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Tepidimonas thermarum sp. nov., a new slightly thermophilic betaproteobacterium isolated from the Elisenquelle in Aachen and emended description of the genus *Tepidimonas* ☆

Luciana Albuquerque[a], Igor Tiago[b], António Veríssimo[b], Milton S. da Costa[a,*]

[a]*Departamento de Bioquímica and Centro de Neurociências e Biologia Celular, Universidade de Coimbra, 3001-401 Coimbra, Portugal*
[b]*Departamento de Zoologia and Centro de Neurociências e Biologia Celular, Universidade de Coimbra, 3004-517 Coimbra, Portugal*

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Abstract

Several non-pigmented bacterial isolates, with an optimum growth temperature of about 50–55 °C, were recovered from the Elisenquelle at Aachen, Germany. Phylogenetic analysis of the 16S rRNA gene sequence of strains AA-1^T and AA-2 indicated that these organisms represent a new species of the genus *Tepidimonas*. The major fatty acids of strains AA-1^T and AA-2 are 16:0 and 16:1 $\omega 7c$. Ubiquinone 8 is the major respiratory quinone, the major polar lipids are phosphotidylethanolamine and phosphotidylglycerol. The new isolates are aerobic; thiosulfate is oxidized to sulfate in the presence of a metabolizable carbon source. The organism assimilated organic acids and amino acids, but did not assimilate carbohydrates or polyols. On the basis of the phylogenetic analyses, physiological and biochemical characteristics, we propose that strains AA-1^T (= LMG 23094^T; = CIP 108777^T) and AA-2 (= LMG 23095; = CIP 108778) represents a new species for which we recommend the name *Tepidimonas thermarum*.

Keywords: *Tepidimonas thermarum*; *β*-subclass; *Proteobacteria*; Slightly thermophilic

Introduction

The species of the genus *Tepidimonas*, namely *Tepidimonas ignava*, *Tepidimonas aquatica* and "*Tepidimonas taiwanensis*", were isolated from hot springs and from an industrial hot water tank, respectively [6,3,13]. These *Betaproteobacteria* have optimum growth temperatures around 50 °C, two of these three species do not utilize carbohydrates as single carbon sources, oxidize reduced sulfur compounds to sulfate and are strictly aerobic. One strain named "*Tepidimonas arfidensis*" was isolated from the bone marrow of a patient with leukemia, but the isolation of this organism may have been due to contamination of the sample [10], which also appears not utilize carbohydrates.

We recently recovered several isolates from the Elisenquelle (Elisa Spring) at Aachen (Aix-la-Chapelle) in Germany. Two of the isolates, AA-1^T and AA-2, were characterized in detail and possessed many

☆ *Nucleotide sequence accession numbers.* The 16S rRNA gene sequences determined in this study were deposited in EMBL data library under the accession numbers: AA-1^T (AM042693) and AA-2 (AM042694).

*Corresponding author. Tel.: +351 239824024; fax: +351 239826798.
E-mail address: milton@ci.uc.pt (M.S. da Costa).

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characteristics similar to the type strains of *T. ignava, T. aquatica* and "*T. taiwanensis*". However, differences in physiological, biochemical and chemotaxonomic characteristics allied to the phylogenetic analysis show that strains AA-1^T and AA-2 belong to a novel species of the genus *Tepidimonas* for which we propose the name *Tepidimonas thermarum* sp.nov.

Material and methods

Isolation and bacterial strains

Strains AA-1^T (T = type strain) and AA-2 were isolated from the Elisenquelle at Aachen, Germany. Water samples were maintained without temperature control for 6 days, and then filtered through membrane filters (Gelman type GN-6; pore size 0.45 μm; diameter 47 mm). The filters were placed on the surface of agar-solidified Degryse medium 162 [4], wrapped in plastic bags and incubated at 50 °C for up to 4 days. Cultures were purified by sub-culturing and the isolates stored at −70 °C in Degryse medium 162 with 15% (w/v) glycerol. The type strains of *Tepidimonas ignava* SPS-1037^T (= DSM 12034^T), *T. aquatica* CLN-1^T (= DSM 14833^T) and "*T. taiwanensis*" I1-1^T (= LMG 22826^T) were used for comparative purposes.

Morphology, growth, biochemical and physiological characteristics

Cell morphology and motility were examined by phase contrast microscopy during the exponential growth phase. Flagella were visualized by light microscopy after staining of the cells with the Ryu stain [8]. The growth temperature range of the strains was examined by measuring the turbidity (610 nm) of cultures incubated in 300 ml metal-capped Erlenmeyer flasks, containing 100 ml Degryse medium in a reciprocal water-bath shaker [13]. The pH range for growth was examined at 50 °C in the same medium by using 50 mM MES, HEPES, TAPS, CAPSO and CAPS over a pH range from 5.5 to 11.0 [13]. Unless otherwise stated, all biochemical and tolerance tests were performed, as described previously [6], in Degryse liquid medium or Degryse agar at 50 °C for up 6 days. Catalase, oxidase and DNAse activities were examined as described previously [13]. Additional enzymatic activities were obtained using the API ZYM system (bioMérieux) at 50 °C. Anaerobic growth was assessed in cultures in Degryse medium containing KNO_3 (1.0 g/l) incubated in anaerobic chambers (GENbox anaer, bioMérieux). Single-carbon source assimilation tests were performed in a minimal medium composed of Degryse basal salts to which filter-sterilized ammonium sulfate (0.5 g/l), vitamin and nucleotide solution [17] and the carbon source (2.0 g/l) were added. Growth of the strains on single carbon sources was examined by measuring the turbidity of cultures incubated at 50 °C in 20 ml screw capped tubes containing 10 ml of medium for up to 6 days. Growth on reduced sulfur compounds was assessed on modified 69 medium (www.dsmz.de/media/med069.htm) containing the following components per liter: $Na_2HPO_4 \cdot 12H_2O$, 10.6 g; KH_2PO_4, 1.5 g; NH_4Cl, 0.3 g; yeast extract, 1.0 g; $MgCl_2$, 0.1 g; trace elements solution of medium 27 (www.dsmz.de/media/med027.htm) without sulfate, 1 ml and containing the vitamin and nucleotide solution [17]. Thiosulfate was added to this media at concentrations that varied between 0.1 and 5.0 g/l. At appropriate intervals, the turbidity of the cultures was measured, the cells were harvested by centrifugation and the levels of thiosulfate and sulfate in the supernatants were determined using the methods described by Westley [22] and Sörbo [18], respectively.

Polar lipid, lipoquinone and fatty acid composition

The cultures used for polar lipid analysis were grown in 11 Erlenmeyer flasks containing 250 ml of Degryse medium at 50 °C in a reciprocal water bath shaker until late exponential phase of growth. Harvesting of the cultures, extraction of the lipids and single dimensional thin-layer chromatography were performed as described previously [1]. Lipoquinones were extracted, purified by thin-layer chromatography and separated by high performance liquid chromatography [21]. Cultures for fatty acid analysis were grown on solidified Degryse medium, in sealed plastic bags submerged in a water bath at 50 °C for 24 h. Fatty acid methyl esters (FAMEs) were obtained from fresh wet biomass and separated, identified and quantified with the standard MIS Library Generation Software (Microbial ID Inc.) as described previously [2].

Determination of G + C content of DNA and 16S rRNA gene sequence determination and phylogenetic analyses

The DNA for the determination of the G + C content of the DNA was isolated as described previously [14]. The G + C content of DNA was determined by high-performance liquid chromatography as described by Mesbah et al. [12].

The extraction of genomic DNA for 16S rRNA gene sequence determination, PCR amplification of the 16S rRNA gene and sequencing of the purified PCR products were carried out as described previously [15]. Purified reactions were electrophoresed using a model 310 Genetic Analyzer (Applied Biosystems, Foster City,