Pyruvatibacter mobilis gen. nov., sp. nov., a marine bacterium from the culture broth of *Picochlorum* sp. 122

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A Gram-stain-negative, aerobic bacterium, designated strain GYP-11T, was isolated from the culture broth of a marine microalga, *Picochlorum* sp. 122. Cells were dimorphic rods; free living cells were motile by means of a single polar flagellum, and star-shaped-aggregate-forming cells were attached with stalks and non-motile. Sodium pyruvate or Tween 20 was required for growth on marine agar 2216. 16S rRNA gene sequence analysis revealed that this isolate shared 94.07 % similarity with its closest type strain, *Parvibaculum hydrocarboniclasticum* EPR92T. Phylogenetic analyses indicated that strain GYP-11T represents a distinct lineage in a robust clade consisting of strain GYP-11T, alphaproteobacterium GMD21A06 and *Candidatus Phaeomarinobacter ectocarpi* Ec32. This clade was close to the genera *Parvibaculum* and *Tepidicaulis* in the order *Rhizobiales*. Chemotaxonomic and physiological characteristics, including cellular fatty acids and carbon source profiles, also readily distinguished strain GYP-11T from all established genera and species. Thus, it is concluded that strain GYP-11T represents a novel species of a new genus in the order *Rhizobiales*, for which the name *Pyruvatibacter mobilis* gen. nov., sp. nov. is proposed. The type strain of *Pyruvatibacter mobilis* is GYP-11T (＝CGMCC 1.15125T＝KCTC 42509T).

Cultivation aims to create an artificial system mimicking *in situ* conditions. Despite extensive studies on ecosystems where sampling is conducted, the conditions used in classical cultivation conditions are often far from the endogenous abiotic and biotic conditions required for microbial growth (Alain & Querellou, 2009). Recently, it has been shown that substrate availability provides a series of ecological niches in which specialized populations can bloom (Sapp *et al.*, 2007; Teeling *et al.*, 2012; Taylor *et al.*, 2014). Thus, addition of particular substrates is essential for the successful cultivation of some uncultured bacteria. The aim of this study was to identify the exact taxonomic standing of strain GYP-11T, a pure culture that requires addition of pyruvate or Tween 20 for growth on marine agar 2216 (MA; BD).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Pyruvatibacter mobilis* GYP-11T is KR078282.

One supplementary table and four supplementary figures are available with the online Supplementary Material.

The microalga *Picochlorum* sp. 122 was isolated from the Indian Ocean, and cultivated outdoors in filtered natural seawater (collected from west of Sanya Bay, China) amended with urea (1 g l⁻¹), NaHCO₃ (1 g l⁻¹), Na₂HPO₄ (8 mg l⁻¹) and FeSO₄·7H₂O (5 mg l⁻¹). The culture broth of *Picochlorum* sp. 122 was collected at the late exponential phase, stored and transported at room temperature. Strain GYP-11T was initially isolated as a mixture of GYP-11T and GYP-15T (the latter representing a member of another new genus, details of which will be published in due course) from this culture broth by spreading serial dilutions on GYP plates (2 g tryptone, 1 g yeast extract, 3 ml glycerol, 18 g agar powder and 1 litre of aged seawater, pH 8.0; auto-claving at 121 °C for 15 min). Finally, strain GYP-11T was purified by spreading the bacterial mixture on a GYP plate. However, strain GYP-11T was unable to grow in pure culture on GYP or on MA. A substrate screen was performed using the Biolog Gen III Microplate, and the results indicated that carboxylates with at least three carbons could be oxidized. Subsequently, viability of strain GYP-11T was restored by addition of propionate to GYP medium. Finally, pyruvate and Tween 20 were shown to be optimal substrates for growth of strain GYP-11T. Thus, subsequent
growth experiments were performed aerobically on MA or in marine broth 2216 (BD) amended with 0.5 % pyruvate (MAP/MBP) in the dark at 30 °C. Bacterial stocks were stored at −70 °C in sterile aged seawater supplemented with 20 % glycerol (v/v).

The complete 16S rRNA gene sequence of strain GYP-11T was obtained by a genome scan (Illumina Hiseq 2000 sequencing and sequence reconstruction using SOAPdenovo v2.04) at Shanghai Majorbio Bio-pharm Technology. 16S rRNA gene sequence similarities were obtained from the GenBank and EzTaxon-e databases (Kim et al., 2012). Alignment of 16S rRNA gene sequences was performed using the SINA software package (Pruesse et al. 2012) in the SILVA rRNA database. Phylogenetic trees were reconstructed using the maximum-likelihood (Felsenstein, 1981), neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Swofford, 1993) algorithms in the software package MEGA version 5.0 (Tamura et al., 2011). The phylogenetic distance matrices were estimated by the Kimura two-parameter model (Kimura, 1980). The topology of the phylogenetic tree was evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The complete 16S rRNA gene sequence (1462 bp) of strain GYP-11T was obtained. Results of 16S rRNA gene sequence global alignment in the EzTaxon-e database revealed that strain GYP-11T was related most closely to Parvibaculum hydrocarboniclasticum EPR92T with a similarity of 94.07 %. This sequence similarity suggested that strain GYP-11T may represent a novel species of a new genus (Yarza et al., 2014) as the recommended genus threshold is 94.5 %.

Phylogenetic analyses based on the maximum-likelihood algorithm indicated that strain GYP-11T represents a distinct lineage in a robust clade (the GYP-11T group) consisting of Candidatus Phaeomarinobacter ectocarpi Ec32, strains GYP-11T and alphaproteobacterium GMD21A06 (Fig. 1). This GYP-11T group is close to the genera Parvibaculum and Tepidicaulis in the order Rhizobiales (Fig. 1).
The maximum-likelihood tree topology was also supported by the neighbour-joining and maximum-parsimony algorithms (Figs S1 and S2, available in the online Supplementary Material). However, the bootstrap confidence level between the GYP-11T group and some other related genera was uncertain. Based on these analyses, *Parvibaculum–Tepidicaulis* group was ≤50 % in this study (Fig. 1, Figs S1 and S2), so the exact taxonomic standing of the GYP-11T group in the order *Rhizobiales* was uncertain. Based on these analyses, *Parvibaculum lavamentivorans* DS-1T (=MCCC 1A03287T=DSM 13023T) and *Parvularcula bermudensis* HTCC 2503T (=MCCC 1A02685T=KCTC 12087T), obtained from the Marine Culture Collection of China (MCCC), were used as reference strains in subsequent studies. These bacteria were grown on MAP or in MBP.

Cellular morphology was determined by transmission electron microscopy (Hitachi TEM System-H7650) and optical microscopy (Olympus BX53). Cell mobility was tested using an optical microscope and the hanging drop technique (Bernardet et al., 2002). The Gram reaction was determined as described by Gerhardt et al. (1994). Catalase activity was determined by observing bubble production in a 5 % (v/v) hydrogen peroxide solution and oxidase activity was determined by using oxidase test strips (Huankai). NaCl requirement and tolerance were tested at 30 °C for 4 days in reconstituted MBP with NaCl concentrations ranging from 0 and 15 % (0, 0.25, 0.5, 1, 2, 3, 5, 6, 8, 10, 13 and 15 %, w/v). Growth at different pH was tested in MBP with stalks and were non-motile (Fig. S3A, B, D). Star-shaped-aggregate-forming cells were attached with stalks and were non-motile (Fig. S3C). Stalked cells were motile with a single polar flagellum (Fig. S3C, B). The ability to form endospores and hydrolysis of starch, casein, chitin, gelatin and Tweens 20, 40 and 80 were tested as described by Dong & Cai (2001). For alkane degradation, 0.25 % n-dodecane was added to the bacterial culture broth (medium: MB amended with 0.5 % Tween 20); after 7 days of shaking incubation at 30 °C in the dark, the alkane residue was extracted in n-hexane and analysed by using GC (Shimadzu 2014C). Additional carbohydrate metabolism was tested using the API 20NE kit according to the manufacturer’s protocol except that cells were suspended in sterile aged natural seawater. Anaerobic growth was determined by using the nitrate reduction hole of the API 20NE system, which was sealed with mineral oil. Other phenotypic characteristics were tested by following the standard procedures compiled by Tindall et al. (2007).

Cells of strain GYP-11T were Gram-stain-negative, aerobic, non-spore-forming, dimorphic rods (Fig. S3). Free living cells were motile with a single polar flagellum (Fig. S3C), and star-shaped-aggregate-forming cells were attached with stalks and were non-motile (Fig. S3A, B, D). Star-shaped aggregation in marine bacteria has been observed in the genera *Rhizobium* and *Agrobacterium* (Rüger & Höfte, 1992). Stalked cells were also observed in *Tepidicaulis*.
Description of Pyruvatibacter mobilis sp. nov.

Pyruvatibacter mobilis (mo‘bi.lis. L. masc. adj. mobilis motile).

The description is as for the genus with the following additional properties. Cells are usually 0.22–0.48 μm wide and 1.3–2.45 μm long. Colonies are pale, tiny and opaque on MAP. Growth occurs at pH 6.0–9.0, at 15–45 °C and in the presence of 0.25–8 % (w/v) NaCl, and optimally at 30 °C, at pH 7.0–8.0 and with 1–3 % NaCl. Gelatin, Tween 20, 40 and 80 are hydrolysed. Production of H₂S does not occur. Protease, β-glucosidase and β-galactosidase are positive, while indole production, D-glucose fermentation, arginine dihydrolase and urease are negative. Utilization of adipic acid, propionic acid, sodium butyrate, β-hydroxybutyric acid, D-fructose 6-phosphate, glucuronamide, L-histidine, sodium lactate, acet acet acid and pyruvate is positive. Utilization of L-arabinose, D-mannose, D-mannitol, lactic acid, trisodium citrate and capric acid is negative. The type strain, GYP-11T (=CGMCC 1.15125T=KCTC 42509T), was isolated from the culture broth of a marine microalga, Picocloruma sp. 122.

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References


