Pleomorphobacterium xiamenense Yin et al. 2013 is a later heterotypic synonym of Oceanicella actignis Albuquerque et al. 2012

Zhaobin Huang,1,2,3,4,5 Qiliang Lai1,2,3,4,5 and Zongze Shao1,2,3,4,5,*

Abstract

Pleomorphobacterium xiamenense CLW\textsuperscript{T} was compared with Oceanicella actignis PRQ-67\textsuperscript{T} to examine the taxonomic relationship between the two organisms. The 16S rRNA gene sequence comparison showed that the two strains had 99.9 % sequence similarity. Phylogenetic analysis showed the two strains formed an independent tight cluster, distinctly branching from the closely related species in the family Rhodobacteraceae. Whole genomic comparison between the two strains revealed a digital DNA–DNA hybridization estimate of 88.4 % and average nucleotide identity of 98.8 %, strongly supporting that the two strains represented a single species. In addition, neither strain displayed any striking difference in biochemical characteristics, fatty acid composition, and polar lipid profile. According to priority, Pleomorphobacterium xiamenense is reclassified as a later heterotypic synonym of Oceanicella actignis based on the phylogenetic relationship, whole genomic comparison, fatty acid composition and polar lipid profile, and other phenotypic and biochemical properties.

The genus Oceanicella was proposed to describe a bacterial group with pleomorphic cell, Gram-stain-negative, obligate aerobic and catalase- and oxidase-positive [1]. The type species is Oceanicella actignis with type strain PRQ-67\textsuperscript{T} (=LMG 25334\textsuperscript{T}=DSM 22673\textsuperscript{T}), isolated from a shallow marine hot spring on a beach in the Azores. Pleomorphobacterium xiamenense, with type strain CLW\textsuperscript{T} (=LMG 26245\textsuperscript{T}=DSM 24423\textsuperscript{T}=CGMCC 1.10808\textsuperscript{T}=MCCC 1A06272\textsuperscript{T}), proposed by Yin et al., was also isolated from a hot spring but terrestrial in origin, in Xiamen, Fujian province, PR China [2]. Interestingly, strain CLW\textsuperscript{T} showed many characteristics of the genus Oceanicella, as mentioned above. More interestingly, the two strains shared a high 16S rRNA gene sequence similarity of 99.9 % (only one base mismatch), suggesting that they represented a single species. Through the phenotypic and genotypic comparison of the two strains, we proposed that P. xiamenense is a later heterotypic synonym of O. actignis.

The 16S rRNA gene sequences of the two strains PRQ-67\textsuperscript{T} (accession number JQ864435) and CLW\textsuperscript{T} (accession number HQ709062) and the closely related taxa in the family Rhodobacteraceae were retrieved from the recently updated EzBiocloud Database [3] and the nucleotide database in the National Center for Biotechnology Information (NCBI) resources. The sequences were aligned using CLUSTALW implemented in MEGA 7.0 [4]. Two different phylogenetic algorithms were employed to determine the phylogenetic relationship, neighbour-joining (NJ) and maximum-likelihood (ML). The node support of the tree topology was evaluated using bootstrapping estimation of 1000 replicates for the two methods. The best substitution model (T92+G+I) for the ML tree was determined under the lowest Bayesian information criterion. The 16S rRNA gene sequence comparison showed that strain CLW\textsuperscript{T} shared 99.9 % similarity with strain PRQ-67\textsuperscript{T}, together with strain PRQ-68 (JQ864436), strain LQ (HQ709063) and an uncultured bacterium clone PNG_Kap3_B439 (JF935191), exhibiting greater than 99.0 % similarity with one another. Phylogenetic analysis of NJ and ML (data not shown) supported that the two strains were tightly grouped together, but distinctly separated from the closely related species in the family Rhodobacteraceae (Fig. 1).

DNA–DNA hybridization (DDH) and average nucleotide identity (ANI) are recognized as the gold standards to classify bacterial species [5, 6]. Therefore, digital DNA–DNA hybridization (dDDH) estimates between the two strains were calculated based on the whole genome sequences using the Genome-to-Genome Distance Calculator (GGDC 2.1) online service (http://ggdc.dsmz.de/distcalc2.php) [7], and the ANI value was calculated using the ANI Calculator online service in the EzBiocloud database (http://www.ezbiocloud.net/tools/ani). The genome sequences were...
obtained from the Genome portal of the Joint Genome Institute (JGI) (http://genome.jgi.doe.gov/), under the genome identifier (ID) 2593339287 for *O. actignis* DSM 22673^T^ and genome IDs 2615840710 and 2663762749 for *P. xiamenense* DSM 24423^T^ and *P. xiamenense* CGMCC 1.10808^T^, respectively. The OrthoANIu value (%) of the two *P. xiamenense* genomes was 99.97 %, and thus we compared genome similarity between strain CGMCC 1.10808^T^ and strain DSM 22673^T^.

The DNA G+C content of strain PRQ-67^T^ was 72.4 mol%, displaying no distinct difference from that of strain DSM 24423^T^ at 72.2 mol% and strain CGMCC 1.10808^T^ at 72.3 mol%.

The physiological and biochemical characteristics were tested in this study using three API test strips (bioMérieux). *O. actignis* PRQ-67^T^ and *P. xiamenense* CLW^T^ were obtained from the BCCM/LMG Bacteria Collection, Belgium, and the Marine Culture Collection of China, respectively. The two strains demonstrated the same colony morphology cultured on Marine Agar 2216 (BD) at 45 °C. To obtain the cell biomass of the two strains, they were inoculated in Marine Broth 2216 (BD) at 45 °C for 24 h and collected by centrifugation at 3214 g for 10 min. Three kinds of API test strips, API ZYM, API 20E and API 20NE incubated at 45 °C were used to compare the physiological and biochemical characteristics following the manufacturer’s instructions. The results showed that except for the utilization of mannitol, strains PRQ-67^T^ and CLW^T^ showed completely identical biochemical characteristics as follows: both positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and acid phosphatase;
both weakly positive for valine arylamidase, cystine arylamidase and naphthol AS-BI-phosphohydrolase; nitrate can be reduced to nitrite; positive for hydrolysis of gelatin; Voges–Proskauer reaction is positive; adipic acid can be utilized as sole carbon source.

In addition, the two strains PRQ-67T and CLW T displayed a similar fatty acid profile, consisting of C18:1ω7c and 11-methyl C18:1ω7c (both <5% difference) [1, 2]. What is more, the two strains had identical polar lipid composition, both consisting of phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol and two aminolipids [1, 2].

Thus, combined with the phenotypic and genotypic evidence analysed above, we propose that Pleomorphobacterium xiamenense Yin et al. 2012 is a later heterotypic synonym of Oceanicella actignis Albuquerque et al. 2012. The type strain is PRQ-67T (=LMG 25334T=DSM 22673T); strain CLW (=LMG 26245=DSM 24423=CGMCC 1.10808=MCCC 1A06272) is another strain of the species.

Funding information
This work was financially supported by the National Infrastructure of Microbial Resources of China (NIMR-2017-9) and the project of Xiamen Southern Oceanographic Centre (14CZP034HJ08).

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

References