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TAXONOMIC DESCRIPTION
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# *Actibacterium pelagium* sp. nov., a novel alphaproteobacterium, and emended description of the genus *Actibacterium*

Li-Li Guo,[1,2] Yue-Hong Wu,[2] Xiao-Wu Xu,[3] Chao-Jie Huang,[3] Yun-Yun Xu,[3] Hong Cheng,[2] Chun-Sheng Wang,[2] Min Wu[1,*] and Xue-Wei Xu[2,*]

The genus *Actibacterium*, belonging to the family *Rhodobacteraceae* of the class *Alphaproteobacteria*, was first proposed by Lucena *et al.* [1]. Currently, the genus *Actibacterium* consists of three species with validly published names, *A. mucosum* (type species), *A. atlanticum* and *A. ureilyticum* [1–3]. The natural habitats from which *Actibacterium* species have been isolated are marine seawater collected from the Mediterranean [1], the Atlantic Ocean [2] and the western Pacific Ocean [3]. Members of the genus *Actibacterium* are Gram-negative, ovoid to rod-shaped, strictly aerobic and chemo-organotrophic with complex ionic requirements. The genus *Actibacterium* possesses ubiquinone 10 (Q-10) as sole respiratory quinone, and phosphatidylglycerol, an unidentified aminolipid and an unidentified lipid as major polar lipids [4]. This study focuses on the description of a novel strain named JN33$^T$, which was isolated from the seawater of the western Pacific Ocean.

During a study on bacterial diversity of the seawater of the western Pacific Ocean, seawater samples were collected by a rosette sampler connected with a CTD system (SBE911 Plus; Sea-Bird Electronics) from the western Pacific Ocean (at a depth of 100 m) in December 2015. Aboard the ship, an approximately 100 µl seawater sample was immediately spread on natural seawater agar (natural seawater supplemented with 0.05 % peptone, 0.01 % yeast extract and agar 1.5 %, w/v, pH 7.2–7.4). After a month of incubation at 25 °C, approximately 100 colonies appeared. One-tenth of these colonies were picked and purified. Among the obtained strains, only one strain, designated JN33$^T$, belonging to the genus *Actibacterium* was isolated and subjected to polyphasic study. The purity of strain JN33$^T$ was confirmed by the uniformity of cell morphology by phase-contrast microscopy (DM 5000B; Leica). The reference strains *A. atlanticum* LMG 27158$^T$ and *A. mucosum* DSM 28448$^T$ were obtained from the LMG (Collection of the Laboratorium voor Microbiologie en Microbiele Genetica) and DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen), respectively. The reference strain *A. ureilyticum* LS-811$^T$ was obtained from Young's lab [3]. Unless otherwise stated, these strains were routinely cultured in marine broth 2216 (MB; BD) or on marine agar 2216 (MA; BD) at 30 °C and maintained at −80 °C with 25 % (v/v) glycerol.

Cell morphology, ultrastructure, size and motility were observed using transmission electron microscopy (JEM-1230; JEOL) for strain JN33$^T$that had been grown on MA for 36 h. The hanging drop method was used for motility testing. Growth at different NaCl concentrations (0, 0.5, 1.0, 3.0, 5.0, 7.5, 10.0 and 15.0 %, w/v) was tested in NaCl-free MB (prepared according to the MB formula, but without NaCl). The requirement of sea salts for growth (0, 0.5, 1.0, 2.0, 3.0, 4.0, 4.5 and 5.0 %, w/v; Sigma) was also tested in PY medium (peptone 5.0 g, yeast extract 1.0 g and distilled water 1 L, pH 7.2). The pH range for growth in MB was measured from pH 5.0 to pH 10.0 at intervals of 0.5 pH units at 30 °C. MES (50 mM, pH 5.0–6.0), PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5) and CAPSO [3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid] (pH 9.0–10.0) were used as biological buffers. The temperature range for growth was investigated at various temperatures (4, 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C). Growth was monitored by measuring $OD_{600}$ in a UV/visible spectrophotometer (Ultrospec 6300 Pro; Amersham Biosciences). Upper and lower limits for growth were confirmed when no growth was observed after 1month's incubation. Anaerobic growth was carried out with AnaeroPack (Mitsubishi). Sodium nitrate (20 mM) and sodium nitrite (20 mM) were used as potential electron acceptors.

Unless stated otherwise, physiological and biochemical tests were performed in MB at 30 °C. Oxidase activity was tested by oxidation of *p*-aminodimethylaniline oxalate solution

**Author affiliations:** [1]College of Life Sciences, Zhejiang University, Hangzhou 310058, PR China; [2]Key Laboratory of Marine Ecosystem and Biogeochemistry, Second Institute of Oceanography, State Oceanic Administration, Hangzhou 310012, PR China; [3]Department of Gastrointestinal and Pancreatic Surgery, Zhejiang Provincial People Hospital, Peoples Hospital of Hangzhou Medical College, Hangzhou 310014, PR China.
***Correspondence:** Min Wu, wumin@zju.edu.cn; Xue-Wei Xu, xuxw@sio.org.cn
**Keywords:** *Actibacterium*; *Alphaproteobacteria*; whole genome sequencing.
**Abbreviations:** ANI, average nucleotide identity; DDH, DNA-DNA hybridization; Q-10, ubiquinone 10.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JN33$^T$ is KY273602. The GenBank accession numbers for the whole genome sequences of strain JN33$^T$ and *Actibacterium ureilyticum* LS-811$^T$ are NSBU00000000 and NSBT00000000, respectively.
Two supplementary figures are available with the online Supplementary Material.

(1 %, w/v). Catalase activity was determined by bubble production in hydrogen peroxide solution (3 %, v/v). Hydrolysis of starch and Tweens 20, 40 and 80 was performed according to Dong and Cai [5]. The concentration of Tweens 20, 40 and 80 was modified to 0.5 % (w/v). To determine which compounds could be used as sole carbon and energy sources, BM medium [6] was used and 0.2 % (w/v) sugars, 0.2 % (w/v) alcohols, 0.1 % (w/v) organic acids or 0.1 % (w/v) amino acids were added. The utilization of carbohydrates was observed after inoculation for 3, 5 and 7 days. Acid production was determined using marine oxidation–fermentation medium [7] supplemented with 1.0 % sugars. API 20NE strips (bioMérieux) and API ZYM strips (bioMérieux) were used to test additional physiological and biochemical characteristics according to the manufacturer's instructions. Sea salts (2 %, w/v; Sigma) were added to the AUX medium provided by the manufacturer. Susceptibility to antibiotics was tested using a two-layer plate method [8] and the diameter of the antibacterial circles was measured. Susceptibility to antibiotics was determined following the criteria of > 3 mm as susceptible, 1–3 mm as weakly susceptible and < 1 mm as resistant [9]. Three reference strains, *A. atlanticum* LMG 27158$^T$, *A. mucosum* DSM 28448$^T$ and *A. ureilyticum* LS-811$^T$, were used for comparison.

For fatty acid analysis, the cellular fatty acids of strain JN33$^T$ and the reference strains were determined under identical conditions in parallel. Cells were collected from the third quadrant (at the late exponential phase) along the streaking axis after growth on MA at 30 °C for 3 days. Cellular fatty acid methyl esters were obtained and analysed according to the instructions of the Microbial Identification System (MIDI) using the RTSBA6 database. Chloroform/methanol (2 : 1, v/v) was used to extract isoprenoid quinones from cells of strain JN33$^T$. Extracts were evaporated to dryness at 35 °C, and then were resuspended in chloroform/methanol (2 : 1, v/v). Isoprenoid quinone was purified by TLC on GF254 silica gel plates (Branch of Qingdao Haiyang Chemical Company) with n-hexane/ether (17 : 3, v/v) and then analysed by HPLC-MS (Agilent 1200 chromatograph and Thermo Finnigan LCQ DECA XP MAX mass spectrometer) [10]. Total lipids were extracted and separated by two-dimensional TLC on silica gel 60 F254 plates (Merck). Four kinds of spray reagents were used to detect the corresponding lipids, comprising molybdophosphoric acid for total lipids, molybdenum blue for phosphorus-containing lipids, ninhydrin reagent for lipids containing free aminolipids and *p*-anisaldehyde reagent for glycolipids [11].

Genomic DNA was obtained by means of the Bacterial Genomic DNA Fast Extraction kit (DongSheng Biotech). The 16S rRNA gene of strain JN33$^T$ was amplified and sequenced using bacterial domain-specific primers 27F (5′-GAGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGAC-3′) [12]. Then, the 16S rRNA gene sequence was compared with closely related sequences of reference organisms via the EzBioCloud online database (www.ezbiocloud.net). The genomes of strain JN33$^T$ and *A. ureilyticum* LS-811$^T$ were sequenced by Solexa PE150 sequencing technology with the HiSeq platform (Novogene Technology Company). The sequencing generated approximate 1 Gbyte of clean data (approximate 250-fold genome coverage). The *de novo* assembly of the reads was performed using ABySS 1.5.2 [13]. The assembly *k*-value was tested from 35 to 64 to find the optimal *k*-value using abyss-pe script. The quality of microbial genomes was assessed using the bioinformatic tool CheckM [14]. The genome sequences of *A. atlanticum* 22II-S11-z10$^T$ (AQQY00000000) and *A. mucosum* KCTC 23349$^T$ (JFKE00000000) were retrieved from the GenBank database. The average nucleotide identity (ANI) was calculated using the OrthoANIu algorithm of the Chun lab's online Average Nucleotide Identity calculator [15]. *In silico*DNA–DNA hybridization (DDH) values were calculated by genome-to-genome distance calculator (GGDC) [16].

The complete sequence of the 16S rRNA gene was annotated via the RNAmmer 1.2 Server [17] and was used for pairwise sequence alignment by the EzTaxon-e service [18]. Based on EzTaxon-e results, 17 species were selected for phylogenetic analysis and *Oceanibaculum indicum* P24$^T$ was used as the outgroup. Multiple sequence alignment based on the 16S rRNA gene sequence data was performed using CLUSTAL W [19] and phylogenetic trees were reconstructed by neighbour-joining [21], maximum-parsimony [22] and maximum-likelihood methods [23] using the MEGA 5 program package [20]. Bootstrap analysis (1000 resample datasets) was used to evaluate the topology of the trees. Kimura's two-parameter model [24] was used for phylogeny construction and evolutionary distances analysis.

Strain JN33$^T$ was Gram-stain-negative, non-spore-forming, non-motile, rod-shaped, 0.6–1.2 µm in width and 1.5–2.5 µm in length (Fig. S1, available in the online Supplementary Material). Flagella were not observed. Colonies on MA agar plates were cream in colour, circular, convex, smooth and approximately 1–2 mm in diameter after incubation for 3 days at 30 °C. Strain JN33$^T$ grew well on MA, but not on TSB agar (BD) or Luria–Bertani (LB) agar (BD). The pH and temperature range for growth were pH 6.5–8.5 (optimum at pH 7.0) and 10–35 °C (optimum at 30 °C). No growth occurred at 4 or 40 °C after 1 month of incubation. The NaCl concentration range for growth was 0.5–5.0 % (w/v, optimum 3.0 %). Sea salts were necessary for growth. Strain JN33$^T$ was positive for catalase activity, hydrolysis of aesculin and gelatin, susceptible to the antibiotics (µg per disc unless otherwise stated) ampicillin (10), amoxicillin (20), carbenicillin (100), cefoxitin (30), chloramphenicol (30), erythromycin (15), kanamycin (30), nitrofurantoin (300), novobiocin (30), penicillin (10), polymyxin B (300 IU), rifamcin (5), tetracyline (30), tobramycin (10) and vancomycin (30), and resistant to bacitracin (0.04 U), gentamicin (10), nystatin (100) and streptomycin (10). Detailed phenotypic characteristics are given in the species description and Table 1.

The complete 16S rRNA gene sequence of strain JN33$^T$ (1456 nt) was obtained from the whole genome sequence. Based on the EzTaxon service and CLUSTAL W results, strain JN33$^T$ showed high 16S rRNA gene sequence similarity to *A. atlanticum* 22II-S11-z10$^T$ (97.3 %), *A. mucosum* KCTC 23349$^T$ (96.6 %) and *A. ureilyticum* LS-811$^T$ (95.7 %) and exhibited less than 97.0 % 16S rRNA gene sequence similarity with respect to the other type strains of the *Rhodobacteraceae* species. The topologies of neighbour-joining, maximum likelihood and maximum-parsimony phylogenetic trees indicated that the genus *Actibacterium* formed a monophyletic clade and strain JN33$^T$ clustered together with *A. atlanticum* and *A. ureilyticum* (Fig. 1). Phylogenetic analysis indicated strain JN33$^T$ represents a novel member of the genus *Actibacterium*.

The genome completeness of strain JN33$^T$ and *Actibacterium ureilyticum* LS-811$^T$ were 99.4 and 99.2 %, with 0.5 and 1.1 % contamination, respectively. Genome sequences estimated to be of $\geq$ 95 % completeness, with $\leq$ 5 % contamination, were considered as excellent reference genomes for deeper analyses [14]. The DNA G+C content of strain JN33$^T$ was 57.8 mol%, a value a little below the range reported for members of the genus *Actibacterium*, i.e. 59.0–61.3 mol% [1–3], and the values calculated from genome sequences, i.e. 59.0–64.3 mol% (Table 1). The ANI values between strain JN33$^T$ and the type strains of the *Actibacterium* species were 73.1–73.8 %. The *in silico* DDH values (the recommended results from formula 2) indicated that strain JN33$^T$ and the type strains of the *Actibacterium* species shared 19.8–20.1 % DNA relatedness. The ANI values and *in silico* DDH values were strikingly lower than the threshold values of the species boundary (ANI 94–96 % and *in silico* DDH 70 %) [25, 26], revealing a low taxonomic relatedness between strain JN33$^T$ and the three type strains of the *Actibacterium* species. These values indicate that strain JN33$^T$ represents a different genomic species.

The chemotaxonomic data supported the results of the phylogenetic analysis. The sole respiratory quinone found in strain JN33$^T$ was Q-10, which is a typical characteristic of the genus *Actibacterium* [4]. Fatty acid analysis revealed strain JN33$^T$ contained predominantly summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), which was similar to the reference strains (Table 2). The percentages of $C_{10:0}$ 3-OH, $C_{18:0}$ and $C_{20:1}\omega7$c were similar in all four strains (Table 2). Strain JN33$^T$ possessed phosphatidylglycerol and an unidentified aminolipid as the major polar lipids, similarly to the three reference strains (Fig. S2) [1–3].

The chemotaxonomic data results also clearly showed some differences in fatty acid compositions and polar lipid profiles among strain JN33$^T$ and the type strains of the *Actibacterium* species. Strain JN33$^T$ contained iso-$C_{18:0}$ (5.8 %), which was not detected in the reference strains (Table 2). $C_{19:0}$ cyclo $\omega8c$ was detected in the reference strains (in amounts of 3.4–9.5 %), but was not found in strain JN33$^T$. $C_{16:0}$ was detected in strain JN33$^T$ as a major fatty acid (11.3 %), while it was found as a minor fatty acid in *A. atlanticum* (2.0 %) and *A. ureilyticum* (1.4 %). 11-Methyl $C_{18:1}\omega7c$ was present in moderate amounts in strain JN33$^T$ (at 4.8 %), but was found in minor amounts in *A. ureilyticum* (1.0 %). In addition, one unidentified glycolipid (GL3) was present in strain JN33$^T$ in moderate or minor amounts, while it was present in *A. ureilyticum* LS-811$^T$ as the major lipid (Fig. S2 and reference [3]). Phosphatidylcholine was found in *A. atlanticum* LMG 27158$^T$ [2] in major amounts, but was not detected in strain JN33$^T$ (Fig. S2).

In conclusion, strain JN33$^T$ exhibits the typical characteristics of the genus *Actibacterium*, such as having Q-10 as the sole respiratory quinone, summed feature 8 as the predominant fatty acid, and phosphatidylglycerol as the major polar lipid. However, strain JN33$^T$ could also be distinguished from the type strains of the *Actibacterium* species by differences in phenotypic characteristics such as NaCl range and optimum, temperature range and optimum, hydrolysis of aesculin and gelatin, enzyme activities, carbohydrate utilization and acid production (Table 1). For instance, strain JN33$^T$ showed a growth maximum at 5.0 % NaCl concentration, while growth of *A. atlanticum* occurred even at 9.0 % NaCl concentration. Strain JN33$^T$ could not hydrolyse Tweens 40 and 80, while *A. atlanticum* LMG 27158$^T$ could. L-Arabinose, sodium gluconate and sucrose were only used by strain JN33$^T$ as carbon sources. On the basis of the phylogenetic analysis, genomic data and chemotaxonomic results, as well as phenotypic characteristics, strain JN33$^T$ represents a novel species of the genus *Actibacterium*, for which the name *Actibacterium pelagium* sp. nov. is proposed.

## DESCRIPTION OF *ACTIBACTERIUM PELAGIUM* SP. NOV.

*Actibacterium pelagium* (pe.la′gi.um. L. neut. adj. *pelagium*, of or belonging to the sea).

Cells are Gram-stain-negative, non-spore-forming, non-motile, rod-shaped, 0.6–1.2 μm in width and 1.5–2.5 μm in length. Colonies are cream in colour, 2 mm in diameter, circular, smooth and flat after 3 days' incubation at 30 °C on MA. No growth occurs in the absence of NaCl and sea salts. Grows on NaCl-free MB supplemented with 0.5–5.0 % (w/v) NaCl (optimum 3.0 %). The pH and temperature ranges for growth are pH 6.5–8.5 and 10–35 °C (optimum at pH 7.0 and 30 °C). No anaerobic growth occurs on MA supplemented with sodium nitrate or sodium nitrite. Positive for oxidase, weakly positive for catalase. Negative for nitrate reduction, indole production from tryptophan, arginine dihydrolase and urease. Aesculin and gelatin are hydrolysed. Tween 20, Tween 40, Tween 80 and starch are not hydrolysed. The following compounds are utilized as sole carbon and energy source: L-alanine, L-arabinose, cellobiose, glucose, glycerol, isoleucine, mannitol, D-mannose, sodium acetate, sodium gluconate and sucrose. The following compounds are not utilized as sole carbon and energy source: L-cysteine, lactose, sodium malate and trehalose. Ethanol, maltose, raffinose, L-rhamnose and L-sorbose are weakly

**Table 1.** Differential characteristics of strain JN33$^T$ and the type strains of *Actibacterium* species

Strains: 1, strain JN33$^T$; 2, *A. atlanticum* LMG 27158$^T$; 3, *A. mucosum* DSM 28448$^T$; 4, *A. ureilyticum* LS-811$^T$. All data was obtained from this study under identical growth conditions, except where indicated otherwise. +, Positive; −, negative; W, weakly positive; ND, no data available.

| Characteristic | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Growth in NaCl (%, w/v): | | | | |
| Range | 0.5–5.0 | 0.5–9.0* | 1.7–5.0† | 1.0–6.0‡ |
| Optimum | 3.0 | 1.0~7.0* | ND | 3.0‡ |
| Growth temperature (°C): | | | | |
| Range | 10–35 | 10–41* | 15–37† | 15–40‡ |
| Optimum | 30 | 28* | ND | 30‡ |
| Catalase | W | + | + | + |
| Nitrate reduction | − | W | + | + |
| Urease | − | + | + | + |
| Hydrolysis of: | | | | |
| Aesculin and gelatin | + | + | + | − |
| Tween 40 and Tween 80 | − | + | − | − |
| Enzyme activities: | | | | |
| *N*-Acetyl-$\beta$-glucosaminidase | − | − | − | + |
| Cystine arylamidase | − | − | + | + |
| Naphthol-AS-BI-phosphohydrolase | + | − | + | + |
| Trypsin | − | − | + | − |
| Valine arylamidase | − | − | + | + |
| Assimilation of: | | | | |
| L-Arabinose | + | − | − | − |
| Cellobiose | + | + | + | − |
| Ethanol | W | + | − | + |
| DL-Isoleucine | + | − | + | W |
| Lactose | − | − | + | − |
| Maltose | W | − | − | − |
| D-Mannitol | + | + | − | + |
| D-Mannose | + | + | + | W |
| Raffinose | W | − | − | − |
| L-Rhamnose | W | − | − | − |
| L-Sorbose | W | − | − | − |
| Sucrose | + | − | − | − |
| Trehalose | − | + | − | − |
| Sodium gluconate | + | − | − | − |
| Sodium malate | − | − | − | + |
| Acid production from: | | | | |
| Cellobiose | − | + | + | − |
| Ethanol | − | − | − | + |
| D-Galactose | − | − | + | − |
| D-Inositol | W | − | + | − |
| Lactose | − | − | + | − |
| D-Mannitol | + | W | − | + |
| D-Ribose | − | − | + | + |
| D-Sorbitol | + | − | − | + |
| D-Xylose | + | − | + | + |
| Susceptibility to (μg per disc): | | | | |
| Erythromycin (15) | + | + | + | − |
| Novobiocin (30) | + | + | − | + |
| Polymyxin B (300 IU) | + | + | − | − |
| Tobramycin (10) | + | W | − | W |
| DNA G+C content (mol%) (by genome) | 57.8 | 59.0 | 60.8 | 64.3 |

*Data were taken from Li *et al*. [2].
†Data were taken from Lucena *et al*. [1].
‡Data were taken from Lin *et al*. [3].

used. Acid is produced from glucose, D-mannitol, D-sorbitol and D-xylose, weakly produced from D-inositol and not produced from cellobiose, ethanol, D-galactose, lactose, maltose, melezitose, raffinose, L-rhamnose, ribitol, D-ribose, L-sorbose and trehalose. Acid and alkaline phosphatases, esterase (C4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase activities are present, whereas *N*-acetyl-$\beta$-glucosaminidase, cystine arylamidase, $\alpha$-chymotrypsin, $\alpha$-fucosidase, $\alpha$- and $\beta$-galactosidases, $\alpha$- and $\beta$-glucosidases, $\beta$-glucuronidase, lipase (C14), $\alpha$-mannosidase, valine arylamidase and trypsin activities are absent. The sole respiratory quinone is ubiquinone 10 (Q-10). The major fatty acids are summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and $C_{16:0}$. The major polar lipids are phosphatidylglycerol, one unidentified phospholipid and two unidentified aminolipids. In addition, moderate to minor amounts of three unidentified glycolipids, one unidentified phospholipid and eleven unidentified lipids are also present.

The type strain, JN33$^T$ (= CGMCC 1.16012$^T$= KCTC 52653$^T$ ) was isolated from seawater of the western Pacific Ocean. The DNA G+C content is 57.8 mol% (by genome).

## EMENDED DESCRIPTION OF *ACTIBACTERIUM* LUCENA *ET AL.* 2012

In addition to the characteristics reported by Lucena *et al.* [1], the following properties are observed. The G+C content of the DNA varies between 57.8 and 64.3 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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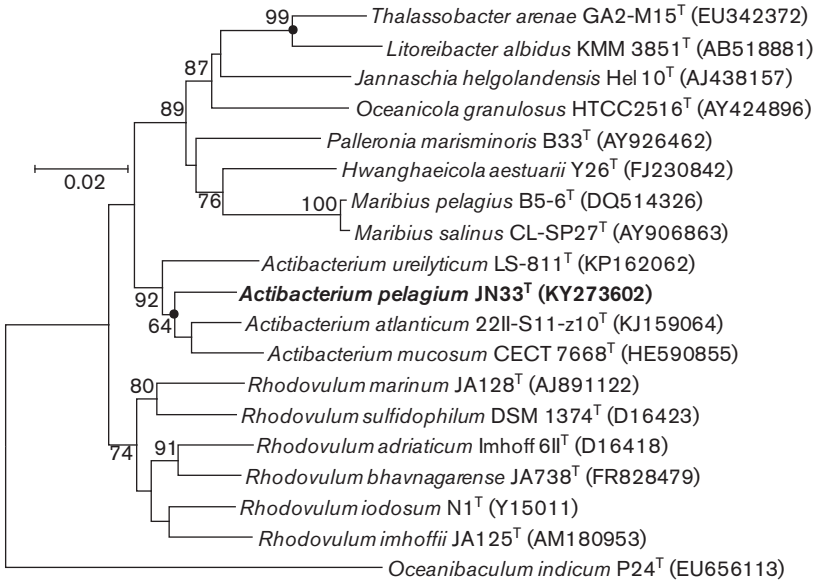


**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationships of the isolate and its related taxa. Bootstrap values (> 60 %) based on 1000 replications are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with maximum-likelihood and maximum-parsimony algorithms. Bar, 0.02 substitutions per nucleotide position.

**Table 2.** Comparison of cellular fatty acid composition of strain JN33$^T$ and the type strains of *Actibacterium* species

Strains: 1, strain JN33$^T$; 2, *A. atlanticum* LMG 27158$^T$; 3, *A. mucosum* DSM 28448$^T$; 4, *A. ureilyticum* LS-811$^T$. All data was obtained from this study. –, Not detectable; TR, trace (< 0.5 %).

| Fatty acid (%) | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Straight-chain | | | | |
| $C_{10:0}$ | – | 4.2 | – | – |
| $C_{12:0}$ | – | – | 0.9 | – |
| $C_{16:0}$ | 11.3 | 2.0 | 14.4 | 1.4 |
| $C_{17:0}$ | TR | – | – | TR |
| $C_{18:0}$ | 4.6 | 6.6 | 5.4 | 6.6 |
| Branched-chain | | | | |
| iso-$C_{18:0}$ | 5.8 | – | – | – |
| Unsaturated | | | | |
| 11-Methyl $C_{18:1}\omega7c$ | 4.8 | 6.5 | 9.1 | 1.0 |
| $C_{19:0}$ cyclo $\omega8c$ | – | 3.4 | 9.5 | 7.9 |
| $C_{20:1}\omega7c$ | 0.5 | 0.6 | 1.3 | 0.6 |
| Hydroxy | | | | |
| $C_{10:0}$ 3-OH | 4.6 | 6.4 | 7.6 | 3.9 |
| $C_{12:0}$ 3-OH | – | 2.5 | 5.7 | – |
| Summed feature* | | | | |
| 3 | 0.7 | – | – | TR |
| 8 | 66.9 | 67.8 | 46.1 | 77.8 |

*Summed features represent groups of two fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contains $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 8 contains $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.


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