, some strains do not belong to the above two lineages, such as *P. pachastrellae*, *Pseudomonas luteola*, *Pseudomonas pertucinogena* and *Pseudomonas oryzihabitans* (Mulet et al., 2010). A new group, the *P. pertucinogena* group, was reported by Mulet et al. (2012b), including *P. sabulinigri* KCTC 22137<sup>T</sup>, *P. xiangenensis* JCM 13530<sup>T</sup>, *P. baumannii* DSM 22558<sup>T</sup>, *P. litoralis* CECT 7670<sup>T</sup>, *P. pertucinogena* LMG 1874<sup>T</sup>, *P. pelagia* JCM 15562<sup>T</sup>, *P. pachastrellae* JCM 12285<sup>T</sup> and *P. aestusigni* CECT 8317<sup>T</sup> (Sánchez et al., 2014).

Strain KX 20<sup>T</sup> was isolated from the deep seawater (depth of 1390 m) in Okinawa Trough, northwestern Pacific Ocean. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain KX 20<sup>T</sup> was related to members of the genus *Pseudomonas* and shares the highest sequence identities with *Pseudomonas aestsusigni* CECT 8317<sup>T</sup> (99.4 %) and *Pseudomonas pachastrellae* JCM 12285<sup>T</sup> (98.5 %). The 16S rRNA gene sequence identities between strain KX 20<sup>T</sup> and other members of the genus *Pseudomonas* were below 96.6 %. The gyrB and rpoD genes of strain KX 20<sup>T</sup> shared 82.0 to 89.3 % sequence identity with the gyrB and rpoD genes of the closest phylogenetic neighbours of KX 20<sup>T</sup>. The predominant cellular fatty acids of strain KX 20<sup>T</sup> were summed feature 8 (C<sub>18</sub>:1ω7c and/or C<sub>18</sub>:1ω6c) (29.2 %), C<sub>16</sub>:0 (24.5 %), summed feature 3 (C<sub>16</sub>:1ω7c and/or C<sub>16</sub>:1ω6c) (21.5 %) and C<sub>12</sub>:0 (8.2 %). The major polar lipids of strain KX 20<sup>T</sup> were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and unknown phospholipids. The genomic DNA G+C content of strain KX 20<sup>T</sup> was 62.9 mol%. On the basis of phylogenetic analysis and phenotypic characteristics, a novel species, *Pseudomonas oceani* sp. nov. is proposed. The type strain is KX 20<sup>T</sup> (=CGMCC 1.15195<sup>T</sup>=DSM 100277<sup>T</sup>).

**Pseudomonas oceani** sp. nov., isolated from deep seawater

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In this study, we identified a novel Gram-stain-negative, aerobic, motile, and rod-shaped bacterium, strain KX 20<sup>T</sup>, isolated from the deep seawater in Okinawa Trough, northwestern Pacific Ocean. Phylogenetic analysis based on 16S rRNA gene sequence showed that strain KX 20<sup>T</sup> was related to members of the genus *Pseudomonas* and shares the highest sequence identities with *Pseudomonas aestsusigni* CECT 8317<sup>T</sup> (99.4 %) and *Pseudomonas pachastrellae* JCM 12285<sup>T</sup> (98.5 %). The 16S rRNA gene sequence identities between strain KX 20<sup>T</sup> and other members of the genus *Pseudomonas* were below 96.6 %. The gyrB and rpoD genes of strain KX 20<sup>T</sup> shared 82.0 to 89.3 % sequence identity with the gyrB and rpoD genes of the closest phylogenetic neighbours of KX 20<sup>T</sup>. The predominant cellular fatty acids of strain KX 20<sup>T</sup> were summed feature 8 (C<sub>18</sub>:1ω7c and/or C<sub>18</sub>:1ω6c) (29.2 %), C<sub>16</sub>:0 (24.5 %), summed feature 3 (C<sub>16</sub>:1ω7c and/or C<sub>16</sub>:1ω6c) (21.5 %) and C<sub>12</sub>:0 (8.2 %). The major polar lipids of strain KX 20<sup>T</sup> were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and unknown phospholipids. The genomic DNA G+C content of strain KX 20<sup>T</sup> was 62.9 mol%. On the basis of phylogenetic analysis and phenotypic characteristics, a novel species, *Pseudomonas oceani* sp. nov. is proposed. The type strain is KX 20<sup>T</sup> (=CGMCC 1.15195<sup>T</sup>=DSM 100277<sup>T</sup>).
Ocean. The seawater was obtained by a Remotely Operated Vehicle (ROV) equipped on the KEXUE vessel during the cruise of April 2014 (Tollefson, 2014). For bacterial isolation, seawater was spread on marine agar 2216 (Kobayashi et al., 2008). After incubation for 5 days at 28 °C under aerobic conditions, strain KX 20 T was selected, and pure culture was obtained after three successive transfers to fresh medium. The strain was stored at −80 °C in liquid medium supplemented with 20 % (v/v) glycerol. In addition to strain KX 20 T, the type strain *P. pachastrellae* JCM 12285 T was obtained from the Japan Collection of Microorganism (JCM) and *P. aestuariigenri* CECT 8317 T was obtained from Colección Española de Cultivos Tipo (CECT), and these were used as reference strains. A polyphasic taxonomic study of strain KX 20 T and the reference strains was performed using phylogenetic and phenotypic characterization. The results of this study indicated that strain KX 20 T represents a novel species of the genus *Pseudomonas*, and belongs to the *P. pertucinogenri* group.

The genomic DNA was extracted from strain KX 20 T using a Tiangen DNA kit. The 16S rRNA gene was amplified by PCR using the universal primers B27F and B1492R (Frank et al., 2008). The gene was cloned into pEASY-T (TransGen Biotech) and confirmed by sequence analysis. 16S rRNA gene sequence analysis (1498 nt) was performed using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). Phylogenetic trees of 16S rRNA gene were reconstructed using MEGA6 (Tamura et al., 2013) after multiple data alignment by CLUSTALW. Phylogenetic trees were also reconstructed by the neighbour-joining and maximum-likelihood methods, and the distances were calculated according to the Kimura two-parameter model (Kimura, 1980). The topology of the phylogenetic tree was estimated by bootstrap analysis of 1000 replicates (Felsenstein, 1981). The results of the phylogenetic analysis indicated that strain KX 20 T belongs to the genus *Pseudomonas* (Fig. 1). Comparison of the 16S rRNA gene sequence of strain KX 20 T with those of the type strains recorded in the EzTaxon-e database indicated that strain KX 20 T was positioned within the genus *Pseudomonas*, with *P. aestuariigenri* CECT 8317 T (Sánchez et al., 2014) being the most closely related species (99.4 % identity), following by *P. pachastrellae* JCM 12285 T (Romanenko et al., 2005a) (98.5 % identity). The sequence identities between KX 20 T and the rest of the species of the genus *Pseudomonas* were below 96.6 %.

To differentiate further between strain KX 20 T and the closely related species, the sequences of the housekeeping genes *gyrB* and *rpoD* were included in phylogenetic analysis. Amplification of *gyrB* and *rpoD* was performed as described by Mulet et al. (2010). The sequences obtained (971 nt for *gyrB* and 894 nt for *rpoD*) were submitted to GenBank. The *gyrB* gene of strain KX 20 T shares 87.4 % and 85.6 % sequence identity with the *gyrB* gene of *P. aestuariigenri* CECT 8317 T and *P. pachastrellae* JCM 12285 T, respectively. The *rpoD* gene of strain KX 20 T shares 89.3 % and 82.0 % sequence identity with the *rpoD* gene of *P. aestuariigenri* CECT 8317 T and *P. pachastrellae* JCM 12285 T, respectively. The phylogenetic tree of concatenated *gyrB* and *rpoD* gene sequences was reconstructed by the neighbour-joining method using MEGA6 (Fig. 2). The concatenated tree showed that the most closely related type strains of KX 20 T were *P. aestuariigenri* CECT 8317 T and *P. pachastrellae* JCM 12285 T (Fig. 2). Based on 16S rRNA, *gyrB* and *rpoD* gene sequences, the similarities of strain KX 20 T with *P. sabulinigri*, *P. xiamenensis*, *P. baumanniseri*, *P. litoralis*, *P. pertucinogenri*, *P. pelagia* and *P. aeruginosa* were 87, 86, 85, 85, 84 and 84 %, respectively. These identities were much lower than the 97 % threshold established for discriminating species in the genus *Pseudomonas* by multilocus sequence analysis of three concatenated genes (Mulet et al., 2010). Therefore strain KX 20 T should be considered as a representative of a previously undescribed species of the genus *Pseudomonas*, and belongs to the *P. pertucinogenri* group.

DNA–DNA hybridization was performed as reported previously (Liu & Shao, 2005). DNA of strain KX 20 T was hybridized with that of *P. aestuariigenri* and *P. pachastrellae*. The results showed that the hybridization values between KX 20 T and *P. aestuariigenri* and *P. pachastrellae* were 56.9 % and 32.6 %, respectively. Since a threshold value of 70 % DNA–DNA relatedness is recommended for definition of a bacterial species (Wayne et al., 1987), strain KX 20 T represents a novel species within the genus *Pseudomonas*.

For base composition analysis, DNA was extracted according to the procedure of Moore et al. (1999). The G+C content of DNA was determined according to the method of Mesbah et al. (1989). The DNA G+C content of strain KX 20 T was 62.9 mol%, which is consistent with the values reported for species of the genus *Pseudomonas* (Toro et al., 2013; Mulet et al., 2012a).

For cellular fatty acid analysis, strains were cultured in trypticase soy broth agar (TSA; Difco) for 3 days at 28 °C. Fatty acid methyl esters were prepared and analysed according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0) and identified by using the TSB6.0 database of the Microbial Identification System (Sasser, 1990). The major fatty acids of strain KX 20 T were summed feature 8 (C18:1ω7c and/or C18:1ω6c) (29.2 %), C16:0 (24.5 %), summed feature 3 (C16:1ω7c and/or C16:1ω6c) (21.5 %) and C12:0 (8.2 %) (Table S1, available in the online Supplementary Material). In strain KX 20 T, both C10:0 3-OH (3.5 %) and C12:0 3-OH (3.4 %) were present, which is in accordance with the characteristics of the genus *Pseudomonas* (Peix et al., 2005; Lang et al., 2010; Wang et al., 2010). The fatty acids of strain KX 20 T showed similarities to those of the reference strains *P. aestuariigenri* and *P. pachastrellae*, but were present in different proportions. For example, strain KX 20 T had more C17:0 cyclo and less summed feature 3 than other type strains.

Polar lipids of strain KX 20 T were extracted according to the protocol of Minnikin (1984) and separated by two-dimensional TLC. Identification of individual lipids was performed by spraying with appropriate detection reagents (Komagata & Suzuki, 1987). The polar lipids found in strain
KX 20<sup>T</sup> were phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and unknown phospholipids (PLs). The presence of PE as predominant phospholipid with smaller amounts of PG, DPG and PLs is consistent with that previously reported for other species of the genus *Pseudomonas* (Romanenko et al., 2005a; C/C<sub>19</sub>amara et al., 2007).

Characteristics of strain KX 20<sup>T</sup> were examined using routine cultivation methods on marine agar at 28°C (except where indicated otherwise). Cell morphology was examined with a transmission electron microscope (JEM-1200; JEOL) using cells of strain KX 20<sup>T</sup> cultured at 28°C for 3 days on marine agar and negatively stained with 1% (w/v) phosphotungstic acid. Gram staining was performed according to the protocol of Doetsch (1981). Motility was observed by the hanging-drop method (Bernardet et al., 2002). The temperature range for growth of strain KX 20<sup>T</sup> was determined on marine agar plates at 4, 8, 16, 28, 37, 40, 41, 42 and 43°C. Salinity and pH ranges for growth of strain KX 20<sup>T</sup> were determined by measuring optical densities at 595 nm after incubation in 96-well microplates as reported previously (Dai et al., 2014). To test anaerobic growth, strain KX 20<sup>T</sup> was cultured at 28°C on marine agar plates with resazurin (0.02%, w/v) added as an indicator of anaerobic conditions. The plates were incubated in an anaerobic incubator (YQX-II; Yuejin) filled with nitrogen. Standard approaches (Tindall et al., 2007) were used to study catalase and oxidase activity and the degradation of starch, gelatin, Tween 20 and Tween 40 with the modification that sterile seawater was used. DNase activity was examined by using DNase agar (Base Bio-Tech) with sterile seawater. API 20NE and API ZYM strips (bioMérieux) as well as the

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic position of strain KX 20<sup>T</sup> within the genus *Pseudomonas*. Bootstrap values (>50%) based on 1000 replications are shown at branch node. Triangles indicate that the corresponding nodes were also recovered in the maximum-likelihood tree. GenBank accession numbers are given in parentheses. *Acinetobacter baumannii* DSM 30007<sup>T</sup> (X81660) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.
Biolog GN2 MicroPlate kit were used to determine the physiological characteristics and activities of constitutive enzymes according to the manufacturers’ instructions. For fluorescent pigment analysis, cells were grown in King B agar and nutrient agar, and pigment production and spectral characteristics were detected as reported by Hildebrand et al. (1994).

Cells of strain KX 20<sup>T</sup> were Gram-stain-negative, non-pigmented, aerobic, rod-shaped (0.4–0.6 µm in width and 2.1–2.6 µm in length) and were mobile by a single polar flagellum (Fig. S1). Cells were positive for catalase and oxidase activity, and negative for fluorescent pigments. The phenotypic characteristics of strain KX 20<sup>T</sup> are reported in the species description, and the differences with respect to the most closely related species of the genus Pseudomonas are listed in Table 1. Phenotypic studies showed that strain KX 20<sup>T</sup> displayed characteristics consistent with those for the genus Pseudomonas, but strain KX 20<sup>T</sup> differed significantly from related type strains in terms of several phenotypic properties. For KX 20<sup>T</sup>, positive carbon assimilations were observed with capric acid, D-mannose, adipic acid, trisodium citrate and malic acid. In contrast, P. aestusnigri and P. pachastrellae could assimilate only some of these carbon sources. All strains were able to assimilate the following substrates: Tween 40, Tween 80, L-alanine, L-asparagine, pyruvic acid methyl ester, sebacic acid, β-hydroxybutyric acid, bromosuccinid acid, succinic acid and D,L-lactic acid. Strain KX 20<sup>T</sup> could assimilate L-alanyl-glycine, but P. aestusnigri and P. pachastrellae could not.

On the basis of phylogenetic analysis, phenotypic characteristics, fatty acid composition and polar lipids, the marine strain KX 20<sup>T</sup> was considered to represent a novel species, for which the name Pseudomonas oceani sp. nov. is proposed.

**Description of Pseudomonas oceani sp. nov.**

*Pseudomonas oceani* (o.ce.a’ni. L. gen. n. *oceani* of the ocean).

Cells of strain KX 20<sup>T</sup> are Gram-stain-negative, strictly aerobic, rod-shaped, non-pigmented, 0.4–0.6 µm in width and 2.1–2.6 µm in length, and are motile by a single flagellum. Growth occurs at 4–41 °C, pH 6.0–10.0 and with 0–10% (w/v) NaCl. The colonies are circular, smooth, non-pigmented, whitish and transparent. No fluorescent pigments are produced in King B medium. Positive for oxidase and catalase activities; positive for hydrolysis of Tween 20 and 40, and negative for hydrolysis of starch, DNA and gelatin. Negative for nitrate reduction, aesculin hydrolysis, gelatin hydrolysis, indole production and glucose fermentation; negative for the activities of arginine dihydrolase, urease and β-galactosidase. Assimilates capric acid, D-mannose, adipic acid, trisodium citrate and malic acid, but not D-glucose, L-arabinose, D-mannitol, N-acetylglucosamine, maltose, potassium gluconate or phenylacetic acid. In the API ZYM strip, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, trypsin, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. When assayed with the Biolog GN2 Microplate, positive for utilization of bromosuccinic acid, L-alaninamide, α-ketovaleric acid, D-alanine, L-phenylalanine, Tween 40, Tween 80, D,L-lactic acid, L-alanine, L-proline, putrescine, propioninic acid, pyruvic acid methyl ester, β-hydroxybutyric acid, L-alanyl-glycine, L-asparagine, L-glutamic acid, sebacic acid, succinic acid monomethyl ester and succinic acid; weakly positive or negative utilization of the other substrates. The
Table 1. Phenotypic characteristics of strain KX 20T and the closely related type strains *P. aestusnigri* CECT 8317T and *P. pachastrellae* JCM 12285T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell width (μm)</td>
<td>0.4–0.6</td>
<td>0.6–0.7*</td>
<td>0.4–0.5†</td>
</tr>
<tr>
<td>Cell length (μm)</td>
<td>2.1–2.6</td>
<td>1.6–2.0*</td>
<td>1.4–1.6†</td>
</tr>
<tr>
<td>API 20NE</td>
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</tr>
<tr>
<td>d-Mannose</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Adipic acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Trisodium citrate</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Malic acid</td>
<td>+</td>
<td>–</td>
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<td>Biolog GN2</td>
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<tr>
<td>Acetate</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<td>Raffinose</td>
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<td>w</td>
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<tr>
<td>α-Ketoglutaric acid</td>
<td>w</td>
<td>–</td>
<td>+</td>
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<tr>
<td>1-Alaninamide</td>
<td>+</td>
<td>w</td>
<td>–</td>
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<td>1-Ketovaleric acid</td>
<td>+</td>
<td>w</td>
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<tr>
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<tr>
<td>1-Proline</td>
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<tr>
<td>Putrescine</td>
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<tr>
<td>1-Alanyl-glycine</td>
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<tr>
<td>D-Glucose 6-phosphate</td>
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<td>–</td>
</tr>
<tr>
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<tr>
<td>1-Arabinose</td>
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<td>+</td>
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<tr>
<td>1-Glutaic acid</td>
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<td>w</td>
<td>+</td>
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<tr>
<td>r-Aminobutyric acid</td>
<td>w</td>
<td>+</td>
<td>–</td>
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</table>

*Data from Sánchez et al. (2014).†Data from Romanenko et al. (2005a).

The stype strain is KX 20T (=CGMCC 1.15195T=DSM 100277T), isolated from deep seawater. The G+C content of the genomic DNA of the type strains is 62.9 mol%.

**References**


**Major polar lipids** diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and unknown phospholipids.

The stype strain is KX 20T (=CGMCC 1.15195T=DSM 100277T), isolated from deep seawater. The G+C content of the genomic DNA of the type strains is 62.9 mol%.

**Acknowledgements**

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