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Short communication

Hyphomonas atlanticus sp. nov., isolated from the Atlantic Ocean and emended description of the genus *Hyphomonas*☆

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ABSTRACT

A taxonomic study was carried out on strains 22II1-22F38^T and 22II-S13e, which were isolated from sea water and sediment from the Atlantic Ocean, respectively. The two strains were Gram-negative, oxidase and catalase positive, oval to pear shaped, and motile by a single polar flagellum. Phylogenetic analysis based on 16S rRNA gene sequences indicated that both strains belonged to the genus *Hyphomonas*, with highest sequence similarity (98.2%) to the type strains *H. jannaschiana* DSM 5153^T and *H. johnsonii* ATCC 43964^T. The genomic ANIm values and DNA-DNA hybridization estimate values between strain 22II1-22F38^T and seven type strains ranged from 82.84% to 84.10% and from 18.0% to 19.1%, respectively. Isolate 22II1-22F38^T had a G + C content of 58.3% and used Q-11 as the predominant respiratory quinone. The combined phenotypic and genotypic data showed that both strains represented a novel species of the genus *Hyphomonas*, for which the name *Hyphomonas atlanticus* sp. nov. is proposed, with the type strain being 22II1-22F38^T (=LMG 27916^T = MCCC 1A09418^T). In addition, we conclude that *Hyphomonas hirschiana* is a later synonym of *Hyphomonas neptunium*.

Introduction

To investigate the phylogenetic diversity of bacteria belonging to *Hyphomonas*, a multilocus sequence analysis (MLSA) was used to distinguish all strains within the genus. It was previously found that six strains isolated from the Atlantic Ocean formed an independent clade and represented a potential novel species [8]. Therefore, this study focused on two isolates, designated as 22II1-22F38^T and 22II-S13e, which were isolated from Atlantic Ocean deep sea water and sediment, respectively. They shared an identical 16S rRNA gene sequence, and comparative analysis indicated that both belonged to the genus *Hyphomonas*, which was proposed by Pongratz [12]. Currently, the genus *Hyphomonas* comprises eight species: *H. polymorpha* [11,12]; *H. neptunium* [11]; *H. oceanitis*, *H. hirschiana* and *H. jannaschiana* [22]; *H. adhaerens*, *H. johnsonii* and *H. rosenbergii* [23]. The genus *Hyphomonas* is a group of Gram-negative, prosthecate, budding, aerobic bacteria. *Hyphomonas* species have a dimorphic life cycle and reproduce by budding from the single polar prosthecae [21]. Until now, all *Hyphomonas* species have been isolated from marine environments. It should be noted, however, that the type strain *H. rosenbergii* ATCC 43869^T purchased from the American Type Culture Collection (ATCC) has been found to be incorrect, since the resequenced 16S rRNA gene, under GenBank accession number KF880383, showed only 92.84% similarity to the original 16S rRNA gene sequence, but highest similarity with *Henriciella marina* Iso4^T (98.4%). The original type strain has thus been shown to be misidentified [7] and, therefore, it was not included in this study.

Materials and methods

Source and cultivation of bacteria

Deep sea water and sediment were sampled by a CTD instrument and television grab, respectively, on February 7th 2011 during

Abbreviation: MCCC, Marine Culture Collection of China.

☆ Nucleotide sequence data for the 16S rRNA genes are available in the DDBL/EMBL/GenBank databases under the accession numbers: 22II1-22F38^T (KF863140), 22II-S13e (KF863142), DSM 2665^T (KF863144), DSM 5152^T (KF863145), DSM 5153^T (KF863146), DSM 5154^T (KF863147), DSM 5155^T (KF863148), ATCC 43964^T (KF863149), ATCC 43965^T (KF863150) and ATCC 43869^T (KF880383).

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cruise DY22II in the South Atlantic Ocean on the R/V "Da-Yang Yi-Hao". The sampling sites were DY22II-(SMAR)-S011-CTD01-22 (W14.4°, S13.9°; depth −2238 m) and DY22II-(SMAR)-S013 (W14.5°, S13.6°; depth −3400 m), respectively. The deep sea water was diluted and spread onto marine agar 2216 medium (BD Difco) plates. The plates were incubated at 28 °C for one week and all resultant colonies were isolated. In the case of sediment, one gram was used for enrichment of oil-degrading bacteria with 1% (v/v) sterilized crude oil. M2 agar medium was used for bacterial isolation as described previously [20]. Among the isolates, two strains with a dull white color were isolated and designated as 22II1-22F38^T and 22II-S13e, respectively. They were subsequently cultivated on marine agar 2216 medium (BD Difco) for morphological and biochemical characterization, unless otherwise indicated. Five type strains (*H. polymorpha* DSM 2665^T, *H. hirschiana* DSM 5152^T, *H. jannaschiana* DSM 5153^T, *H. neptunium* DSM 5154^T and *H. oceanitis* DSM 5155^T) were obtained from the DSMZ. Three type strains (*H. rosenbergii* ATCC 43869^T, *H. johnsonii* ATCC 43964^T and *H. adhaerens* ATCC 43965^T) were obtained from the ATCC.

Phenotypic characterization

The cell size, morphology, and flagellation pattern were observed by transmission electron microscopy (JEM-1230, JEOL) using cells negatively stained with phosphotungstic acid after growth on marine agar at 28 °C for 2 days. Cell motility, catalase and oxidase activities, and starch hydrolysis were tested according to standard methods [3]. The optimal growth temperature was determined over a temperature range of 4–45 °C in marine broth 2216. The pH range for growth was determined in marine broth 2216 adjusted to pH 2.0–10.0, at one pH unit intervals, with citrate/phosphate (pH = 2.0–7.0), Tris/HCl (pH = 8.0–9.0), or sodium carbonate/sodium bicarbonate (pH = 10.0) buffers. Tolerance to NaCl was tested using artificial marine broth 2216 with the addition of NaCl to provide concentrations of 0, 0.5, 1, 2, 3, 5, 7, 9, 12, 15, and 18% (w/v). Antibiotic susceptibility tests were performed using a disc diffusion method as previously described [18]. Other biochemical tests were performed using API 20NE, API 20E and API ZYM strips (bioMérieux) according to the manufacturer's instructions, with modification of the NaCl concentration in all tests to 3.0%. Seven type strains were tested at the same time as strains 22II1-22F38^T and 22II-S13e.

G + C content calculation and phylogenetic analysis of 16S rRNA

The G + C contents of all strains were directly calculated from their genome sequences. Chromosomal DNA was extracted using the SBS extraction kit (SBS Genetech Co., Ltd., Shanghai, China); and amplification of the 16S rRNA gene was conducted according to the method previously described [9]. Sequence similarity was determined using the EzTaxon-e server [6]. Sequences of related taxa were obtained from the GenBank database. Phylogenetic trees were constructed on the basis of neighbor-joining [16], minimum evolution [14,15] and maximum likelihood [5] algorithms by using the MEGA5 program [19] with bootstrap resampling analysis of 1000 replications.

Determination of fatty acid and isoprenoid ubiquinone

The fatty acid methyl esters of whole cells, cultivated on marine agar at 28 °C for 72 h, were extracted and determined according to the standard protocol of the MIDI system [17]. Respiratory quinones were extracted from freeze-dried cells (200 mg) with chloroform/methanol (2:1) and analyzed by reversed-phase HPLC [2].

Average nucleotide identity (ANI) and DNA–DNA hybridization (DDH) estimation

The average nucleotide identity (ANI) and DNA–DNA hybridization (DDH) estimate values between two genomes were calculated using JSpecies (V1.2.1) [13] and the genome-to-genome distance calculator (GGDC2.0) [10].

Results and discussion

Strains 22II1-22F38^T and 22II-S13e were Gram-negative, non-spore-forming, oval to pear shaped, prosthecate, budding bacteria, 0.4–0.5 μm wide and 0.9–1.4 μm long, and motile by a single polar flagellum (see supplementary materials Fig. S1). On marine agar, both strains formed dull white, round, convex, opaque colonies with regular margins. Colonies were 1–2 mm in diameter after 72 h incubation at 28 °C. Growth was observed at 0.5–12% NaCl (optimum 0.5–7%), pH 5–9 (optimum pH 6–8) and 10–37 °C (optimum 25–37 °C). The antibiotic susceptibilities of both strains and seven type strains of the genus *Hyphomonas* are shown in Table S1. Other characteristics are given in the species description.

The almost full-length 16S rRNA gene sequences of strains 22II1-22F38^T and 22II-S13e (1419 bp) and eight type strains of genus *Hyphomonas* were directly obtained by PCR amplification. Strains 22II1-22F38^T and 22II-S13e shared an identical 16S rRNA gene sequence. As indicated in Fig. 1, a phylogenetic tree based on 16S rRNA gene sequences showed that 22II1-22F38^T and 22II-S13e formed a separate clade within the genus *Hyphomonas*. They had the highest sequence similarity (98.2%) to type strains *H. jannaschiana* DSM 5153^T and *H. johnsonii* ATCC 43964^T, followed by *H. adhaerens* ATCC 43965^T (98.0%) and *H. oceanitis* DSM 5155^T (97.8%), while another three type strains had similarities that ranged from 96.1% to 96.6%. The Rep-PCR patterns of the two strains were similar but varied in a few bands (Fig. S2), which indicated they were different strains of the same species. Moreover, multilocus sequence analysis (MLSA) of the five housekeeping genes *leuA*, *clpA*, *pyrH*, *gatA* and *rpoD* showed that 22II1-22F38^T and 22II-S13e had high similarity (99.8%) [8], and therefore should be classified into the same species.

The draft genome sequences of novel strain 22II1-22F38^T (AWFH00000000) and six type strains (*H. polymorpha* DSM 2665^T (ARYM00000000), *H. hirschiana* DSM 5152^T (ARYI00000000), *H. jannaschiana* DSM 5153^T (ARYJ00000000), *H. oceanitis* DSM 5155^T (ARYL00000000), *H. johnsonii* ATCC 43964^T (ARYK00000000) and *H. adhaerens* ATCC 43965^T (ARYH00000000)) have been sequenced by our laboratory and are available in NCBI [8]. The complete genome sequence of *H. neptunium* DSM 5154^T (CP000158.1) has already been reported [1]. The ANIm values of strain 22II1-22F38^T with the seven type strains are shown in Table S2, and they ranged from 82.84% to 84.10%. These values were below standard ANI criteria for species identity (95–96%) [13], indicating that strain 22II1-22F38^T represented a novel species in the genus *Hyphomonas*. In addition, the ANIm values between type strains *H. hirschiana* DSM 5152^T and *H. neptunium* DSM 5154^T were 99.94–99.96%, which demonstrated that they should be classified into one species.

The estimated DDH values of strain 22II1-22F38^T and seven type strains were calculated using GGDC2.0 with the BLAST+ alignment method (Table S3) and they were 18.0–19.1%, which was below the standard criteria (70%) for species cut-off. This result reconfirmed that strain 22II1-22F38^T represented a novel species of the genus *Hyphomonas*. On the other hand, the DDH values between type strains *H. hirschiana* DSM 5152^T and *H. neptunium* DSM 5154^T were 100.0%. In addition, a previous study also showed that these two strains shared a similar electrophoretic pattern of cell envelope proteins [4]. These results