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Elioraea

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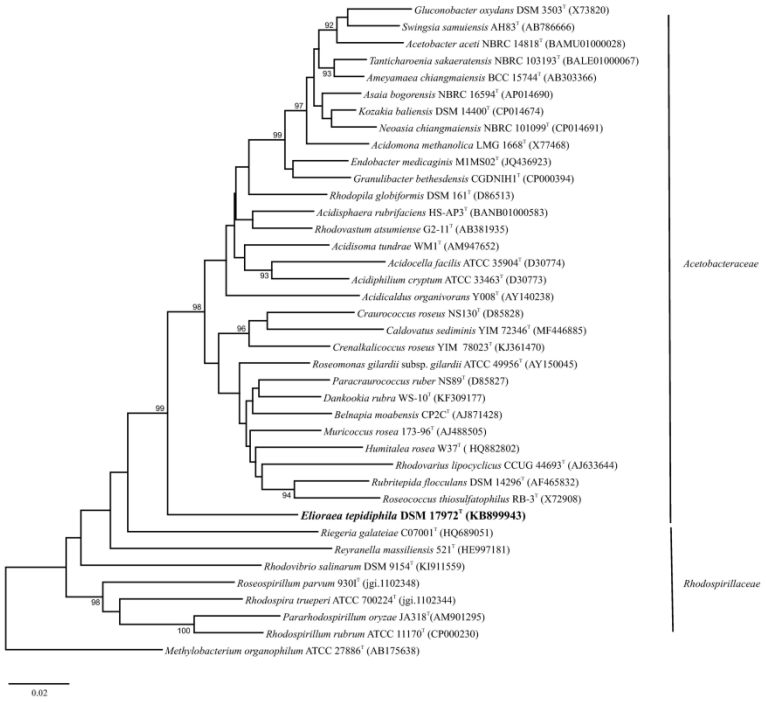

Figure 1. 16S rRNA gene sequence neighbor-joining tree demonstration the position of the genus Elioraea within the radiation of genera of the family Acetobacteraceae. The scale bar represents 2 inferred substitutions per 100 nucleotides. The numbers at branching points represent bootstrap values from 1000 replications. The tree was rooted using the sequence of Methylobacterium organophilum.

269x205mm (300 x 300 DPI)

Proteobacteria / *Alphaproteobacteria* / *Rhodospirillales* / *Acetobacteraceae*

Elioraea

Albuquerque, Rainey, Nobre and da Costa 2008, 776[VP]

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E.li.o'ra.e.a. N.L. fem. n. *Elioraea,* named in honour of Israeli microbiologist Eliora Z. Ron.

Abstract:

Rod-shaped cells, 0.5–1.0 µm in width and 1.0–1.5 µm in length. Motile by one polar flagellum. Endospores are not observed. Stain Gram-negative. Colonies are non-pigmented. Slightly thermophilic and slightly alkaliphilic. Strictly aerobic. Cytochrome c oxidase and catalase positive. Facultatively mixotrophic. Thiosulfate is oxidized to sulfate with the enhancement of growth. Organic acids, proline and glutamine are used as carbon and energy sources; sugars and polyols are not used for growth. Bacteriochlorophyll *a* and *puf* genes are not present. Major respiratory quinone is ubiquinone 10. Major polar lipids are phosphatidylcholine, phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and two unidentified aminolipids. Major fatty acids are straight chain saturated and unsaturated fatty acids including hydroxy derivatives. 16S rRNA gene sequence affiliates this genus to family *Acetobacteraceae*. Isolated from a hydrothermal area.

DNA G+C content (mol %): 70.9 (HPLC).

Type species: ***Elioraea tepidiphila*** Albuquerque, Rainey, Nobre and da Costa 2008, 776^{VP}

Keywords: *Alphaproteobacteria*, *Rhodospirillales*, *Acetobacteraceae*, slightly thermophilic, slightly alkaliphilic.

Rod-shaped cells, 0.5–1.0 µm in width and 1.0–1.5 µm in length. **Motile** by one polar flagellum. Endospores are not observed. Stain Gram-negative. Colonies are **nonpigmented**. **Slightly thermophilic** and **slightly alkaliphilic**. **Strictly aerobic**. Cytochrome c oxidase and catalase positive. **Facultatively mixotrophic. Thiosulfate is oxidized to sulfate** with the enhancement of growth. **Organic acids**, proline and

glutamine are used as carbon and energy sources; sugars and polyols are not used for growth. Bacteriochlorophyll *a* and *puf* genes are not present. Major respiratory quinone is **ubiquinone 10**. Major polar lipids are phosphatidylcholine, **phosphatidylethanolamine**, **diphosphatidylglycerol**, **phosphatidylglycerol** and **two unidentified aminolipids**. Major fatty acids are **straight chain saturated** and **unsaturated fatty acids** including **hydroxy** derivatives. 16S rRNA gene sequence affiliates this genus to family *Acetobacteraceae.* Isolated from a **hydrothermal area**.

DNA G+C content (mol %): 70.9 (HPLC).

Type species: ***Elioraea tepidiphila*** Albuquerque, Rainey, Nobre and da Costa 2008, 776^{VP}

Number of species with validated names: 1.

Family classification: *Acetobacteraceae* (fbm00174).

Further descriptive information

Phylogeny

16S rRNA gene sequence analysis shows the genus *Elioraea* to fall within the radiation of the order *Rhodospirillales* (obm00073) and results of bootstrap analysis demonstrate a strong affiliation with the cluster of taxa comprising the family *Acetobacteraceae* (Fig 1). Pairwise similarity values to the type species of genera of the family *Acetobacteraceae* are in the range 90-91% supporting the distinct lineage status of the species *Elioraea tepidiphila*.

Cell morphology and colony characteristics

Elioraea tepidiphila forms rod-shaped cells 0.5–1.0 µm in width by 1.0–1.5 µm in length that are motile by one polar flagellum. The organism produces translucent non-pigmented colonies. Carotenoid pigments were not detected in extracts under any experimental conditions.

Nutrition and growth conditions

This slightly thermophilic species has an optimum growth temperature of about 45–50ºC and the temperature range for growth is between 30ºC and 52.5ºC. With the exception of *Rubritepida flocullans* (Alarico et al., 2002), *Caldovatus sediminis* (Habib et al., 2017) and *Acidicaldus organivorus* (Johnson et al., 2006), other closely related genera have lower optimum growth temperatures. The optimum pH for growth of the type strain of *E. tepidiphila* is in the range of pH 8.0–8.5, but does not grow below pH 6.0 or above pH 9.5 making the organism also slightly alkaliphilic. Growth occurs in *Thermus* medium, Degryse medium 162 and R3A medium, however, the growth rate increases in R3A medium to which starch and glucose have not been added. Yeast extract is necessary for growth in minimal medium (Albuquerque et al., 2008).

Elioraea tepidiphila was unable to grow on any of the carbohydrates and polyols examined, but this organism assimilates the majority of the organic acids tested, proline and glutamine.

Metabolism and metabolic pathways

Cytochrome c oxidase and catalase are present. The organism reduces nitrate to nitrite but anaerobic growth with nitrate as the electron acceptor was not observed. The addition of thiosulfate to modified medium 27 lacking magnesium sulfate, vitamin B12 solution, L-cysteinium chloride and resazurin, and containing yeast extract, succinate and acetate

(http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium27.pdf) led to an increase in the biomass of *E. tepidiphila* indicating that thiosulfate was used as an energy source in the presence of organic substrates. The determination of the levels of sulfate and thiosulfate in the medium during growth indicated that thiosulfate was completely oxidized to sulfate. Autotrophic growth with H_2, thiosulfate, tetrathionate, sulfite, sulfide as well as photoautotrophic growth with thiosulfate and sulfite was not observed. *E. tepidiphila* appears to be mixotrophic in the presence thiosulfate and organic carbon sources. Bacteriochlorophyll *a* is not detected in *E. tepidiphila* under aerobic or anaerobic growth conditions and the presence of *pufL* and *pufM* genes were are not observed.

The genome sequence indicates that *E. tepidiphila* has genes for the metabolism of glucose through the Embden-Meyerhof-Parnas pathway, namely a putative hexokinase (EC 2.7.1.2) as well as phosphofructokinase (EC 2.7.1.11). However, this organism does not grow on sugars as single carbon sources, but the organism degrades starch assessed by iodine clearing on starch plates. Starch degradation was also predicted by the identification of a 1,4-α-glucan branching enzyme (EC 2.4.1.18) and α-amylase (EC 3.2.1.1) that can convert starch to maltose. However, ABC or PTS transport systems for sugars, including maltose, could not found in the genome. The organism possesses all genes for gluconeogenesis. One of the core enzymes of the Entner-Doudoroff pathway, namely 6-phosphogluconolactonase (EC 3.1.1.31) leading to the synthesis of 6-phosphogluconate is not encountered in the genome, thus precluding the use of this pathway for the catabolism of hexoses. The tricarboxylic acid cycle (Krebs cycle) is complete. Oxidative phosphorylation proceeds via NADH dehydrogenase, succinate dehydrogenase, cytochrome c oxidase cbb3 and an F-type ATPase. This organism lacks *sox*A and *sox*X, so that the oxidation of thiosulfate to sulfate proceeds via thiosulfate sulfurtransferase (EC 2.8.1.1) to sulfite and then to sulfate via sulfite oxidase (EC 1.8.3.1)

or sulfite dehydrogenase (EC 1.8.2.1) to sulfate corroborating the phenotypic results which indicate that the organism is mixotrophic. Nitrate reduction to nitrite is confirmed by genome parameters that predict a nitrate transporter (NRT) as well as the nitrate reductase NarGHI (EC 1.7.5.1).

Polar lipids, respiratory lipoquinones and fatty acids

Phosphatidylcholine, phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and two unidentified aminolipids dominate among the polar lipids of *E. tepidiphila* on thin-layer chromatography. Ubiquinone 10 is the major respiratory quinone, which along with ubiquinone 9 is the most common quinone in the family *Acetobacteraceae*. The fatty acid composition of *E. tepidiphila* was dominated by $C_{18:1}$ ω7*c*, $C_{18:0}$, $C_{19:0}$ cyclo ω8*c* and a rare fatty acid with an equivalent chain length (ECL) of 20.195, tentatively identified as $C_{19:0}$ 2-OH cyclo ω8 (Δ11:12) (Table 1) (Alarico et al., 2002; Albuquerque et al., 2008).

Ecology and habitats

This bacterium has only been isolated from a geothermal spring in the Furnas area of the Island of S. Miguel in the Azores with a temperature of 70ºC and a pH of 7.5. An isolate of *Truepera radiovictrix,* strain TU-8, that is also slightly thermophilic and alkaliphilic was recovered from the same hot spring, but belongs to the phylum "*Deinococcus-Thermus*" (pbm00012) (Albuquerque et al., 2005), Two isolates of *Tepidicella xavieri*, TU-16^T and TU-18 that are also slightly thermophilic but belong to the class *Betaproteobacteria* (cbm00042) (França et al., 2006) were also recovered from the same site. Uncultured environmental clones closely related to the type strain of *E. tepidiphila* and showing 99% pairwise similarity have been recovered from a drilling fluid in China

(AY820713) (Zhang et al., 2005), activated sludge (EF648061) and soil contaminated with chromium in Mexico (KR779695). There are two additional sequences showing 97% 16S RNA gene sequence similarity and representing sequences recovered from thermal environments in China (KM221313) and USA (MH555907). The clone MH555907 was proposed as a *Candidatus* species of the genus *Elioraea* (Tank et al., 2017).

Genome features

A draft genome under the accession number NZ_ARKI01000000 has been produced by DOE Joint Genome Institute that clarifies and extends many of the phenotypic characteristics of *E. tepidiphila*. The genome is 4,304 kb with a G+C of 71.3% (70.9% by HPLC). The organism possesses one 5S, one 16S and one 23S rRNA gene.

Enrichment and isolation procedures

Water samples were filtered through membrane filters (Gelman type GN-6; pore size 0.45 µm; diameter 47 mm). These filters were placed on the surface of *Thermus* medium agar plates (Albuquerque and da Costa 2014), wrapped in plastic bags and incubated at 50°C until colonies appeared on the filters. Later it was observed that the organism had a higher growth rate in R3A medium to which starch and glucose were not added.

Thermus medium (https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium1033.pdf) contains (per liter of water) 1 g yeast extract (Difco), 1 g tryptone (Difco), 100 ml of a macroelements solution (10x concentrated), 10 ml of a trace elements solution (100x concentrated) and 10 ml of 0.17 mM $FeCl_3.6H_2O$, pH adjusted to 8.2 before autoclaving. The 10x concentrated macroelements solution contains per liter of water: 1 g nitrilotriacetic acid, 0.6 g $CaSO_4.2H_2O$, 1 g $MgSO_4.7H_2O$, 0.08 g NaCl, 1.03 g KNO_3,

6.89 g $NaNO_3$, 1.11 g Na_2HPO_4. The 100x concentrated trace elements solution contains per liter of water: 0.22 g $MnSO_4.H_2O$, 0.05 g $ZnSO_4.7H_2O$, 0.05 g H_3BO_3, 0.0025 g $CuSO_4.5H_2O$, 0.0025 g $Na_2MoO_4.2H_2O$, 0.0046 g $CoCl_2.6H_2O$ (Albuquerque and da Costa 2014; Castenholz 1969; Williams and da Costa, 1992).

Degryse medium 162 contains (per liter of water) 2.5 g yeast extract (Difco), 2.5 g tryptone (Difco), 100 ml of a macroelements solution (10x concentrated), 5 ml of a trace elements solution (100x concentrated) (identical to the trace elements of *Thermus* medium), 15 ml of 0.2 M $Na_2HPO_4.12H_2O$, 10 ml of 0.2 M KH_2PO_4 and 0.5 ml of 0.01 M ferric citrate, pH adjusted to 7.5 before autoclaving. The 10x concentrated macroelements solution contained per liter of water: 1 g nitrilotriacetic acid, 0.4 g $CaSO_4.2H_2O$ and 2 g $MgCl_2.6H_2O$ (Degryse et al., 1978, Kristjánsson et al., 1986; Williams and da Costa, 1992). The concentration of $Na_2HPO_4.12H_2O$ and KH_2PO_4 has been reduced in this medium because the growth of some isolates was inhibited by the level of phosphate described in the original composition (Degryse et al., 1978). All concentrated solutions of the both media can be stored at 4ºC. A minimal medium derived from Degryse medium 162 can be used to assess assimilation of organic compounds by replacing tryptone by ammonium sulfate (0.5 g/l) and decreasing the amount of yeast extract to 0.1 g/l.

Modified R3A medium contains (per liter of water) 1.0 g yeast extract (Difco), 1.0 g protease peptone No.3 (Difco), 1.0 g casamino acids (Difco), 0.6 g sodium pyruvate, 0.6 g KH_2PO_4 and 0.1 g $MgSO_4.7H_2O$ (Reasoner and Geldreich, 1985).

Maintenance procedures

Strains of *E. tepidiphila* do not require special procedures for maintenance and long-term storage. Generally, the organisms are maintained on R3A medium without starch and

glucose at 4°C for a few days and can be stored frozen at –70°C in R3A medium without starch and glucose, in *Thermus* or Degryse medium 162 containing 15% glycerol without loss of viability for several years. Long-term preservation is by freeze drying or storage in liquid nitrogen.

Taxonomic comments

The species *E. tepidiphila* is not closely related to any other known alphaproteobacterium, The closest relatives show less than 91% 16S rRNA gene sequence similarity placing this organism at the base of the family *Acetobacteraceae*, a position supported by a 99% bootstrap value. As a new strain and species of this genus are isolated a novel family designation for the genus *Elioraea* may be appropriate. The very rare fatty acid $C_{19:0}$ 2-OH cyclo ω8 (Δ11:12) could also indicate that this organism belongs to a novel family, nevertheless the species *Rubritepida flocculans,* which belongs to the family *Acetobacteraceae* also possesses this fatty acid and thus, it cannot be used as a diagnostic characteristic of a putative new family (Alarico et al., 2002). *Elioraea tepidiphila* has been assigned to the family *Acetobacteraceae* that comprises a very large number of species with diverse phenotypic characteristics. A few species, such as *Caldovatus sediminis* (Habib et al., 2017), *Acidicaldus organivorus* (Johnson et al., 2006) and *Rubritepida flocculans* (Alarico et al., 2002) are slightly thermophilic like *E. tepidiphila*, but all other species in this family are mesophilic. Moreover, these slightly thermophilic organisms utilize sugars for growth. Many species of this family possess bacteriochlorophyll *a* and *puf* genes, but many other species such as *E. tepidiphila* do not. *Elioreae tepidiphila* can be distinguished from all other closely related species by the inability to grow on sugars, the limited ability to grow on amino acids (glutamine and proline) and the ability to grow at elevated temperatures.

Characteristics of the species of the genus *Elioraea*

Elioraea tepidiphila

Albuquerque, Rainey, Nobre and da Costa 2008, 776^{VP}

te.pi.di.phi´la. L. adj. *tepidus*, warm; G. adj. *philos*, loving; N. L. fem. adj. *tepidiphila*, an organism loving warmth.

Forms short rod-shaped cells about 0.5–1.0 μm in width and 1.0–1.5 μm in length. The cells stain Gram-negative and are motile by one polar flagellum. Endospores are not formed. Colonies on *Thermus* medium, Degryse medium 162 and R3A medium are nonpigmented. Carotenoid pigments were not detected. The optimum growth temperature is about 45–50ºC; growth occurred in the range of 30–50ºC; the optimum pH is between 8.0 and 8.5; the pH range for growth is 6.0–9.5. Optimum growth occurs without added NaCl; growth occurs in media with NaCl up to 1.5% (w/v). Aerobic with a strictly respiratory type of metabolism. Facultatively mixotrophic. Autotrophic growth with H_2, thiosulfate, tetrathionate, sulfite and sulfide were not observed. Phototrophic growth growth with thiosulfate and sulfide were not observed. Bacteriochlorophyll *a* is not present; *pufL* and *pufM* genes are not detected. Thiosulfate is oxidized to sulfate. Nitrate is reduced to nitrite. Cytochrome c oxidase and catalase positive. DNAse positive. Alkaline phosphatase, esterase (C 4), esterase lipase (C 8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase test positive in API ZYM; other activities are negative. Starch, xylan and hippurate are hydrolyzed. Gelatin, casein, aesculin, arbutin and elastin are not hydrolyzed. Yeast extract is necessary for growth. DL-lactate, α-ketoglutarate, pyruvate, succinate, malate, citrate, fumarate, L-glutamate, D-gluconate, L-proline and L-glutamine are assimilated; D-glucose, D-

fructose, D-galactose, D-mannose, L-rhamnose, L-fucose, L-sorbose, D-ribose, D-xylose, D-arabinose, L-arabinose, sucrose, maltose, lactose, D-cellobiose, D-trehalose, D-raffinose, D-melibiose, D-melezitose, glycerol, ribitol, xylitol, sorbitol, D-mannitol, *myo*-inositol, L-erythritol, acetate, formate, D-glucoronate, aspartate, L-alanine, L-asparagine, glycine, L-histidine, L-lysine, L-arginine, L-serine, L-valine, L-phenylalanine, L-leucine, L-isoleucine, L-ornithine, L-methionine, L-threonine and L-cysteine are not used as carbon and energy sources. Acid is produced from the following substrates using the API 50CH system: D-ribose, D-fructose, L-sorbose, D-tagatose and potassium 5-ketogluconate. Major respiratory quinone is ubiquinone 10 (U-10). Major polar lipids are phosphatidylcholine (PC), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and two unidentified aminolipids. The major fatty acids are $C_{18:0,}$ $C_{18:1}$ ω7*c*, $C_{19:0}$ 2-OH cyclo ω8 (Δ11:12) and $C_{19:0}$ cyclo ω8*c*. This bacterium was isolated from a hot spring in the Furnas geothermal area in the Island of São Miguel, Portugal.

Source: Hydrothermal water.

DNA G+G content (mol %): 70.9 (HPLC).

DNA G+G content (mol %): 71.3 (Genome).

Type strain: TU-7, DSM 17972, CIP 109115.

GenBank/EMBL/DDBJ/SILVA SSU accession number (16S rRNA gene): EF519867.

GenBank/EMBL/DDBJ/SILVA SSU accession number (genome): NZ_ARKI01000000.

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TABLE 1. Fatty acid composition of *Elioraea tepidiphila,* strain TU-7^T, grown on modified R3A medium agar plates at 50ºC for 48 h

Fatty acids	**ECL**	***Elioraea tepidiphila*[a] TU-7^T**
Unknown 14.960	14.960	0.8 ± 0.1
$C_{16:0}$	16.000	5.4 ± 0.2
$C_{16:0}$ 2-OH	17.048	1.9 ± 0.1
$C_{18:1}$ ω7*c*	17.823	19.0 ± 0.5
Unknown 17.893	17.893	2.5 ± 0.1
$C_{18:0}$	18.000	24.8 ± 0.2
$C_{18:1}$ ω7*c* 11-methyl	18.081	8.0 ± 0.1
$C_{19:0}$ cyclo ω8*c*	18.902	12.4 ± 0.6
$C_{18:0}$ 2-OH	19.089	0.6 ± 0.1
Unknown 19.343	19.343	1.2 ± 0.1
$C_{18:0}$ 3-OH	19.550	3.8 ± 0.1
$C_{19:0}$ 2-OH cyclo ω8 (Δ11:12)	20.195	18.6 ± 0.1

Results are the percentage of the total fatty acids; ±, results are the mean plus the standard deviation of two to four analyses; values for fatty acids present at less than 0.5% are not shown. ECL, equivalent chain length.

[a]Albuquerque et al. (2008).

Figure 1. 16S rRNA gene sequence neighbor-joining tree demonstration the position of the genus *Elioraea* within the radiation of genera of the family *Acetobacteraceae*. The scale bar represents 2 inferred substitutions per 100 nucleotides. The numbers at branching points represent bootstrap values from 1000 replications. The tree was rooted using the sequence of *Methylobacterium organophilum*.

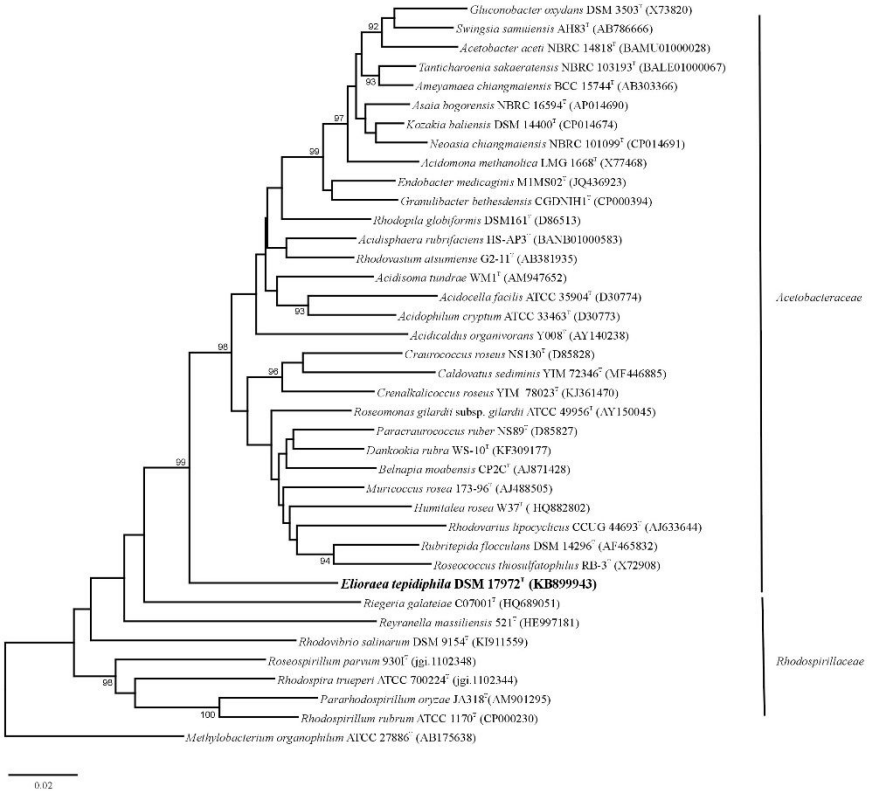
Gluconobacter oxydans DSM 3503T (X73820)
Swingsia samuiensis AH83T (AB786666)
Acetobacter aceti NBRC 14818T (BAMU01000028)
Tanticharoenia sakaeratensis NBRC 103193T (BALE01000067)
Ameyamaea chiangmaiensis BCC 15744T (AB303366)
Asaia bogorensis NBRC 16594T (AP014690)
Kozakia baliensis DSM 14400T (CP014674)
Neoasia chiangmaiensis NBRC 101099T (CP014691)
Acidomona methanolica LMG 1668T (X77468)
Endobacter medicaginis M1MS02T (JQ436923)
Granulibacter bethesdensis CGDNIH1T (CP000394)
Rhodopila globiformis DSM161T (D86513)
Acidisphaera rubrifaciens HS-AP3T (BANB01000583)
Rhodovastum atsumiense G2-11T (AB381935)
Acidisoma tundrae WM1T (AM947652)
Acidocella facilis ATCC 35904T (D30774)
Acidophilum cryptum ATCC 33463T (D30773)
Acidicaldus organivorans Y008T (AY140238)
Craurococcus roseus NS130T (D85828)
Caldovatus sediminis YIM 72346T (MF446885)
Crenalkalicoccus roseus YIM 78023T (KJ361470)
Roseomonas gilardii subsp. gilardii ATCC 49956T (AY150045)
Paracraurococcus ruber NS89T (D85827)
Dankookia rubra WS-10T (KF309177)
Belnapia moabensis CP2CT (AJ871428)
Muricoccus roseus 173-96T (AJ488505)
Humitalea rosea W37T (HQ882802)
Rhodovarius lipocyclicus CCUG 44693T (AJ633644)
Rubritepida flocculans DSM 14296T (AF465832)
Roseococcus thiosulfatophilus RB-3T (X72908)
Elioraea tepidiphila DSM 17972T (KB899943)
Riegeria galateiae C07001T (HQ689051)
Reyranella massiliensis 521T (HF997181)
Rhodovibrio salinarum DSM 9154T (KI911559)
Roseospirillum parvum 930IT (jgi.1102348)
Rhodospira trueperi ATCC 700224T (jgi.1102344)
Pararhodospirillum oryzae JA318T(AM901295)
Rhodospirillum rubrum ATCC 1170T (CP000230)
Methylobacterium organophilum ATCC 27886T (AB175638)
Acetobacteraceae
Rhodospirillaceae
0.02

Figure 1.