Henriciella pelagia sp. nov., isolated from seawater

Yue-Hong Wu, Hong Cheng, Ying-Yi Huo, Xiong-Bin Jin, Chun-Sheng Wang and Xue-Wei Xu*

Abstract

Strain LA220T, isolated from seawater of the Eastern Pacific Ocean, was subjected to a polyphasic taxonomic study. Cells of the strain were Gram-stain-negative, aerobic, motile and short rod-shaped. On the basis of 16S rRNA gene sequence analysis, strain LA220T showed high similarity to Henriciella litoralis SD10T (98.5%), Henriciella marina DSM 19595T (98.3%) and Henriciella aquimarina P38T (97.5%), and exhibited less than 97.0% 16S rRNA gene sequence similarity with respect to the type strains of other Hyphomonadaceae species. Phylogenetic analyses revealed that strain LA220T fell within the cluster of the genus Henriciella. The average nucleotide identity and in silico DNA–DNA hybridization values between strain LA220T and the type strains of Henriciella species were 74.8–76.8 and 18.4–20.8%, respectively. The sole respiratory quinone was ubiquinone-10 (Q-10). The principal fatty acids were summed feature 8 (C18:1ω7c and/or C18:1ω6c) and C16:0. The major polar lipids were three unidentified glycolipids. The DNA G+C content was 59.9 mol%. Phylogenetic distinctiveness, chemotaxonomic differences and phenotypic properties revealed that strain LA220T could be differentiated from recognized Henriciella species. Therefore, strain LA220T is considered to represent a novel species of the genus Henriciella, for which the name Henriciella pelagia sp. nov. (type strain LA220T=CGMCC 1.15928T=KCTC 52577T) is proposed.

The genus Henriciella, belonging to the family Hyphomonadaceae in the class Alphaproteobacteria, was proposed by Quan et al. [1], and the description was emended by Lee et al. [2]. Currently, the genus Henriciella consists of three species with validly published names, Henriciella marina (type species), Henriciella litoralis and Henriciella aquimarina. The natural habitats from which Henriciella species have been isolated are marine environments, including coastal seawater, tidal flat and deep seawater [1–3]. Members of the genus Henriciella are Gram-negative, aerobic and rod-shaped, and possess ubiquinone-10 (Q-10) as the major respiratory quinone. In this study, we present a polyphasic study describing a novel strain isolated from seawater of the Eastern Pacific Ocean.

Strain LA220T was isolated from seawater collected at a depth of 200 m in the Eastern Pacific Ocean (146°59.2867′ E 08°28.5584′ N). The seawater sample was collected by a rosette sampler connected to a CTD (conductivity, temperature and depth) system (SBE911 plus; Sea-Bird Electronics) from the vessel HAI YANG LIU HAO. Aboard the ship, the seawater sample was immediately subjected to culturing. Natural seawater agar (pH 7.2–7.4) supplemented with 0.05% (w/v) peptone (BD Difco) and 0.01% (w/v) yeast extract (BD Difco) was used for isolation. The seawater sample was diluted using the standard ten-fold dilution plating technique and spread on natural seawater agar. After 10 days of aerobic incubation at 30°C, one cream-coloured colony, designated as LA220T, was picked and purified by repeated restreaking. Purity was confirmed by the uniformity of cell morphology. The reference strains H. litoralis DSM 22014T and H. marina DSM 19595T were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) and H. aquimarina LMG 24711T was obtained from the LMG (Collection of the Laboratorium voor Microbiologie en Microbiele Genetica). Unless otherwise stated, strain LA220T and the reference strains were routinely cultured in marine broth 2216 (MB; BD Difco) or on marine agar 2216 (MA; BD Difco) at 30°C and maintained at −80°C with 30% (v/v) glycerol.

Cell morphology and motility were examined using confocal laser scanning microscopy (TCS SP5; Leica) and transmission electron microscopy (JEM-1230; JEOL). The hanging drop method was used for motility testing. The temperature range for growth was determined by incubating at 4, 10, 15, 20, 25, 28, 30, 34, 37, 40, 45 and 50°C. The pH range for growth was determined in MB that was adjusted to pH 5.0–4.0.

Author affiliation: Key Laboratory of Marine Ecosystem and Biogeochemistry, Second Institute of Oceanography, State Oceanic Administration, Hangzhou 310012, PR China.

*Correspondence: Xue-Wei Xu, xuxw@sio.org.cn

Keywords: Henriciella; Hyphomonadaceae; Alphaproteobacteria; phylogenetic analysis; whole genome sequencing.

Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LA220T is KX881660. Those for the whole genome sequences of strain LA220T, H. litoralis DSM 22014T and H. aquimarina LMG 24711T are NC5U00000000, NC5S00000000 and NCST00000000, respectively.

Four supplementary figures and two supplementary tables are available on the online Supplementary Material.

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10.5 (in 0.5 pH unit intervals) using appropriate biological buffers (MES for pH 5.0–6.0, PIPES for pH 6.5–7.0, Tricine for pH 7.5–8.5 and CAPSO for pH 9.0–10.5) at a concentration of 50 mM. Evaluation of the pH values after autoclaving revealed only minor changes. The optimal conditions with NaCl for growth were investigated by using NaCl-free MB (prepared according to the MB formula, but without NaCl) with different NaCl concentrations (0, 0.5, 1.0, 2.0, 3.0, 5.0, 7.5, 10.0, 12.0 and 15.0 %, w/v). Sea salts requirement for growth was determined by incubating in the following medium: 5.0 g peptone, 1.0 g yeast extract and 1 litre distilled water, pH 7.6, supplemented with various concentrations of sea salts (Sigma) (0, 0.5, 1.0, 2.0, 3.0, 4.0, 4.5 and 5.0 %) as final concentration, w/v). Growth was monitored by measuring OD590 in a UV/visible spectrophotometer (Ultrospec 6300 pro; Amersham Biosciences). Upper and lower limits for growth were confirmed when no growth was observed after the inoculated cultures were incubated for 30 days. Anaerobic growth was carried out with AnaeroPack (Mitsubishi) using sodium nitrate or sodium nitrite as a potential electron acceptor.

The Gram reaction, oxidase and catalase activities, and hydrolysis of starch and Tweens 20, 40 and 80 were tested according to Dong and Cai [4]. The utilization of carbohydrates as sole carbon and energy sources was determined in BM medium [5]. The corresponding filter-sterilized complex nutrients (yeast extract, peptone or tryptone, 0.2 %, w/v), sugars (0.2 %, w/v), alcohols (0.2 %, w/v), organic acids (0.1 %, w/v) or amino acids (0.1 %, w/v) were added to the medium. Acid production was tested using marine oxidation-fermentation (MOF) medium supplemented with 1 % sugars [6]. API ZYM and API 20NE tests (bioMérieux) were used to determine additional physiological and biochemical characteristics according to the manufacturer’s instructions. Strips were inoculated with a heavy bacterial suspension (MacFarland 5 standard) in AUX medium supplemented with 2 % (w/v) sea salts (Sigma) [7]. The three reference strains, _H. litoralis_ DSM 22014T, _H. marina_ DSM 19595T and _H. aquimarina_ LMG 24711T, were used as controls in the above tests.

The cellular fatty acids of strain LA220T and the reference strains were determined under identical conditions in parallel. The quadrant streak method was used to dilute the inoculum so that quadrant 3 exhibited confluent growth (at the late exponential phase) along the streaking axis. Cellular fatty acid methyl esters were obtained from cells grown on MA at 30 °C for 4 days from quadrant 3 and analysed according to the instructions of the Microbial Identification System (MIDI). Isoprenoid quinones were extracted from freeze-dried cells (200 mg) with chloroform/methanol (2:1) and analysed by LC-MS (Agilent) [8]. Total lipids were extracted and identified by two-dimensional TLC [9]. Molybdophosphoric acid was used for the detection of all lipids, ninhydrin reagent for lipids containing free amino lipids, ammonium molybdate reagent for phosphorus-containing lipids and α-naphthol reagent for glycolipids.

High-quality genomic DNA was obtained via an AxyPrep Bacterial Genomic DNA Miniprep Kit (Axygen Corning) according to the recommended protocol. The 16S rRNA gene was amplified and analysed as described previously [10]. The genomes of strain LA220T, _H. litoralis_ DSM 22014T and _H. aquimarina_ LMG 24711T were sequenced by Solexa paired-end sequencing technology with a HiSeqXTen platform (Anoroad Gene Technology). One paired-end library was constructed with 500 bp insert size. The sequencing generated approximately 1 Gb of clean data, representing approximately 200-fold genome coverage. Reads were de novo assembled into contigs and subsequently joined into scaffolds using SOAPdenovo (version 2.0.1) [11]. Completeness of the genome sequence was addressed using the bioinformatic tool CheckM (http://ecogenomics.github.io/CheckM/) [12]. The complete sequence of the 16S rRNA gene was annotated via the RNAmer 1.2 Server [13], and was compared with related sequences of reference organisms by the EzTaxon-e service [14]. The genome of _H. marina_ DSM 19595T (AQXT00000000) was retrieved from the GenBank database. The average nucleotide identity (ANI) between two genomes was calculated using the algorithm [15] of the EzGenome web service and the Orthologous Average Nucleotide Identity Tool (OAT software) [16]. The _in silico_ DNA–DNA hybridization (DDH) values was calculated by GGDC [17].

Phylogenetic analysis was performed in _ARB_ release 6.0.2 [18] in the All-Species Living Tree Project database (LTPs123, September 2015) [19]. Based on the obtained phylogenetic resolution and the EzTaxon-e results, further trees were reconstructed by using the _MEGA_ 5 program package [20]. Sequence data were aligned with _CLUSTAL W_ [21]. Phylogenetic trees were reconstructed by the neighbour-joining [22], maximum-parsimony [23] and maximum-likelihood [24] methods with the _MEGA_ 5 program package. Evolutionary distances were calculated according to the algorithm of the Kimura two-parameter model [25] for the neighbour-joining method.

Cells of strain LA220T were Gram-stain-negative, motile, short rod-shaped, 0.5–0.8 µm in width and 0.8–1.5 µm in length (Fig. S1, available in the online Supplementary Material). Colonies were cream-coloured, circular, convex, smooth and 1–2 mm in diameter after 1 day of incubation at 30 °C on MA. Optimal growth occurred at 30–37 °C and pH 6.5. Sea salts were required for growth. Strain LA220T was positive for catalase, oxidase and hydrolysis of aesculin and Tweens 20, 40 and 80. Detailed phenotypic characteristics are given in the species description and Table 1.

The complete 16S rRNA gene sequence of strain LA220T (1442 nt) was obtained. According to the EzTaxon service as well as the _CLUSTAL W_ results, 16S rRNA gene sequence comparisons with _reference_ bacteria with validly published names indicated that strain LA220T shared highest 16S rRNA gene sequence similarity with _H. litoralis_ SD10T (98.5 %), _H. marina_ DSM 19595T (98.3 %) and _H. aquimarina_ P38T (97.5 %) and exhibited less than 97.0 % 16S rRNA
Table 1. Differential phenotypic characteristics between strain LA220\textsuperscript{T} and its most closely related species

Strains: 1, LA220\textsuperscript{T}; 2, H. litoralis DSM 22014\textsuperscript{T}; 3, H. marina DSM 19595\textsuperscript{T}; 4, H. aquimarina LMG 24711\textsuperscript{T}. All data were obtained from this study, except where indicated. +, Positive; –, negative; w, weakly positive.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>Colony colour</td>
<td>Cream</td>
<td>Yellow</td>
<td>Cream</td>
<td>Cream</td>
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<td>Growth in NaCl (% w/v):</td>
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<tr>
<td>Range</td>
<td>0.5–12</td>
<td>1.0–10*</td>
<td>1.0–15†</td>
<td>2.0–12†</td>
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<tr>
<td>Optimum</td>
<td>1.0</td>
<td>2.0–5.0*</td>
<td>1.0–2.0†</td>
<td>3.0–10†</td>
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<td>Growth temperature (°C):</td>
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<tr>
<td>Range</td>
<td>15–40</td>
<td>15–40*</td>
<td>10–37†</td>
<td>10–42†</td>
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<tr>
<td>Optimum</td>
<td>37</td>
<td>25–30*</td>
<td>20†</td>
<td>25–37†</td>
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<tr>
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<tr>
<td>Aesculin</td>
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<td>+</td>
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<td>Tween 80</td>
<td>+</td>
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<td>Enzyme activities:</td>
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<tr>
<td>Esterase (C4)</td>
<td>+</td>
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<td>α-Glucosidase</td>
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<tr>
<td>β-Glucosidase</td>
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<td>Utilization of:</td>
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<tr>
<td>L-Arabino</td>
<td>–</td>
<td>+</td>
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<td>L-Aspartic acid</td>
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<td>D-Galactose</td>
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<td>–</td>
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<td>L-Glucamic acid</td>
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<td>w</td>
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<td>Succinic acid</td>
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<td>w</td>
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<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Ribose</td>
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<td>–</td>
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<tr>
<td>Sucrose</td>
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<tr>
<td>G+C content (mol%)</td>
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<tr>
<td>(by genome)</td>
<td>59.9</td>
<td>58.9</td>
<td>59.9§</td>
<td>62.2</td>
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</table>

*Data were taken from Lee et al. [2].
†Data were taken from Quan et al. [1].
‡Data were taken from Lai et al. [3].
§Data were obtained from the GenBank database (AQXT00000000).

The quality of the microbial genomes was assessed by the bioinformatic tool CheckM. The results indicated that the genome completeness of strain LA220\textsuperscript{T}, H. litoralis DSM 22014\textsuperscript{T} and H. aquimarina LMG 24711\textsuperscript{T} was 99.4, 99.4 and 99.2 %, with a contamination percentage of 0.5, 0.1 and 1.1 %, respectively. Genome sequences estimated to be ≥95 % complete, with ≤5 % contamination, are considered as excellent reference genomes for deeper analyses [12]. The DNA G+C content of strain LA220\textsuperscript{T} was 59.9 mol%, a value in the range reported for members of the genus Henriciella, i.e. 55.2–61 mol% [1–3]. The ANI values between strain LA220\textsuperscript{T} and the reference strains were 74.8–76.8 % (Table S1). The ANI values were far below the threshold of the species boundary (94–96 %) [26], indicating a low taxonomic relatedness between strain LA220\textsuperscript{T} and the reference type strains. The recommended results (formula 2) of the in silico DDH calculations revealed that strain LA220\textsuperscript{T} and the reference strains shared 19.5–20.8 % DNA relatedness (Table S2). The values were below the 70 % recommended for strains to be assigned to different genomic species [27].

The chemotaxonomic data support the results of the phylogenetic analysis. The sole respiratory quinone found in strain LA220\textsuperscript{T} was Q-10, in line with all members of the genus Henriciella [1–3]. Fatty acid analysis revealed strain LA220\textsuperscript{T} contained high proportions of summed feature 8 (C\textsubscript{18:1}ω7c and/or C\textsubscript{18:1}ω6c) and C\textsubscript{16:0} (40.8 and 28.3 %, respectively), which was similar to the reference strains (33.6–50.5 and 20.4–30.8 %, respectively; Table 2). Strain LA220\textsuperscript{T} possessed glucuronopranoseyl diglyceride (GUDG) and monoglycosyldiglyceride (MGDG) as the major compounds, which were similar with respect to the reference strains (Fig. S4 and Quan et al. [1]). In addition, strain LA220\textsuperscript{T} possessed phosphatidylglycerol (PG) and one unidentified glycolipid (GL7), which were found in all three reference strains (Fig. S4 and Quan et al. [1]).

The chemotaxonomic data also revealed some clear differences in the fatty acid composition and polar lipid profiles among strain LA220\textsuperscript{T} and its closest relatives. The percentage of C\textsubscript{16:1}ω5c of strain LA220\textsuperscript{T} (4.5 %) was higher than that of the reference strains (2.3–2.8 %). In addition, strain LA220\textsuperscript{T} contained C\textsubscript{20:4}ω6,9,12,15c (1.6 %), which was detected as a trace (<0.5 %) component in H. litoralis DSM 22014\textsuperscript{T} and H. marina DSM 19595\textsuperscript{T}, but not detected in H. aquimarina LMG 24711\textsuperscript{T} (Table 2). One unidentified glycolipid (GL5) and one unidentified phospholipid (PL2) were present in strain LA220\textsuperscript{T} at moderate or minor amounts, but were not found in the reference strains (Fig. S4 and Quan et al. [1]). In addition, one unidentified lipid (L3) was found in the reference strains (Fig. S4 and Quan et al. [1]) at moderate or minor amounts, but was not detected in strain LA220\textsuperscript{T}.

In conclusion, 16S rRNA gene sequence analyses and chemotaxonomic characterizations supported the placement of strain LA220\textsuperscript{T} within the genus Henriciella. However, strain LA220\textsuperscript{T} could also be distinguished from the type strains of closely related species based on phenotypic differences such as maximum-likelihood and maximum-parsimony phylogenetic trees indicated that strain LA220\textsuperscript{T} formed a cluster with the genus Henriciella (Figs S2 and S3). Phylogenetic analysis thus indicated that strain LA220\textsuperscript{T} may represent a novel member of the genus Henriciella.
as colour, NaCl range and optimum, temperature range and optimum, hydrolysis of aesculin and Tween 80, enzyme activities, carbohydrate utilization and acid production (Table 1). On the basis of the phylogenetic analysis, genomic data and chemotaxonomic results, as well as phenotypic characteristics, strain LA220T represents a novel species of the genus Henriciella, for which the name Henriciella pelagia sp. nov. is proposed.

**DESCRIPTION OF HENRICIELLA PELAGIA SP. NOV.**

*Henriciella pelagia* (pe.la'gi.a. L. fem. adj. pelagia of or belonging to the sea).

Cells are Gram-stain-negative, motile, short rod-shaped, 0.5–0.8 µm in width and 0.8–1.5 µm in length. Colonies are cream-coloured, circular, convex, smooth and 1–2 mm in diameter after 3 days of incubation at 30 °C on MA. Grows in NaCl-free MB supplemented with 0.5–12.0 % (w/v) NaCl (optimum 1.0 %). The pH and temperature ranges for growth are pH 6.0–9.0 and 15–40 °C (optimum at pH 6.5 and 37 °C). Requires sea salts for growth. No anaerobic growth occurs on MA supplemented with sodium nitrate or sodium nitrite. Positive for catalase and oxidase. Negative for arginine dihydrolase, β-galactosidase, glucose fermentation, nitrate reduction and urease. Tweens 20, 40 and 80 and aesculin are hydrolysed. Gelatin and starch are not hydrolysed. According to the API ZYM system, acid phosphatase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase activities are present. The following compounds are utilized as sole carbon and energy source: adipic acid, L-aspartic acid, capric acid, D-glucose, L-glutamic acid, malate, malonate, peptone, phenylacetic acid, succinic acid, tryptone and yeast extract. The following compounds are not utilized as sole carbon and energy source: N-acetyl-glucosamine, L-alanine, L-arabinose, D-fructose, D-galactose, glycerol, glycin, D-myo-inositol, L-ribose, D-ribose, D-mannitol, D-mannose, melezitose, raffinose, sodium gluconate, trehalose and trisodium citrate. Acid is produced from maltose and D-ribose. Acid is not produced from cellobiose, ethanol, D-fructose, D-galactose, D-glucose, D-myo-inositol, α-D-lactose, D-mannitol, D-mannose, melezitose, ribitol, L-rhamnose, raffinose, D-sorbitol, sucrose, L-sorbose, trehalose or D-xylene. The principal fatty acids (>10 %) are summed feature 8 (C18:1ω7c and/or C18:1ω6c) and C16:0. The sole respiratory quinone is Q-10. The sole respiratory quinone is Q-10.
major polar lipids are glucuronopyranosylglyceride, monoglcosyldiglyceride and one unidentified glycolipid. In addition, moderate to minor amounts of phosphatidylethanolamine, phosphatidylglycol, six unidentified glycolipids, two unidentified phospholipids and two unidentified lipids are also present.

The type strain, LA220\textsuperscript{T} (=CGMCC 1.15928\textsuperscript{T}=KCTC 52577\textsuperscript{T}), was isolated from seawater. The DNA G+C content of the type strain is 59.9 mol\% (by genome).

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

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