Tepidiphilus

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2. KEYWORDS: *Tepidiphilus margaritifer*; aerobic digester; thermophilic; chemo-organotroph;

3. ABSTRACT:

Rods with poly-beta-hydroxybutyrate granules, non-spore forming, **Gram-negative** with a **single polar flagellum**. Aerobic, able to grow anaerobically in the presence of nitrate. **Thermophilic**, with the ability to grow between 25 and 61 ºC, with optimum at 50-55 ºC, pH 6-8 and no special growth requirements. **Chemo-organotroph**. Organic acids and amino acids, but not sugars, can be used as single carbon sources. Catalase- and cytochrome c oxidase positive. **Ubiquinone 8** is the major respiratory quinone and the predominant fatty acids are **$C_{16:0}$, cyclo-$C_{17:0}$, cyclo-$C_{19:0}$ *ω8c*, $C_{18:1}$ *ω6c*/*ω7c*,** and **$C_{17:0}$**. Major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidyl coline. Isolated from thermophilic digester, oil well or terrestrial hot spring.

4. DEFINING PUBLICATION:

Tepidiphilus, Manaia, Nogales and Nunes, 2003, 1409^{VP} *emend*. Poddar, Lepcha and Das, 2014, 232.

5. ETYMOLOGY:

Tepidiphilus (Te.pi.di'phi.lus. L. adj. *tepidus* lukewarm; Gr. adj. *philos* friendly to; N.L. masc. n. *Tepidiphilus* liker of lukewarm conditions)

6. GENERIC DEFINITION:

Rods with poly-beta-hydroxybutyrate granules, non-spore forming, **Gram-negative** with a **single polar flagellum**. Aerobic, able to grow anaerobically in the presence of nitrate. **Thermophilic**, with the ability to grow between 25 and 61 ºC, with optimum at 50-55 ºC, pH 6-8 and no special growth requirements. **Chemo-organotroph**. Organic acids and amino acids, but not sugars, can be used as single carbon sources. Catalase- and cytochrome c oxidase positive. **Ubiquinone 8** is the major respiratory quinone and the predominant fatty acids are **$C_{16:0}$, cyclo-$C_{17:0}$, cyclo-$C_{19:0}$ *ω8c*, $C_{18:1}$ *ω6c/ω7c,*** and **$C_{17:0}$**. Major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidyl coline. Isolated from thermophilic digester, oil well or terrestrial hot spring.

The DNA G+C content (mol %) is 58.6 – 66.1 (HPLC method).

Type species: *Tepidiphilus margaritifer*, Manaia, Nogales and Nunes 2003, 1409^{VP} *emend.* Poddar, Lepcha and Das, 2014, 233.

Number of species with validated names: 3.

7. FAMILY CLASSIFICATION:

Hydrogenophilaceae (fbm00184)

8. FURTHER DESCRIPTIVE INFORMATION:

8.1. Cell morphology:

Three validly named species are described within the genus *Tepidiphilus*: *T. margaritifer*, *T. succinatimandens* and *T. thermophilus* (Manaia et al., 2003, Poddar et al., 2014). Cells are Gram-negative non-spore forming rods, with 0.3-0.7 µm width and 1.5-2.0 µm long, motile by means of a single polar flagellum. Poly-beta-hydroxybutyrate intracellular inclusions were described in the type strain of the type species.

8.2. Colonial and cultural characteristics:

On nutritive agar media, form cream round colonies, with 1-2 mm of diameter, after up to 2 days for *T. margaritifer* and *T. thermophilus* or up to 10 days for *T. succinatimandens* at temperature of 50-55ºC. Under a strong light source, *T. margaritifer* colonies present a nacre-like (or pearl-like) appearance, which is whitish and shines with different colors.

8.3. Nutrition and growth conditions:

None of the type strains of the validly named species of the genus *Tepidiphilus* are able to oxidize sugars. In contrast, all the strains are able to use organic acids and amino acids as single carbon sources, namely malate, caproate, succinate, fumarate, pyruvate, glutamic acid and aspartic acid. The range of organic acids and amino acids that can be used as carbon sources vary for the different species with acetate, adipate, benzoate, and L-proline being used only by *T. margaritifer*, i-erythritol, xylitol, L-aspartic acid, hydroxy-L-proline, L-leucine, L-serine or glycerol being used only by *T. succinatimandens*, and cis-aconitic acid, D-galacturonic acid, alpha-ketoglutaric acid, DL-lactic acid, D-alanine and D-glucose 6-phosphate used only by *T. thermophilus* (Table 1).

Able to grow in the temperature range of 25-35 to 60-61 ºC, at a pH range of 5.5-6.0 to 7.5-8.0, and at NaCl concentrations up to 3% (w/v) (for *T. margaritifer* and *T. succinatimandens*, no information available for *T. thermophilus*), with optimal at 50-55ºC and pH 6.5 -7.

<Table 1 near here>

8.4. Metabolism:

Aerobic, with anaerobic growth in the presence of nitrate. Nitrate is reduced to nitrite by *T. thermophilus* and to nitrous oxide by *T. succinatimandens* and probably by *T. margaritifer*, whose nitrate reduction does not produce nitrite. Non-fermentative, chemo-organotroph with the ability to use organic acids and amino acids as single carbon sources. Sugars are not used as carbon sources, with the oxidation of D-glucose 6-phosphate an exception observed in *T. thermophilus*. Tests positive for gelatinase, alkaline phosphatase, and nitrate reductase production, among others, and for DNA and aesculin hydrolysis. Reacts negatively for indole, methyl red and Voges–Proskauer tests, and production of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, and tryptophan deaminase, among others.

8.5. Chemotaxonomic characteristics:

The predominant fatty acids are $C_{16:0}$ (22-30%), cyclo-$C_{17:0}$ (9-17%), cyclo-$C_{19:0}$ *ω8c* (18-30%), $C_{18:1}$ *ω6c*/*ω7c* (7-14%), and $C_{17:0}$ (1-10%). Hydroxyl fatty acids detected in the three species are the components $C_{16:0}$ 3-OH (1.5-1.9%) and the $C_{10:0}$ 3-OH (0.9-1.3%) (Poddar et al., 2014). Major polar lipids observed in the three species are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylcholine. Other minor and

variable among species lipids comprised amino-, glyco- and unknown lipids. The peptidoglycan tetrapeptide contains meso-diaminopimelic acid.

8.6. Genome:

Two genome sequences are available, for *Tepidiphilus thermophilus* JHK30^T (JCM 19170^T) deposited under the DDBJ/EMBL/GenBank accession no. LIPU00000000 (Poddar et al., 2016) and for *T. margaritifer* DSM 15129, deposited under the DDBJ/EMBL/GenBank accession no. NZ_AUDR00000000.1. The *T. thermophilus* draft genome assembly resulted in 37 contigs and 35 scaffolds, with an *N*50 contig size of 141.2 kb and the largest contig 363.6 kb long. The estimated genome size was of 2.3 Mb and the DNA G+C content (mol %) was of 66.1%. The draft genome sequence had 2,186 candidate protein-coding genes (CDSs) and 68 RNAs, specifically 46 tRNAs and seven rRNAs. The *T. margaritifer* draft genome assembly resulted in 22 contigs and 19 scaffolds, with an *N*50 contig size of 277 kb. The estimated genome size was of 2.2 Mb and the DNA G+C content (mol %) was of 64.8%. The draft genome sequence had 2,067 candidate protein-coding genes (CDSs).

8.7. Ecology:

Members of the three *Tepidiphilus* species were isolated from natural or man-made environments with temperatures above 50ºC, in different regions of the globe – Portugal (Porto), India (Jharkhand), Australia (Queensland). *T. margaritifer* was isolated from an enrichment culture obtained from a thermophilic aerobic digester of a domestic wastewater treatment plant (Manaia et al., 2003). *T. thermophilus* was isolated from a sediment sample from a terrestrial hot spring (Poddar et al., 2014). *T. succinatimandens* was isolated from an oil sample collected from the Riverslea oilfield (Salinas et al., 2004).

9. ENRICHMENT/ISOLATION PROCEDURES:

The type strain of *T. margaritifer* was enriched from a thermophilic aerobic digester of a domestic wastewater-treatment plant that reaches temperatures of 60 ºC, using the polycaprolactone diol polymer (Solvay) with a molecular mass of 1000 Da as carbon source in mineral medium (medium A) (Manaia and Moore, 2002) at 50ºC. Purification was made by subculturing the mixed culture on Luria–Bertani (LB) broth containing 20 g agar L^{-1} (LB agar) (Carlton and Brown, 1981).

The type strain of *T. thermophilus* was isolated from sediment samples collected from a terrestrial hot spring at Surajkund, Jharkhand, India, with a surface temperature of 60 ºC and pH 7.2. Sediment samples (2.5 g, wet wt) were used to inoculate 50 mL of nutrient broth, pH 7.2 (Difco), in a 250 mL conical flask, and incubated with agitation at 55 ºC. The resultant overnight culture was serially diluted and plated onto nutrient agar medium that was incubated for 2 days at 55 ºC. The strain JHK30^T, representative of a set of similar isolates, was further characterized after purification by sub-culturing.

The type strain of *T. succinatimandens* was isolated from an oil sample collected from the Riverslea oilfield in the Bowen–Surat basin of Eastern Australia (Queensland), which was stored at 4 ºC until being enriched anaerobically (Fardeau et al., 2000). Enrichment took place in mineral medium supplemented with 1g L^{-1} yeast extract and with a H_2/CO_2 mixture (2 bars) supplied as possible energy and carbon sources. After three enrichment series at 50 ºC, it was isolated heterotrophic bacteria that used organic acids, aerobically or anaerobically.

10. MAINTENANCE PROCEDURES:

Preservation can be made on nutritive media, on agar at 4 ºC for short periods, or in broth supplemented with 15% (v/v) glycerol at -80 ºC for long periods.

11. DIFFERENTIATION OF THE GENUS *TEPIDIPHILUS* FROM OTHER GENERA:

The family *Hydrogenophilaceae* comprises at the moment of writing two genera of moderate thermophiles - the genus *Hydrogenophilus* (see gbm00968) and the genus *Tepidiphilus*. Although the DNA G+C content (mol %) and the fatty acids profile are similar in both genera, a clear distinction is observed at the metabolic level, with *Tepidiphilus* including obligatory chemo-organotrophic bacteria and *Hydrogenophilus* comprising facultative hydrogen-oxidizing autotrophic bacteria. The 16S rRNA gene based phylogenetic analysis support the differentiation of these two genera from other closely related, like *Thauera* (see gbm01004) or *Azoarcus* (gbm00994).

<Figure 1 near here>

12. TAXONOMIC COMMENTS:

The genus *Tepidiphilus* comprises three species each represented by a single strain. The genus was proposed in 2003 based on the description of a single strain representative of the species *Tepidiphilus margaritifer* (Manaia et al., 2003). The species *Petrobacter*

succinatimandens, validly named in 2004, was later considered a member of the genus *Tepidiphilus*, that at the time of its description could not be compared with the type species *Tepidiphilus margaritifer*. The reclassification was proposed by Poddar *et al*. (2014), who proposed a third *Tepidiphilus* species, *T. thermophilus*. The 16S rRNA gene sequence identity within the genus is of 98.9% and 98.7% between *T. margaritifer* and *T. succinatimandens* and *T. thermophilus*, respectively; and of 99.7% identity between the two latter species, *T. succinatimandens* and *T. thermophilus* (Poddar et al., 2014). While the similarity of chemotaxonomic features support that all are members of the same genus, the distinctive phenotypic features and the DNA-DNA hybridization values below 70% (35-58%) give space for the definition of three species, in spite of the high 16S rRNA gene sequence identity values.

13. LIST OF SPECIES OF THE GENUS *TEPIDIPHILUS*:

1. ***Tepidiphilus margaritifer*** Manaia, Nogales and Nunes 2003, 1409^{VP} *emend*. Poddar, Lepcha and Das, 2014, 233.

margaritifer (mar.ga.ri´ti.fer. L. n. *margarita* pearl; L. masc. suffix *-fer* carrying; N.L. masc. adj. *margaritifer* pearl-carrying, referring to the nacre-like appearance of the colonies).

In addition to the genus description, the species can be characterized by the following traits: forms rod-shaped cells, 2.0 µm long and 0.7 µm wide. On LB agar, colonies are nacre-like and 1–2 mm in diameter after 36–48 h. Growth occurs between 25 °C and 61 °C, with an optimum at approximately 50 °C. Growth occurs between pH 6 and 8, and up to 3% NaCl. Nitrate is reduced into to a compound more reduced than nitrite. Hydrogenase-positive. The DNA G+C content (mol %) is 64.8 (HPLC).

Type strain: N2-214 (=DSM 15129=LMG 21637).

GenBank accession number (16S rRNA): AJ504663.

GenBank accession number (genome): NZ_AUDR00000000.1

2. ***Tepidiphilus succinatimandens*** Poddar, Lepcha and Das, 2014, 234^{VP} (*Petrobacter succinatimandens* Salinas, Fardeau, Cayol, Casalot, Patel, Thomas, Garcia and Ollivier, 2004, 648)

succinatimandens (suc.ci.na.ti.man´dens. N.L. n. *succinatum* succinate; L. part. adj. *mandens* eating, consuming; N.L. masc. adj. *succinatimandens* consuming succinate).

In addition to the genus description, the species can be characterized by the following traits: forms rod-shaped cells, 2.0 µm long and 0.3–0.4 µm wide. On roll tubes, colonies are yellowish and 1–2 mm in diameter after one week at 50 °C. Growth occurs between 35 °C and 60 °C, with an optimum at approximately 55 °C. Growth occurs between pH 5 and 8, and up to 3% NaCl. Nitrate is reduced to nitrous oxide.

The DNA G+C content (mol %) is 58.6 (HPLC).

Type strain: 4BON (=DSM 15512=CIP 107790).

GenBank accession number (16S rRNA): AY219713.

3. ***Tepidiphilus thermophilus*** Poddar, Lepcha and Das, 2014, 234^{VP}

thermophilus (ther.mo´phi.lus. Gr. n. *thermê* heat: Gr. adj. *philos* friend, loving; N.L. masc. adj. *thermophilus* heat-loving).

In addition to the genus description, the species can be characterized by the following traits: forms irregular rods, 1.5-1.8 µm long and 0.6–0.65 µm wide. On nutrient agar colonies are creamish and 1–2 mm in diameter. Growth occurs between 30 °C and 60 °C,

with an optimum at 50-55 °C. Growth occurs between pH 5.5 and 7.5. Nitrate is reduced to nitrite.

The DNA G+C content (mol %) is 66.1 (HPLC).

Type strain: JHK30 (=JCM 19170=LMG 27587= DSM 27220).

GenBank accession number (16S rRNA): HM543264.

GenBank accession number (genome): LIPU00000000

RELATED ARTICLES:

gbm00968

gbm01004

gbm00994

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277 **TABLES:**

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279 **Table 1**. Differentiating characteristics between the type strains of the three *Tepidiphilus* species – *T.*
280 *margaritifer* DSM 15129^T, *T. succinatimandens* DSM 15512^T and *T. thermophilus* JHK30^T. (adapted
281 from Poddar et al., 2014).

282 The three strains tested positive for catalase, oxidase, nitrate reductase, gelatinase, alkaline
283 phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, acid phosphatase
284 and naphthol-AS-BI-phosphohydrolase, and hydrolysis of DNA and aesculin. All assimilate malate,
285 caproate, succinate, fumarate, pyruvate, glutamic acid, aspartic acid, and phenylacetic acid.

286 All the strains were negative for indole, methyl red and Voges–Proskauer tests, arginine dihydrolase,
287 lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, tryptophan deaminase, α-
288 chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-
289 mannosidase and α-fucosidase, H2S production, starch hydrolysis, and citrate and malonate
290 utilization. No growth was observed with butyrate, propionate, lactate, 4-aminobenzoate, L-serine,
291 valine or L-alanine. No sugars were oxidized in the API 20E and API50CHB systems.

Characteristics	***T. margaritifer*** **DSM 15129^T**	***T. succinatimandens*** **DSM 15512^T**	***T. thermophilus*** **JHK30^T**
Biochemical tests			
Urea hydrolysis	-	+	-
Reduction of nitrite	+	+	-
Activity of Enzymes (API ZYM)			
Valine arylamidase	+	-	+
Cystine arylamidase	-	-	+
Trypsin	-	-	+

α-Glucosidase	-	-	+
β-Glucosidase	+	-	-
Growth in the presence of			
Trimethoprim (5 μg)	+	-	+
Sulfamethoxazole-trimethoprim (25 μg)	+	+	-
Nalidixic acid (30 μg)	-	+	-
Assimilation (as sole source of carbon)			
Acetate	+	-	-
Adipate	+	-	-
Benzoate	+	-	-
L-Proline	+	-	-
Oxidation of carbon sources (Biolog)			
Tween 40	-	+	+
Tween 80	-	+	+
i-Erythritol	-	+	-
Xylitol	-	+	-
Succinic acid monomethyl ester	-	+	+
cis-Aconitic acid	-	-	+
D-Galacturonic acid	-	-	+
D-Glucuronic acid	-	+	+
α-Ketoglutaric acid	-	-	+
α-Ketovaleric acid	+	+	-
DL-Lactic acid	-	-	+
Bromosuccinic acid	+	+	-
Glucuronamide	-	+	+
D-Alanine	-	-	+
L-Alanine	+	+	-

L-Asparagine	+	+	-
L-Aspartic acid	-	+	-
L-Glutamic acid	+	-	+
Hydroxy-L-proline	-	+	-
L-Leucine	-	+	-
L-Serine	-	+	-
L-Threonine	-	+	+
2,3-Butanediol	+	+	-
Glycerol	-	+	-
D-Glucose 6-phosphate	-	-	+
DNA G+C content (mol%)	64.8	58.6	66.1
Habitat	thermophilic digester	oil well	terrestrial hot spring

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FIGURES:

Figure 1. Phylogenetic tree, based on 16S rRNA gene sequences, showing the position of the *Tepidiphilus* species in relation to the closest phylogenetic genera *Hydrogenophilus*, *Thauera* and *Azoarcus*. The dendrogram was generated by the Neighbor-Joining method. Bootstrap values, generated from 1000 re-samplings, are indicated at branch points. Bar, 1 substitution per 50 nucleotide positions.