Taonella mepensis gen. nov., sp. nov., a member of the family Rhodospirillaceae isolated from activated sludge

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A novel Gram-negative, non-spore-forming, rod-shaped strain, H1T, was isolated from activated sludge by micromanipulation. No close relatives among cultured bacterial isolates were found; phylogenetic analysis based on 16S rRNA gene sequences revealed that strain H1T forms a deep single branch in the family Rhodospirillaceae. Cells of strain H1T were slightly curved to straight rods (1.2–1.4×1.5–1.7 μm) and motile by a single polar flagellum. Strain H1T was able to grow in the presence of 0–4% NaCl and grew optimally at 37°C and pH 6.0–7.0. Chemotaxonomic analysis revealed that strain H1T possessed Q-10 as the predominant ubiquinone and C18:1ω7c, C16:0 and C19:0 cyclo ω8c as the major fatty acids. The DNA G+C content of strain H1T was 65.1 mol%. Comparative analysis of 16S rRNA gene sequences, and phenotypic and chemotaxonomic data, indicate that strain H1T should represent a novel genus and species of the family Rhodospirillaceae. The name Taonella mepensis gen. nov., sp. nov. is proposed. The type strain of Taonella mepensis is H1T (=CICC 10529T = CCTCC AB 2012861T = KACC 16940T).

In an attempt to investigate the fenoxaprop-P-ethyl herbicide-degrading bacteria, several strains were isolated from activated sludge. One of the strains was able to degrade the downstream degradation product of fenoxaprop-P-ethyl, (R)-2-(4-hydroxyphenoxy)propionic acid. We characterized the novel degradative strain named H1T. Comparative 16S rRNA analysis indicated that strain H1T forms a new branch within the family Rhodospirillaceae, members of which are thought to play an important role in aquatic and marine environments (Liu et al., 2010). The exact taxonomic position of strain H1T was determined in the present study using a polyphasic approach that included an analysis of phenotypic properties and a detailed phylogenetic analysis based on 16S rRNA gene sequences.

At the time of manuscript preparation, the family Rhodospirillaceae comprises 29 genera (http://www.bacterio.cict.fr/classifgenerafamilies.html#Rhodospirillaceae). Due to the abundance of genera, members of this family have been isolated from various environments; for example, members of the genera Rhodocista (Kawasaki et al., 1992), Rhodospirillum (Imhoff et al., 1998), Phaeospirillum (Imhoff et al., 1998), Azospirillum (Tarrand et al., 1978), Skermanella (Sly & Stackebrandt, 1999) and Magnetospirillum (Schleifer et al., 1991) were isolated from freshwater; members of the genera Rhodovibrio (Imhoff et al., 1998), Thalassospira (López-López et al., 2002), Thalassobaculum (Zhang et al., 2008), Nisaea (Urios et al., 2008) and Oceanibaculum (Lai et al., 2009) were isolated from seawater, and these genera are represented by strains from marine environments; and strains representing the genus Rhodospira (Pfenning et al., 1997) and Tistlia consotensis (Díaz-Cárdenas et al., 2010) were isolated from salt water and collected separately from salt marsh and saline spring environments. Similar to members of the genera Defluviicoccus (Maszenan et al., 2005), Caenispirillum (Yoon et al., 2007) and Dongia (Liu et al., 2010), strain H1T was isolated from activated sludge collected from a pesticide chemical factory (31° 51′ 08.36″ N 120° 56′ 37.22″ E, Nantong, Jiangsu, China). On the basis of the phenotype and genotype, we found that strain H1T could not be classified into any known genus and, therefore, strain H1T should be considered to represent a novel species in a new genus in the family Rhodospirillaceae, for which the name Taonella mepensis gen. nov., sp. nov. is proposed.
The sludge sample was suspended in normal saline. Using the standard dilution plating technique, strain H1\textsuperscript{T} was isolated from a BSM plate [laboratory prepared, containing (per litre distilled water) 1 g NH\textsubscript{4}NO\textsubscript{3}, 1.5 g KH\textsubscript{2}PO\textsubscript{4}, 0.5 g KH\textsubscript{2}PO\textsubscript{4}, 1 g NaCl, 0.2 g MgSO\textsubscript{4}, 15 g agar; adjusted to pH 7.0] after incubation at 30 °C for 5 days. Cells were preserved at −70 °C in LB supplemented with 15 % (v/v) glycerol.

Several bacteriological agar media, including R2A [laboratory prepared, containing (per litre distilled water) 0.5 g tryptone (Difco), 0.5 g yeast extract (Difco), 0.5 g Casamino acids, 0.5 g glucose, 0.5 g soluble starch, 0.3 g sodium pyruvate, 0.3 g K\textsubscript{2}HPO\textsubscript{4}, 0.05 g MgSO\textsubscript{4}.7H\textsubscript{2}O, 15 g agar; adjusted to pH 7.0] and YP [1 %, 6 g tryptone (Difco), 3 g yeast extract (Difco), 2 g beef extract, 3 g NaCl, 0.01 g FeCl\textsubscript{3}, 15 g agar, pH 7.0] and TYB [1 %, 3.0 g tryptone (Difco), 3.0 g yeast extract (Difco), 0.5 g MgSO\textsubscript{4}.7H\textsubscript{2}O, 0.3 g NaCl, 15 g agar; pH 7.0], were tested. Abundant growth was observed on R2A and YP, while little growth was observed on TYB.

Cell morphology was determined by transmission electron microscopy (Hitachi H-7650). Cells of strain H1\textsuperscript{T} occurred singly, were slightly curved to straight rods (1.2–1.4 \(\mu\)m) and motile by means of a polar flagellum (Fig. 1). Spores were not observed and cells were not resistant to heating at 90 °C for 5 min. Conditions for growth were tested in TY medium containing 2.5 g tryptone \(1^{-1}\) and 1.25 g yeast \(1^{-1}\) extract. Growth at various NaCl concentrations (0, 0.5 and 1.0–10.0 %, w/v, increments of 1.0 %) and pH (pH 4.0–10.0, increments of 0.5 pH units) were tested at 28 °C. The optimum growth conditions for strain H1\textsuperscript{T} were determined to be 0.5 % NaCl, 37 °C (range 25–40 °C) and pH 6.0–7.0 (range 5.0–8.0).

Standard methods were used to test catalase activity, oxidase activity and nitrate reduction (McCarty & Cross, 1984). Assimilation of various substrates, enzyme activities and other physiological and biochemical properties were analysed using the Biolog GN2 system and the API 20NE and API 20E identification systems (bioMérieux) according to the manufacturers’ instructions. Antibiotic susceptibility was tested on TY plates (with 0.5 % NaCl) using antibiotic discs containing one of the following: polymyxin B, 30 \(\mu\)g; streptomycin, 10 \(\mu\)g; ofloxacin, 5 \(\mu\)g; neomycin, 15 \(\mu\)g; rifampicin, 5 \(\mu\)g; ampicillin, 10 \(\mu\)g; erythromycin, 15 \(\mu\)g; kanamycin, 30 \(\mu\)g; gentamicin, 10 \(\mu\)g; tetracycline, 30 \(\mu\)g; chloramphenicol, 30 \(\mu\)g or penicillin G, 10 IU. Growth under anaerobic conditions was determined on R2A agar supplemented with or without 0.1 % (w/v) nitrate by using the GasPak Anaerobic System (BBL) according to the manufacturer’s instructions. Strain H1\textsuperscript{T} was sensitive to kanamycin, streptomycin, ofloxacin, neomycin and erythromycin. Oxidase, catalase and nitrate reductase activities were positive. The results of the Biolog GN2, API 20E, API 20NE and antibiotic susceptibility tests are listed in the species description.

Respiratory quinone composition analysis, performed by the identification service of the DSMZ (Braunschweig, Germany), showed that ubiquinone Q-10 was the dominant quinone (75 %) in strain H1\textsuperscript{T} and the remaining quinones (25 %) were unknown. These data support the affiliation of strain H1\textsuperscript{T} to the class Alphaproteobacteria because the possession of ubiquinone Q-10 as the major quinone is a characteristic of this group (Xie & Yokota, 2005). Fatty acid analysis, performed by the identification service of the DSMZ, showed that the major cellular fatty acids of strain H1\textsuperscript{T} were C\textsubscript{18:1}\text{\textomega}7\text{c} (42.9 %), C\textsubscript{16:0} (24.9 %) and C\textsubscript{19:0} cyclo \(\omega\)8\text{c} (17.6 %). The fatty acids C\textsubscript{18:0} (3.4 %) and C\textsubscript{16:1}\text{\textomega}7\text{c}/C\textsubscript{16:1}\text{\textomega}6\text{c} (1.9 %) were also present in lower concentrations. The hydroxy fatty acids identified were C\textsubscript{14:0} 3-OH/ iso-C\textsubscript{16:1} I (2.7 %), C\textsubscript{18:0} 3-OH (2.5 %) and C\textsubscript{16:0} 3-OH (2.5 %). These data suggest that strain H1\textsuperscript{T} possesses a unique fatty acid profile among members of the family Rhodospirillaceae. The polar lipids consisted of phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and phosphatidylmonomethylethanolamine (PMME) (Fig. 2). The inability of strain H1\textsuperscript{T} to grow photoheterotrophically under anoxic conditions was similar to members of the genera recently established in the family Rhodospirillaceae (Liu et al., 2010, 2011). Aerobic liquid cultures of strain H1\textsuperscript{T} were colourless. Spectra of acetone: methanol (7:2) extracts from cells grown on TY agar plates for six days were analysed with a UV2450 spectrometer (Shimadzu) to determine whether strain H1\textsuperscript{T} possessed any pigments (Biebl et al., 2005). No spectrum corresponding to the presence of pigments could be observed (data not shown), indicating that bacteriochlorophyll \(a\) is absent.

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**Fig. 1.** Transmission electron micrograph of a negatively stained cell of strain H1\textsuperscript{T}. Bar, 1 \(\mu\)m.
The G+C content of strain H1\textsuperscript{T} genomic DNA was determined by thermal denaturation (Mandel & Marmur, 1968), with Escherichia coli K-12 used as a standard, and was calculated to be 65.1 mol\%. Chromosomal DNA was isolated and purified according to the method described by Yoon et al. (1996). The 16S rRNA gene was amplified using two bacterial universal primers, 27F and 1492R (Lane, 1991), and sequenced as described by Zhang et al. (2003). The nearly full-length H1\textsuperscript{T} 16S rRNA gene sequence comprised 1408 nt. Phylogenetic trees were reconstructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods in the MEGA 5 program (Tamura et al., 2011) with bootstrap values based on 1200 replications (Felsenstein, 1985) (Fig. 3). When subjected to BLAST analysis (http://blast.ncbi.nlm.nih.gov/blast), strain H1\textsuperscript{T} showed the highest 16S rRNA gene sequence similarity (99\%) with bacterial strain HE616173). The nearly full-length H1\textsuperscript{T} 16S rRNA gene sequence similarity between strain H1\textsuperscript{T} and all other members of the family Rhodospirillaceae, together with the differential phenotypic properties (data not shown), suggest that this isolate represents a novel species of a new genus for which the name Taonella mepensis gen. nov., sp. nov. is proposed.

**Description of Taonella gen. nov.**

Taonella (Ta.o.nel’la. N.L. fem. dim. n. Taonella named after Jian Tao, who discovered the organism).

Cells are Gram-negative, non-spore-forming, motile, slightly curved to straight rods. Strictly aerobic and heterotrophic. Internal membrane system and bacteriochlorophyll \(a\) are absent. Never phototrophic. Catalase- and oxidase-positive. Nitrate is reduced to nitrite. Major fatty acids are \(C_{18:1}\)\textsubscript{\( \Delta 9\)}C, \(C_{16:0}\) and \(C_{19:0}\) cyclo \(\omega 8\)c. The major ubiquinone is Q-10. The type species is *Taonella mepensis*.

**Description of Taonella mepensis sp. nov.**

*Taonella mepensis* (me.pen’sis. N.L. fem. adj. *mepensis* of or belonging to Mepe, short for the Laboratory of Microbial Ecology & Protein Engineering).

Cells are 1.2–1.4 \(\mu\)m wide and 1.5–1.7 \(\mu\)m long with a single polar flagellum. NaCl is not required for growth but up to 4\% NaCl is tolerated. The optimum pH for growth is 6.0–7.0 (range, 5.0–8.0). Starch and gelatin are hydrolysed; aesculin is not hydrolysed. \(H_2\)S and indole are not produced. Urease, \(\beta\)-galactosidase and arginine dihydrolase activities are not observed; weak glucose fermentation is observed. Grow on glucose (weakly), arabinose, gluconate, malic acid, adipic acid, citric acid, monomethylsuccinate, \(\alpha\)-ketoglutarate, \(\beta\)-hydroxybutyric acid, propionic acid, succinic acid, \(L\)-alaninamide, \(L\)-alanine, \(L\)-aspartic acid, \(L\)-glutamic acid, glycyL-L-glutamic acid, \(L\)-ornithine, \(L\)-proline and \(L\)-serine. The predominant cellular fatty acids are \(C_{18:1}\)\textsubscript{\( \Delta 9\)}C, \(C_{16:0}\) and \(C_{19:0}\) cyclo \(\omega 8\)c. The major ubiquinone is Q-10. The polar lipids consist of PG, PE and PMME. Tolerant of polymyxin B, rifampicin, ampicillin, gentamicin, tetracycline, chloramphenicol and penicillin. Sensitive to kanamycin, streptomycin, ofloxacin, neomycin and erythromycin.

The type strain, H1\textsuperscript{T} (=CICC 10529\textsuperscript{T} =CCTCC AB 2012861\textsuperscript{T} =KACC 16940\textsuperscript{T}), was isolated from activated sludge in Nantong, Jiangsu, China. The DNA G+C content of the type strain is 65.1 mol\%.

Fig. 2. Two-dimensional thin-layer chromatography of polar lipids of strain H1\textsuperscript{T}. AL1–AL3, unidentified aminolipids (AL1 is directly underneath the spot labelled PG, but is smaller in size).
Fig. 3. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain H1T within the family Rhodospirillaceae. Bootstrap values (≥70%) based on 1200 replications are shown at branch nodes. The sequence of E. coli ATCC 11775T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

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References


