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

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Research Article

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Abstract

A urea-utilizing bacterium, designated Q2-2^T, was isolated from landfill. Cells of strain Q2-2^T were Gramstain-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 detected was Q-8, and the polar lipids identified were mainly 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 (97.99%), *Pusillimonas soli* MJ07^T (97.54%), *Parapusillimonas granuli* Ch07^T (97.27%), *Pusillimonas ginsengisoli* DCY25^T (97.09%) and *Paracandidimonas soli* IMT-305^T (96.39%). 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 lacteus* sp. nov. (type strain Q2-2^T = CGMCC 1.19179^T = JCM 34906^T) is proposed.

Introduction

Paracandidimonas, one of the members of *Burkholderiales* order and family *Alcaligenaceae*, is mainly isolated from soil and is a genus that has been published in recent years. At present, there are only two species reported in the genus of *Paracandidimonas*, including *Paracandidimonas soli* IMT-305^T from soil in Alabama (Kämpfer P et.al. 2017) and *Paracandidimonas caeni* 24^T from the sludge from a pesticide manufacturing plant (Yao L et.al. 2019). 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, strain Q2-2^T 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 days. After completely mixing the samples, they were spread on R2A plates and incubated at 30°C for 2 days. A translucent milky white colony was isolated and purified which was named Q2-2^T. In order 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 reference strains, which was cited, are from the related original research articles.

Phenotypic characteristic analysis

The colony morphology of strain Q2-2^T was identified during the logarithmic growth phase. The morphology, flagella and length of cells were observed by scanning electron microscopy (Fischer E R et al. 2012). Strain Q2-2^T was inoculated in R2A medium and incubated in a 200 rpm shaker at 30°C for 2 days 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 X et al. 2018). The tubes were

placed in an incubator for static culturing for 2-3 days, and then the bacterial motility was observed. Gram stain testing was carried out according to kit instructions (Qingdao Hopebio Biotechnology Co.,LTD.)(Claus D 1992). 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 G 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 OD_{600 nm} was measured with a UV-visible spectrophotometer (UV756CRT, Yoke Instruments, Shanghai) to determine the optimum growth.

Catalase activity was determined by the 3% (v/v) H₂O₂ foam production method, and oxidase activity was determined using 1% (w/v) tetramethyl-p-phenylenediamine (Cowan S T *et al.* 1966). Single carbon source assimilation tests were performed using Biolog GNIII (Gao JL *et al.* 2016). Other physiological and biochemical tests were performed using API ZYM, API 20NE, and API 50CH strips, which were respectively observed after 4h, 20h, 24h and 48h of incubation at 37°C, as the per manufacturer's instructions.

Chemotaxonomic analysis

Cells were cultured in R2A liquid medium at 30°C for 2 days 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 L D *et al.* 1988), and validated by the Sherlock Microbial Identification System (MIDI version 5.0) (Su Y *et al.* 2016). The identities of the FAMES were verified by capillary GLC, the TSBA60 database (Sasser M 1990) and a 6890 gas chromatograph (Hewlett Packard, USA), Chloroform/methanol (2:1, v/v) was utilized to extract the isoprenoid quinones, which were inside the cells. Polar lipids were extracted with chloroform/methanol (1:2; v/v), then identified by two-dimensional TLC on silica gel 60 F254 (Merck) plates (10cm × 10cm) as described by Tindall (Tindall B J 1990) using chloroform/methanol/water (65:25:3.8, v/v) and chloroform/methanol/acetic acid/water (40:7.5:6:1.8, v/v) as the developing solvents.. 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 27F/1492R (Lane D J 1985) was used for the amplification of the 16S rRNA gene of the genome, according to the established procedure. The PCR protocol consisted of an initial denaturation period of 4 min at 94°C, then 30 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C and a final extension period of 3 min at 72°C. The amplified 16S rRNA gene PCR product was then sequenced by Sangon Biotech (Shanghai) Co., Ltd, and the resulting 16S rRNA gene sequence was compared with the closely related sequence of the reference organism, using the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST/>) to sequence the 16S rRNA gene sequence of strain Q2-2^T with the comparable 16S rRNA gene sequence in the GenBank/EMBL/DDBJ databases (Shiratori H *et al.* 2009). A phylogenetic tree was constructed (Felsenstein J 1989) using the calculation formulas of the Neighbour-joining (Saitou N and Nei M 1987), least squares (Fitch W M and Margoliash E 1967), Maximum Likelihood (Felsenstein J 1981) and Minimum-Evolution (Fitch W M 1971) methods with the MEGA X program package (Kumar S *et al.* 2018). The G + C content of strain Q2-2^T was analyzed with the RAST server based on the complete genome sequence (Aziz RK *et al.* 2008). The ANI values were computed through the Orthologous Average Nucleotide Identity Tool (OAT) (Chun J *et al.* 2015).

Results And Discussion

Strain Q2-2^T grew on the R2A medium. After growth on R2A plates at 30°C for 48h, the colonies were 1.0-2.0 mm in diameter with smooth surface, circular, milky white and convex. Cells of strain Q2-2^T were Gramstain-negative, aerobic, immobile, with no flagella, and short rods of 0.2-0.36 µm width and 0.9-1.8 µm length (Fig. 1). Other physiological and biochemical characteristics of strain Q2-2^T are included in the species description and in Tables 1 and 2.

Table 1
Total fatty acid contents in Q2-2^T

Fatty acids	Proportion
C _{12:0}	0.17
C _{14:0}	4.38
C _{15:0}	/
C_{16:0}	33.95
C _{17:0}	0.62
C_{17:0} cyclo	11.42
C _{18:0}	0.83
C _{14:1} ω5c	0.15
C _{15:1} ω6c	0.64
C _{16:1} ω5c	0.16
C _{18:1} ω9c	0.28
C_{18:1}ω7c	8.75
C _{16:0} 2-OH	0.38
C _{16:0} 3-OH	0.29
^a Summed feature 1	0.17
^aSummed feature 2	13.88
^aSummed feature 3	30.35
^a Summed feature 4	0.42
Fatty acids that represent > 5% are indicated in bold	
^a Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1 contains C _{15:1} iso H/C _{13:0} 3-OH, Summed feature 2 contains C _{14:0} 3-OH/C _{16:1} iso I and/or C _{12:0} aldehyde, Summed feature 3 contains C _{16:1} ω7c/C _{16:1} ω6c, and Summed feature 4 contains C _{17:1} iso I/anteiso B.	

Table 2

Differential phenotypic characteristics between strain Q2-2^T and its closest related type strains

Characteristic	1	2	3	4	5	6
Colony colour	milky white	yellow	yellow	beige	pale yellow	slightly yellow
Cell size(μm)	0.2-0.36×0.9-1.8	0.3-0.5×0.7-1.0	0.3-0.5×0.7-1.0	0.9×1.5	0.5-0.8×0.3-0.6	0.5×1.5
Anaerobic growth	-	-	ND	facultatively anaerobic	-	□
Optimum pH	8.0	7.0	6.5-7.0	7.0	7.0	7.0
Optimum temperature (°C)	30	30	30	30	30	20-25
Optimum NaCl (%)	1.0	0.5	ND	1.3	ND	1.0-2.0
Catalase	□	-	□	□	□	□
Oxidase	w	-	□	□	□	□
Nitrate reduction	□	-	□	-	□	-
Alkaline phosphatase	-	□	□	-	□	-
Urease	□	-	ND	□	-	-
Major polar lipids	DPG,PE,PME,PG	PE,PME,PG,PL	PE,PG	PE,PG	PE,PG,APL,L	PE,PG,APL
Respiratory quinone	ubiquinone Q-8	ubiquinone Q-8	ubiquinone Q-8	ubiquinone Q-8	ubiquinone Q-8	ubiquinone Q-8
DNA G+C content (mol%)	61.10	56.83	59.40	67.90	57.30	63.90
Strains: 1. Strain Q2-2 ^T ; 2. <i>Paracandidimonas caeni</i> 24 ^T (Yao L et al. 2019); 3. <i>Pusillimonas soli</i> MJ07 ^T (Lee M et al. 2010); 4. <i>Parapusillimonas granuli</i> Ch07 ^T (Kim YJ et al. 2010); 5. <i>Pusillimonas ginsengisoli</i> DCY25 ^T (Srinivasan S et al. 2010); 6. <i>Paracandidimonas soli</i> IMT-305 ^T (Kämpfer P et.al. 2017). +, Positive; -, negative; w, weakly positive; ND: no data. All data are from the present study except where indicated otherwise.						

The cellular fatty acid profiles of strain Q2-2^T and related strains are shown in Table 1. There were, to a certain extent, some comparable qualitative and quantitative characteristics of strain Q2-2^T with the interrelated type strains. The major fatty acids of strain Q2-2^T were C_{16:0} (33.95%), C_{17:0} cyclo (11.42%) and C_{18:0} (0.83%), which were the same as those of *Paracandidimonas caeni* 24^T. The major fatty acid C_{14:0} was found in strain Q2-2^T, but not in the related strains except for *Pusillimonas ginsengisoli* DCY25^T. Ubiquinone Q-8, which was consistently detected in all of the related strains, was determined to be the characteristic respiratory quinone. The major polar lipids of strain Q2-2^T were DPG, PE, PME and PG.

The diverse characteristics between strain Q2-2^T and related type strains are shown in Table 2. In the API ZYM system, esterase (C4), lipid esterase (C8), leucine aramide, valine aramidase, acid phosphatase, naphthol AS-BI-phosphohydrolase tested positive, while alkaline phosphatase, esterase (C14), cystine aramidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-glucosaminidase, α-mannosidase, and β-fucosidase were absent.

Comparing the 16S rRNA gene sequences, strain Q2-2^T had the highest similarity (97.99%) with *Paracandidimonas caeni* 24^T, followed by *Pusillimonas soli* MJ07^T (97.54%), *Parapusillimonas granuli* Ch07^T (97.27%), *Pusillimonas ginsengisoli* DCY25^T (97.09%), *Candidimonas nitroreducens* SC-089^T (96.51%), *Pusillimonas harenae* B201^T (96.49%), *Paracaligenes ureilyticus* GR24-5^T (96.48%), *Paracandidimonas soli* IMT-305^T (96.39%), *Pusillimonas caeni* EBR-8-1^T (96.39%), and *Caenimicrobium hargitense* CGII-59m2^T (96.30%). Based on the phylogenetic trees built by NJ and MJ tree construction methods, strain Q2-2^T clustered with the genus *Paracandidimonas* (Fig. 2). The DNA G + C content of strain Q2-2^T was 61.1 mol%. The ANI values between strain Q2-2^T and *Paracandidimonas caeni* 24^T, *Pusillimonas soli* MJ07^T, *Parapusillimonas granuli* Ch07^T, *Pusillimonas ginsengisoli* DCY25^T and *Paracandidimonas soli* IMT-305^T were 71.02%, 73.52%, 74.32%, 74.59% and 72.29%. The genome of strain Q2-2^T contains 4004 protein-coding genes as well as 976 hypothetical proteins which account for 23.38% in the coding genes.

According to the polyphasic evidence described above, it may be concluded that strain Q2-2^T represents a novel species of the genus *Paracandidimonas* in the family *Alcaligenaceae*, for which the name *Paracandidimonas lacteus* sp. nov. is proposed.

Description of *Paracandidimonas lacteus* sp. nov.

Paracandidimonas lacteus (Pa.ra.can.di.di.mo'nas. Gr. pref. para-, like; N.L. fem. n. *Candidimonas*, bacterial genus name; N.L. fem. n. *Paracandidimonas*, a bacterium like *Candidimonas*, lac.te'us. L. masc. adj. lacteus milk-coloured, milky).

Bacterial colonies are smooth, semitransparent, milky white and convex, while the cells were 0.2-0.36 μm × 0.9-1.8 μm in size, immobile, no flagella, short rods. It 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). Esterase (C4), lipid esterase (C8), leucine aramide, valine aramidase, acid phosphatase and naphthol AS-BI-phosphohydrolase could be generated. Test results were positive for nitrate reduction, arginine dihydrolase, urease and aescin hydrolysis. Its assimilates manifold compounds as carbon sources, for instance: D-glucose, D-fructose, D-mannose, sorbitol, amygdalin, esculin, salicin, D-cellobiose, D-sucrose, D-trehalose, pyruvic acid methyl ester, D-xylose, D-galacturonic Acid, L-asparagine, Tween 40, L-phenylalanine, Tween 80, 4-hydroxy benzoic acid, L-serine, D-glucosaminic acid, itaconic acid, glycyl-L-glutamic acid, glucose-1-phosphate, α-ketobutyric acid, α-D-lactose, D,L-α-glycerol, D-malic acid and putrescine. Ubiquinone Q-8, which was consistent with all of the related strains, was determined to be detected the characteristic respiratory quinone. The major fatty acids were C_{16:0}, C_{17:0} cyclo, C_{18:1} ω7c, Summed feature 2 (iso-C_{16:1} I/C_{14:0} 3-OH and/or C_{12:0} aldehyde) and Summed feature 3 (C_{16:1} ω7c/C_{16:1} ω6c). The major polar lipids were DPG, PE, PME, PG. The DNA G + C content of the strain Q2-2^T was 61.1mol%. 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.

Abbreviations

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

Declarations

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

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

Author contributions Xiu-fang Hu and Cong Sun designed the research and project outline. Chu Zhang, Qian Pu, Jun-jie Ying, Hang-tao Wu, Ying Chen and Guo-hong Zeng performed isolation, deposition, and identification. Chu Zhang, Cheng-ze Li, Bin-jie Wang, Yu-han Zhang and Ou Li performed fatty acids analysis. All authors read and approved the final manuscript.

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Figures

Figure 1

Legend not included with this version

Figure 2

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