Paracandidimonas lactea sp. nov., a urea-utilizing bacterium isolated from landfill

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Abstract

A urea-utilizing bacterium, designated Q2-2 T, was isolated from landfill. Cells of strain Q2-2 T were Gram stain-negative, aerobic, short-rod bacteria. Strain Q2-2 T was observed to grow at a temperature range of 15–37 °C (optimum 30 °C), a pH range of 5.5–9.5 (optimum pH 8.0) and 0–4% (w/v) NaCl (optimum 1%). The major respiratory quinone was Q-8, and the major polar lipids were diphosphatidyl glycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, and phosphatidyl glycerol. Based on the 16S rRNA gene sequence, strain Q2-2 T had the highest similarity with Paracandidimonas caeni 24 T (98.0%), followed by Pusillimonas soli MJ07 T (97.5%), Parapusillimonas granuli Ch07 T (97.2%), Pusillimonas ginsengisoli DCY25 T (97.1%) and Paracandidimonas soli IMT-305 T (96.4%). The ANI values between strain Q2-2 T and the above related type strains were 71.02%, 73.52%, 74.32%, 74.59% and 72.29%, respectively. The DNA G+C content of strain Q2-2 T was 61.1%. Therefore, strain Q2-2 T represents a novel species of the genus Paracandidimonas, for which the name Paracandidimonas lactea sp. nov. (type strain Q2-2 T = CGMCC 1.19179 T = JCM 34906 T) is proposed.

Keywords Paracandidimonas lactea · Novel species · Polyphasic taxonomy · Urea-utilizing bacterium

Abbreviations
ANI Average nucleotide identity
DPG Diphosphatidyl glycerol
PE Phosphatidylethanolamine
PME Phosphatidylmethylethanolamine
PG Phosphatidyl glycerol

Introduction

Paracandidimonas is one of the members of the order Burkholderiales and the family Alcaligenaceae. At present, there are only two species reported in the genus of Paracandidimonas, including Paracandidimonas soli IMT-305 T from soil in Alabama (Kämpfer et al. 2017) and Paracandidimonas caeni 24 T from the sludge from a pesticide manufacturing plant (Li et al. 2018). Both species have not been reported as having urease activity.

A bacterial strain utilizing urea, named Q2-2 T, was recently isolated from landfill, which appears to have different properties from the known species of Paracandidimonas. Therefore, this strain was identified by polyphasic taxonomic approaches. Based on the results, it is proposed herein that strain Q2-2 T represents a novel species of the genus Paracandidimonas.

Materials and methods

Isolation and culture conditions

Soils were sampled from a landfill site in Hangzhou, Zhejiang Province. The soils (10 g) were diluted with sterile water, and then placed in a shaker at 30 °C and 200 rpm for 2 d. After mixing, the samples were spread on R2A plates and incubated at 30 °C for 2 d. A translucent milky white colony was isolated and purified which was named Q2-2 T. To preserve it for the subsequent identification experiments,
the culture was maintained as a glycerol suspension (25%, w/v) at −80 °C. The data for the cited reference strains are from the original species descriptions.

**Phenotypic characteristic analysis**

The colony morphology of strain Q2-2ᵀ was identified during the logarithmic growth phase. The morphologies, flagella and lengths of cells were observed by scanning electron microscopy (Elizabeth et al. 2012). The strain was inoculated in R2A medium and incubated in a 200 rpm shaker at 30 °C for 2 d to prepare seed liquid. Semi-solid culture tubes (0.5% agar) were prepared, with a small amount of fresh seed liquid dipped in the inoculation ring, and vertically inserted into the center of the semi-solid tubes (about 3 cm) (Shen et al. 2018). The tubes were then placed in an incubator for static culturing, and the bacterial motility was observed after 2–3 d. Gram stain testing was carried out according to kit instructions (Qingdao Hopebio Biotechnology Co., LTD.). Growth tests were performed at different temperatures (4, 10, 15, 20, 25, 30, 35, 37, 40, 42, 45 °C), pH ranges (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0) (Gomorie 1955) and NaCl concentrations (0–6%, W/V, 0.5% intervals). The pH was adjusted using the following buffers: MES (5.0–5.5), MOPS (6.0–7.5), Tricine buffer (8.0–8.5), and CAPSO (9.0–10.0). The OD₆₀₀ₙₘ was measured with a UV–Vis spectrophotometer (UV756CRT, Yoke Instruments, Shanghai) to determine the optimum growth.

Catalase activity was determined using the 3% (v/v) H₂O₂ foam production method, and oxidase activity was determined with 1% (w/v) tetramethyl-p-phenylenediamine (Cowan et al. 1966). Single carbon source assimilation tests were performed using Biolog GNIII (Jun-Lian et al. 2016). Other physiological and biochemical tests were performed using API ZYM, API 20NE, and API 50CH strips, which were, respectively, observed after 4 h, 20 h, 24 h and 48 h of incubation at 37 °C, as per the manufacturer’s instructions.

**Chemotaxonomic analysis**

Cells were cultured in R2A liquid medium at 30 °C for 2 d until the exponential stage, then centrifuged and the residue was freeze-dried. Fatty acid methyl esters (FAMEs) were extracted and analysed by Kuykendall’s method (Kuykendall et al. 1988), and validated by the Sherlock Microbial Identification System (MIDI version 5.0) (Su et al. 2016). The identities of the FAMEs were verified through capillary GLC using an HP 6890 gas chromatograph (Hewlett Packard, USA) and compared analyse against the TSBA60 database (Sasser 1990). Chloroform/methanol (2:1; v/v) was utilized to extract the isoprenoid quinones. Polar lipids were extracted with chloroform/methanol (1:2; v/v), then identified by two-dimensional TLC on silica gel using 60 F254 (Merck) plates (10 cm × 10 cm) as described (Tindall 1990), the developing solvents were chloroform/methanol/water (65:25:3.8, v/v) and chloroform/methanol/acetic acid/water (40:7.5:6:1.8, v/v). For visualization and detection of total polar lipids, aminolipids, phospholipids and glycolipids, the TLC plates were sprayed with molybdophosphoric acid, ninhydrin reagent, molybdenum blue and a-naphthol/H₂SO₄ reagent, respectively.

**Genomic characteristics and phylogenetic analysis**

The bacterial universal primer pair 27F/1492R was used for the amplification of the 16S rRNA gene of the genome, according to the established procedure (Weisburg et al. 1991). The PCR product was sequenced by Sangon Biotech (Shanghai) Co., Ltd, and the resulting sequence was compared with the closely related sequence of the reference organism using the EzBioCloud server (http://www.ezbiocloud.net) (Yoon et al. 2017). Phylogenetic trees were constructed using the calculation formulas of the neighbour-joining (Saitou and Nei 1987), least squares (Fitch and Margoliash 1967), maximum likelihood (Joseph and Felsenstein 1981) and minimum-evolution (Fitch 1971) methods with the MEGA X program package (Sudhir et al. 2018). The G+C content of strain Q2-2ᵀ was analysed with the RAST server based on the complete genome sequence (Aziz et al. 2008). The ANI values were computed through the Orthologous Average Nucleotide Identity Tool (OAT) (Chun et al. 2015), and the digital DNA-DNA hybridization (dDDH) values were calculated by Genome-to-Genome Distance Calculator (http://ggdc.dsmz.de/) (Meier-Kolthoff et al. 2013). To support and demonstrate the 16S rRNA phylogenetic trees of strain Q2-2ᵀ, multi-locus sequence analysis (MLSA) based on housekeeping genes (gyrB, hisS, recA, rpoB) was performed with related strains (Glaeser and Kämpfer 2015).

**Results and discussion**

**Morphology, physiology and biochemical analysis**

After growth on R2A plates at 30 °C for 48 h, the colonies were 1.0–2.0 mm in diameter with smooth surface, circular, milky white and convex. Cells of strain Q2-2ᵀ were Gram negative, aerobic, immobile, short rods of 0.2–0.36 µm width and 0.9–1.8 µm length with no flagella (Fig. S1). The diverse characteristics between strain Q2-2ᵀ and related type strains are shown in Table 1. All negative traits from commercial kits are listed in the Table S1, and the utilization of carbon sources (Biolog GNIII) are listed in the Table S2.
Phylogenetic and genome sequence analysis

The length of the sequenced 16S rRNA gene of strain Q2-2<sup>T</sup> was 1443 bp. Based on the 16S rRNA gene sequence identity calculations on the EzBioCloud server, strain Q2-2<sup>T</sup> showed the highest similarity with Paracandidimonas caeni<sup>24 T</sup> (98.0%), followed by Pusillimonas soli<sup>MJ07 T</sup> (97.5%), Parapusillimonas granuli<sup>Ch07 T</sup> (97.2%), Paracandidimonas soli<sup>IMT-305 T</sup> (96.4%). The phylogenetic trees showed that strain Q2-2<sup>T</sup> clustered with the genus Paracandidimonas, and formed a lineage separated from the other related species (Fig. 1). The phylogenetic analysis based on housekeeping genes (gyrB, hisS, recA, rpoB) presented the same result (Fig. 2). The DNA G+C content of strain Q2-2<sup>T</sup> was 61.1 mol%.

The ANI values between strain Q2-2<sup>T</sup> and Paracandidimonas caeni<sup>24 T</sup>, Pusillimonas soli<sup>MJ07 T</sup>, Parapusillimonas granuli<sup>Ch07 T</sup>, Pusillimonas ginsengisoli<sup>DC25 T</sup> and Paracandidimonas soli<sup>IMT-305 T</sup> were 71.02%, 73.52%, 74.32%, 74.59%, and 72.29%, respectively. The dDDH values between strain Q2-2<sup>T</sup> and the above strains were 19.70%, 20.60%, 21.20%, 20.80%, and 20.70%, respectively. The genome of strain Q2-2<sup>T</sup> contains 4004 protein-coding genes as well as 976 hypothetical proteins, which account for 23.38% of the coding genes. Through gene annotation, it is concluded that strain Q2-2<sup>T</sup> has multiple urease-related subunits and accessory protein genes such as ureD, ureE and ureF, which support the function of degrading urea using urease.

Chemotaxonomic characterization

The cellular fatty acid profiles of strain Q2-2<sup>T</sup> are shown in Table S3. There were some comparable qualitative and
The major fatty acids of strain Q2-2 T were C16:0 (33.95%), C17:0 cyclo (11.42%) and C18:0 (0.83%), which were the same as those of *Paracandidimonas caeni* 24 T. Compared with *Paracandidimonas soli* IMT-305 T, the main unsaturated fatty acids of strain Q2-2 T had high consistency. The major fatty acid C14:0 was found in strain Q2-2 T, but not in the related strains with the exception of *Pusillimonas ginsengisoli* DCY25 T. The respiratory quinone of strain Q2-2 T was Q-8. The major polar lipids of strain Q2-2 T were DPG, PE, PME and PG, which were similar to those of the reference strains. According to the polyphasic evidence described above, it is concluded that strain Q2-2 T represents a novel species of the genus *Paracandidimonas* in the family *Alcaligenaceae*, for which the name *Paracandidimonas lactea* sp. nov. is proposed.

**Description of Paracandidimonas lactea** sp. nov.

*Paracandidimonas lactea* (lac.te’a. L. fem. adj. lactea, milk-coloured, milky).

The strain grows at a temperature range of 15–37 °C with the optimum temperature of 30 °C and at a pH range of 5.5–9.5 with the optimum pH of 8.0. The NaCl range was 0–4% (W/V), and the optimum tolerance was 1% NaCl (w/v). It is positive for esterase (C4), lipid esterase (C8), leucine aramid, valine aramidase, acid phosphatase, naphthol AS-BI-phosphohydrolase, nitrate reduction, arginine dihydrolase, urease and aescin hydrolysis. It utilizes various carbon sources, such as Tween 40, Tween 80, d-xylose, l-asparagine, l-phenylalanine, d-galacturonic acid, l-serine, d-glucosaminic acid, Patrescine. The characteristic respiratory quinone is Ubiquinone Q-8. The major fatty acids were C16:0, C17:0 cyclo, C18:1 ω7c, Summed feature 2 (iso-C16:1 ω3-OH and/or C12:0 3-OH) and/or C12:0 3-OH) and/or C12:0 aldehyde) and Summed feature 3 (C16:1 9c/16:1 9c). The major polar lipids were DPG, PE, PME, and PG. The DNA G + C content of the strain Q2-2 T was 61.1 mol%.

The type strain was Q2-2 T (= CGMCC 1.19179 T = JCM 34906 T), isolated from landfill soil in Hangzhou, China.

The GenBank/EMBL/DDBJ accession numbers of the 16S rRNA gene sequence and complete genome sequence of strain Q2-2 T are MZ520859 and JAJJOZ000000000, respectively.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00203-022-03190-4.

**Author contributions** X-fH and CS: designed the research and project outline. CZ, QP, J-jY, H-tW, YC and G-hZ: performed isolation, deposition and identification. CZ, C-zL, B-jW, Y-hZ and OL: performed fatty acids analysis. All authors read and approved the final manuscript.

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**Declarations**

**Conflict of interest** The authors declare that there are no conflicts of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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