***Tepidimonas ignava* gen. nov., sp. nov., a new chemolithoheterotrophic and slightly thermophilic member of the β -Proteobacteria**

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A bacterial isolate with an optimum growth temperature of about 55 °C was recovered on a medium composed of one part Kligler's iron agar and four parts of *Thermus* Agar from the hot spring at São Pedro do Sul in central Portugal. Phylogenetic analyses using the 16S rRNA gene sequence of strain SPS-1037^T indicated that the new organism represented a new genus and species of β -Proteobacteria. The major fatty acids of strain SPS-1037^T are C16:0 and C17:0. Ubiquinone 8 is the major respiratory quinone, and the major polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The new isolate is aerobic and chemolithoheterotrophic. Thiosulfate and tetrathionate were oxidized to sulfate. The growth yield of the organism was improved by the addition of thiosulfate to media containing organic carbon sources, but the organism did not grow autotrophically under the conditions examined. Heterotrophic growth of strain SPS-1037^T occurs on amino acids and organic acids, but this organism does not assimilate carbohydrates. On the basis of the phylogenetic analyses, and physiological and biochemical characteristics, it is proposed that strain SPS-1037^T represents a new genus and a new species for which the name *Tepidimonas ignava* is proposed.

Keywords: *Tepidimonas ignava*, β -Proteobacteria, thermophile

INTRODUCTION

The deeply branching lines of descent within the domain *Bacteria* are, in contrast to the most recent lineages, primarily composed of thermophilic species. Thus far, the phylum *Aquifex*–*Hydrogenobacter*, and the order *Thermotogales*, for example, contain hyperthermophilic, thermophilic and slightly thermophilic species. Many of the species of the green non-sulfur bacteria and of the *Deinococcus*–*Thermus* phyla are also thermophilic. On the other hand, the vast majority of the species belonging to the most recent lines of descent, such as the *Proteobacteria*, are mesophilic. There are, of course, exceptions and slightly thermophilic, moderately thermophilic and even extremely

thermophilic species have been described from these bacterial lineages. The β -subclass of the *Proteobacteria*, for example, includes many mesophilic species, but it also includes a few slightly thermophilic species, namely *Thiomonas thermosulfata* (Shooner *et al.*, 1996; Moreira & Amils, 1997), and the thermophilic species *Thermothrix thiopara* (Caldwell *et al.*, 1976) and *Thermothrix azorensis* (Odintsova *et al.*, 1996). Many β -Proteobacteria oxidize inorganic sulfur compounds and it may not come as a surprise that the thermophilic species of this subclass also share this characteristic. Nevertheless, strict chemo-organotrophs, capable of growth at high temperatures, have not yet been described within the β -Proteobacteria. The species of the genus *Thermothrix* are obligately or facultatively chemolithoautotrophic, while the species of the genus *Thiomonas* are facultative chemolithoautotrophs. The species of these genera are not closely related to each other, and there may be no relationship between sulfur chemolithotrophy and the ability of organisms to grow at high temperatures.

Abbreviations: FAME, fatty acid methyl ester; GYM, glutamine/yeast extract medium.

The EMBL accession number for the 16S rRNA sequence of *Tepidimonas ignava* strain SPS-1037^T is AF177943.

We recently isolated one slightly thermophilic organism from the hot spring runoff at São Pedro do Sul on a medium composed of a mixture of *Thermus* medium and Kligler's iron agar. The organism is aerobic and chemolithoheterotrophic with an optimum growth temperature of about 55 °C. Phylogenetic analysis showed that the strain belonged to the β -*Proteobacteria*, but was unrelated to any of the known genera. On the basis of these results and of the biochemical, physiological and chemotaxonomic characteristics we propose that strain SPS-1037^T represents a new genus and species which we name *Tepidimonas ignava*.

METHODS

Isolation and bacterial strains. Strain SPS-1037^T was isolated from an artificial runoff of the hot spring located near São Pedro do Sul in central Portugal. Water samples were transported without temperature control and filtered the same day through membrane filters (Gelman type GN-6, pore size 0.45 µm, diameter 47 mm); the filters were placed on the surface of a solid medium composed of four parts *Thermus* medium (Williams & da Costa, 1992) and one part of Kligler's iron agar (Difco). These preparations were wrapped in plastic bags and incubated at 60 °C for up to 4 d. Cultures were purified by subculturing on medium 162 (Degryse *et al.*, 1978) containing 2.5 g tryptone l⁻¹ and 2.5 g yeast extract l⁻¹ and were stored at -70 °C in the same medium with 15 % (w/v) glycerol. *Bosea thiooxidans* (DSM 9653^T) and *Thiobacillus tepidarius* (DSM 3134^T) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany and used as controls for the utilization of reduced sulfur compounds by strain SPS-1037^T.

Morphological, biochemical and tolerance characteristics. Electron microscopy was performed on exponential phase cultures. Cells were fixed with aqueous 4 % paraformaldehyde, 1.25 % glutaraldehyde and 10 mM calcium chloride fixative at room temperature. The cells were washed after 4 h with 50 mM cacodylate buffer supplemented with 10 mM calcium chloride (pH 6.4) and fixed at room temperature for 2 h with osmium tetroxide in veranol acetate buffer containing 10 mM calcium chloride (Silva & Macedo, 1983, 1987). The samples were embedded in Epon after dehydration in ethanol. The sections were routinely contrasted with uranyl acetate followed by lead citrate. Observations and micrographs were performed with a Zeiss EM10C electron microscope. Cells dimensions and motility were determined by phase-contrast microscopy. The number and the position of flagella were visualized by light microscopy after staining of the cells with the Ryu stain (Heimbrook *et al.*, 1989).

Unless otherwise stated, all biochemical and tolerance tests were performed as described previously (Santos *et al.*, 1989, Nunes *et al.*, 1992) in Degryse 162 liquid medium or Degryse 162 agar at 55 °C for up to 5 d. The growth temperature range of strains SPS-1037^T 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. The pH range for growth was examined at 55 °C in the same medium using 20 mM MES for pH values between 5.0 and 6.5, 20 mM Tris for pH values between 7.0 and 8.5, and 20 mM CAPSO [3-

(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid] for pH values between 9.0 and 10.5; the pH of each buffer was adjusted with HCl or NaOH. The pH values of the cultures were determined at room temperature. Control media, containing each buffer adjusted to pH 7.5, were used to assess possible inhibitory effects of the buffering agents.

Single-carbon-source assimilation tests were performed in a minimal medium composed of Degryse 162 basal salts containing a filter-sterilized vitamin/amino acid solution (Sharp & Williams, 1988), ammonium sulfate (0.5 g l⁻¹) and the carbon sources (2.0 g l⁻¹). Growth was examined daily by measuring the turbidity of cultures incubated at 55 °C in 20 ml screw-capped tubes containing 10 ml medium for a total of 7 d. Positive and negative control cultures were grown in medium 162 and in minimal medium without carbon source.

Growth on reduced sulfur compounds. Strain SPS-1037^T was grown in glutamate/yeast extract medium (GYM) previously used for the growth of *Bosea thiooxidans* (Das *et al.*, 1996), containing 2.5 g succinate l⁻¹ and the vitamin/amino acid solution of Sharp & Williams (1988), and medium 69 (Deutsche Sammlung von Mikroorganismen und Zellkulturen, 1993) containing 1 g yeast extract l⁻¹. Thio-sulfate was added to each of these media at concentrations that varied between 0.5 and 5.0 g l⁻¹. *Bosea thiooxidans* was cultivated at 30 °C and strain SPS-1037^T was cultivated at 55 °C. At appropriate intervals, the turbidity of the cultures was determined and the cells were harvested by filtration through Gelman type GN-6 membrane filters. The levels of thiosulfate and sulfate in the filtrates were determined using the methods described by Westley (1987) and Sörbo (1987), respectively.

Polar lipid, lipoquinone and fatty acid composition. The cultures used for polar lipid and lipoquinone analyses were grown on Degryse 162 medium at 55 °C until the exponential phase of growth. The cells were removed from the surface of the Petri dishes and the extraction of lipids was performed as described previously (Prado *et al.*, 1988). The individual polar lipids were separated by mono-dimensional TLC on silica gel G plates (Merck; 0.25 mm thickness) with a solvent system consisting of chloroform/methanol/acetic acid/water (80:12:15:4, by vol.).

Lipoquinones were extracted from freeze-dried cells and purified by TLC as described by Tindall (1989). They were separated with a Gilson HPLC apparatus by using a reverse-phase column (RP18, Spherisorb, S5 ODS2) with methanol/heptane (10:2, v/v) as the mobile phase and were detected at 269 nm.

Cultures for fatty acid analysis were grown on Degryse 162 agar plates in sealed plastic bags submerged in a water bath at 55 °C for 24 h. Fatty acid methyl esters (FAMES) were obtained from fresh wet biomass by saponification, methylation and extraction as described by Kuykendall (1988). The FAMES were separated using a Hewlett Packard model 5890 gas chromatograph equipped with a flame-ionization detector fitted with a 5 % phenylmethyl silicone capillary column (0.2 mm × 25 m; Hewlett Packard). The carrier gas was high purity H₂, the column head pressure was 60 KPa, the septum purge was 5 ml min⁻¹, the column split ratio was 55:1 and the injection port temperature was 300 °C. The temperature of the oven was programmed from 170–270 °C at a rate of 5 °C min⁻¹. Identification and quantification of the FAMES, as well as the numerical analysis of the fatty acid profiles, were performed by using

Fig. 1. Ultrastructure of strain SPS 1037^T. The arrow shows a polyphosphate inclusion. Bar, 0.20 µm.

the standard MIS Library Generation Software (Microbial ID).

Determination of G + C content of DNA and 16S rRNA gene sequence, and phylogenetic analysis. DNA was isolated as described by Cashion *et al.* (1977). The G + C content of DNA was determined by HPLC as described by Mesbah *et al.* (1989).

Extraction of genomic DNA for 16S rRNA gene sequence determination, PCR amplification of the 16S rRNA gene and sequencing of the purified PCR products was carried out as described previously (Rainey *et al.*, 1996). Purified sequencing-reaction products were electrophoresed using a model 310 Genetic Analyzer (Applied Biosystems). The 16S rRNA gene sequence obtained in this study was aligned against the previously determined *β-Proteobacteria* sequences available from the public databases using the ae2 editor (Maidak *et al.*, 1999). The method of Jukes & Cantor (1969) was used to calculate evolutionary distances. Phylogenetic dendrograms were generated using various treeing algorithms contained in the PHYLIP package (Felsenstein, 1993).

Nucleotide sequence accession numbers. The 16S rRNA gene sequence determined in this study was deposited with EMBL under accession number AF177943. The accession numbers of the reference sequences used in the phylogenetic analyses are as follows: *Alcaligenes faecalis*, M22508; *Alcaligenes xylosoxidans*, M22509; *Azoarcus indigens*, L15531; *Brachymonas denitrificans*, D14320; *Bordetella bronchiseptica*, U04948; *Burkholderia cepacia*, M22518; *Chromobacterium violaceum*, M22510; *Comamonas testosteroni*, M11224; *Eikenella corrodens*, M22512; *Escherichia coli*, J01695; *Gallionella ferruginea*, L07897; *Ideonella dechloratans*, X72724; *Iodobacter fluviatilis*, M22511; *Janthinobacterium lividum*, Y08846; *Kingella kingae*, M22517; *Leptothrix discophora*, Z18533; *Nitrosomonas europaea*, M96399; *Ralstonia eutropha*, D88000; *Rhodocyclus purpureus*, M34132; *Rhodoferrax fermentans*, D16211; *Simonsiella muelleri*, M59071; *Sphaerotilis natans*, Z18534; *Spirillum volutans*, M34131; *Taylorella equigenitalis*, X68645; *Telluria mixta*, X65589; *Telluria chitinolytica*, X65590; *Vitreoscilla stercoraria*, M22519; strain ac-15, U46749; strain ac-16, U46748; strain BrG4, U51104.

RESULTS

Isolation, morphological and biochemical characteristics of strain SPS-1037^T

Different proportions of Kligler's iron agar, a medium generally used for the differentiation of enteric bacteria based on fermentation of dextrose and lactose, and production of hydrogen sulfide, were added to *Thermus* medium to make the latter more selective. One Gram-negative organism was isolated, among several colonies of spore-forming bacteria after incubation of a 4:1 mixture of *Thermus*/Kligler's iron agar. Higher proportions of Kligler's iron agar led to the isolation of spore-forming bacteria alone or did not lead to the isolation of any organisms after 4 d incubation at 60 °C. The temperature of the site at São Pedro do Sul from which the organism was recovered was 58 °C and the pH was 8.4. Two further attempts to isolate this organism on the same medium from the same site failed.

Strain SPS-1037^T formed very short, Gram-negative, rod-shaped cells and possessed one polar flagellum. Colonies were non-pigmented and translucent. Strain SPS-1037^T had an envelope composed of an outer membrane with a triple-layer structure and a very thin peptidoglycan layer visible between the cytoplasmic membrane and the outer membrane (Fig. 1). Polyphosphate inclusions were also visible in the electron micrographs.

The optimum growth temperature of strain SPS-1037^T in Degrise medium 162 was between 50 and 55 °C, with a maximum growth temperature of between 60 and 65 °C (Fig. 2). The optimum pH for growth was about pH 7.5–8.5. Strain SPS-1037^T was strictly aerobic and did not reduce nitrate or nitrite under anaerobic conditions. Yeast extract or a vitamin/amino acid solution was required for growth on single carbon sources. With the exception of hide powder azure, proteins were not degraded, but urease was

Fig. 2. Effect of the temperature on the growth of strain SPS 1037^T.

present. One of the most interesting characteristics of this organism was its inability to utilize any of the hexoses, pentoses, disaccharides or polyols tested. In

fact, strain SPS-1037^T was able to grow only on amino acids and organic acids (Table 1). The G + C content of the DNA was 69.7 mol %.

Initial observations indicated that strain SPS-1037^T produced sulfate from thiosulfate and tetrathionate, but not from cysteine or thioglycolate. To investigate the role of thiosulfate as an electron donor we used *Bosea thiooxidans* and *Thiobacillus tepidarius*, known to be chemolithoheterotrophic or facultatively chemolithoautotrophic on reduced sulfur compounds, respectively, as controls. In contrast to *Thiobacillus tepidarius*, strain SPS-1037^T was incapable of growth on mineral medium containing thiosulfate, supplemented with yeast extract (100 mg l⁻¹), or without yeast extract, added as a source of growth factors. The addition of thiosulfate (1.0 g and 5.0 g l⁻¹) to medium 69 containing 1 g yeast extract l⁻¹ lead to an increase in the biomass of strain SPS-1037^T indicating that thiosulfate was used as an energy source in the presence of organic substrates (Table 2). In contrast to *Bosea thiooxidans* (Das *et al.*, 1996), an increase in the final biomass of strain SPS-1037^T was not observed in GYM containing 2.5 g succinate l⁻¹ to which thiosulfate was added. The determination of the levels of

Table 1. Characteristics of strain SPS-1037^T

+, Positive result or growth; w, weak positive result; -, negative result or no growth.

Presence of:	Growth in:	Utilization of carbon source:							
Catalase	w	1 % NaCl	+	D-Arabinose	-	Ethanol	-	Glycine	-
Oxidase	+	2 % NaCl	-	L-Arabinose	-	Glycerol	-	L-Alanine	+
DNase	-			D-Fructose	-	i-Erythritol	-	L-Cysteine	-
Xylanase	-	Hydrolysis of:		D-Ribose	-	D-Arabitol	-	L-Serine	+
α -Galactosidase	-	Arbutin	-	D-Xylose	-	Ribitol	-	L-Asparagine	+
β -Galactosidase	-	Aesculin	-	L-Fucose	-	Xylitol	-	L-Aspartate	+
α -Glucosidase	-	Gelatin	-	D-Galactose	-	D-Mannitol	-	L-Threonine	w
β -Glucosidase	-	Elastin	-	D-Glucose	-	D-Sorbitol	-	L-Glutamate	+
<i>p</i> -nitrophenyl palmitate	+	Fibrin	-	D-Mannose	-	<i>myo</i> -Inositol	-	L-Glutamine	+
<i>p</i> -nitrophenyl laurate	+	Hide Powder Azure	+	L-Rhamnose	-	Acetate	+	L-Leucine	+
PHB†	-	Casein	-	L-Sorbose	-	Glycerate	-	L-Methionine	-
Indole	-	Starch	-	Sucrose	-	Lactate	+	L-Proline	+
Urease	+	Hippurate	-	D-Cellobiose	-	Pyruvate	+	L-Valine	-
		Tween 20	-	Lactose	-	Fumarate	+	L-Arginine	-
Reduction of nitrate	-	Tween 40	-	Maltose	-	Malate	+	L-Histidine	-
		Tween 60	-	D-Melibiose	-	Succinate	+	L-Isoleucine	w
Anaerobic growth with nitrate	-	Tween 80	-	D-Trehalose	-	Oxaloacetate	+	L-Lysine	-
MRVP*	-			D-Raffinose	-	α -Ketoglutarate	+	L-Phenylalanine	-
				<i>N</i> -Acetyl-glucosamine	-			L-Tryptophan	-
								L-Ornithine	+

* Methyl Red Voges-Proskauer.

† Poly- β -hydroxybutyrate.

Table 2. Effect of the medium and the initial concentration of thiosulfate on the growth of *Bosea thiooxidans* and strain SPS-1037^T

The maximum turbidity of *Bosea thiooxidans* was reached after 50 and 90 h growth at 30 °C in GYM and medium 69, respectively. The maximum turbidity of strain SPS-1037^T was reached after 55 and 30 h at 55 °C in GYM and medium 69, respectively.

Medium	Na ₂ S ₂ O ₃ (g l ⁻¹)	Maximum turbidity	
		<i>Bosea thiooxidans</i>	SPS-1037 ^T
GYM	0	0.62	0.55
	1	0.98	0.47
	5	1.33	0.48
69	0	0.74	0.19
	1	0.79	0.26
	5	1.12	0.41

Fig. 3. TLC of polar lipids. Lanes: 1, *Burkholderia cepacia* DSM 7288^T; 2, strain SPS 1037^T; 3, *Delftia acidovorans* DSM 39^T; 4, *Chromobacterium violaceum* DSM 30191^T. PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

sulfate and thiosulfate in the medium during growth of strain SPS-1037^T indicated that thiosulfate was oxidized to sulfate.

Polar lipid, respiratory quinone and fatty acid composition

The polar lipid pattern on TLC revealed the presence of phosphatidylethanolamine and phosphatidylglycerol among other minor phospholipids (Fig. 3).

Table 3. Mean fatty acid composition of strain SPS-1037^T after growth at 55 °C

Fatty acid	Proportion (%)
8:0 3-OH	0.8
15:1 ω 6c	1.3
15:0	5.3
16:1 ω 7c	12.4
16:1 ω 7	1.9
16:1 ω 5c	0.4
16:0	34.7
iso 17:1 ω 9c	0.4
17:0 cyclo	11.8
17:0	18.0
18:1 ω 7c	6.1
18:0	2.7
Unknown*	2.6
19:0 cyclo ω 8c	0.9

* Unknown fatty acid with equivalent chain length of 18.841.

The same pattern was found in other β -*Proteobacteria* examined during this study, namely the type strains of *Delftia acidovorans* (DSM 39^T), *Burkholderia cepacia* (DSM 7288^T) and *Chromobacterium violaceum* (DSM 30191^T). The major respiratory lipoquinone was ubiquinone 8. The fatty acid composition was fairly simple and was dominated by 16:0 (35%) and 17:0 (18%), but unsaturated fatty acids were present in large relative proportions at the optimum growth temperature (Table 3).

Phylogenetic analysis: gene sequence comparison

An almost complete 16S rRNA gene sequence of 1493 nt in length was determined for the new isolate strain SPS-1037^T. Phylogenetic analyses based on a data set comprising 1096 unambiguous nucleotides between positions 38 and 1400 [*Escherichia coli* positions (Brosius *et al.*, 1978)] showed the new isolate to cluster within the radiation of the β -subclass of the *Proteobacteria* (Fig. 4). Pairwise 16S rRNA gene sequence comparisons with representatives of validly described taxa within the β -subclass of the *Proteobacteria* gave similarity values in the range 86.9 to 93.4%. The highest 16S rRNA gene sequence similarity value between the sequence of strain SPS-1037^T and 16S rRNA gene sequences available in the public databases was found to strain ac-15 at 96.6% when 1369 positions were directly compared. The 16S rRNA gene sequence of strain SPS-1037^T was compared to the partial sequences of other known thermophiles within the β -subclass of the *Proteobacteria*. Although these reference sequences were as short as 142 nt, values of ~85% similarity were obtained for the comparisons to *Thermothrix azorensis* (U59127), *Thermothrix thiopara* (U61284) and *Thiomonas cuprina* (U67162).

Fig. 4. 16S rRNA gene sequence-based neighbour-joining phylogenetic tree showing the position of the new isolates within the radiation of representative species of the β subclass of the *Proteobacteria*. The position of the root was determined by the inclusion of *Escherichia coli* as an outgroup sequence. The scale bar represents 10 inferred nucleotide substitutions per 100 nucleotides.

DISCUSSION

Many thermophilic species were initially isolated on media containing low concentrations of yeast extract and tryptone, but the repeated use of these media generally results in the isolation of organisms that are already known. Aerobic spore-formers and species of the genera *Thermus* and *Meiothermus* had been isolated before from the hot spring at São Pedro do Sul on yeast extract/tryptone media (Santos *et al.*, 1989; Nobre *et al.*, 1996). γ -Irradiation of samples resulted in the isolation of bacteria of the genera *Deinococcus* and *Rubrobacter* from the same site (Ferreira *et al.*, 1997, 1999). Recently, we began using media normally recommended for other purposes in an attempt to isolate different aerobic thermophilic bacteria from this hot spring. Strain SPS-1037^T was isolated on a medium composed of a mixture of *Thermus*/Kligler's iron agar but several attempts to recover other isolates

from the same site failed, so the isolation of this organism may have been fortuitous.

Phylogenetic analysis of strain SPS-1037^T shows that this organism and a strain isolated several years ago, designated ac-15 (Nold *et al.*, 1996), from Octopus Spring in Yellowstone National Park, cluster together within the β -*Proteobacteria* (Fig. 4). These two strains share low (< 94.0%) 16S rRNA gene sequence similarity with any of the previously described taxa within the β -subclass of the *Proteobacteria* and can therefore be considered to represent a lineage of genus status within this group. 16S rRNA gene sequence similarity values ~85% excluded strains SPS-1037^T and ac-15 from membership of the genera with previously described thermophilic species within the β -subclass of the *Proteobacteria*, namely *Thermothrix* and *Thiomonas*.

The characteristics of strain ac-15 have not been

described and so are not available for comparison with those of strain SPS-1037^T, its nearest known relative. With a 16S rRNA gene sequence difference of ~3% these two strains could represent distinct species of a novel genus. Although both strains represent a distinct lineage within the β -*Proteobacteria*, and strain SPS-1037^T has a high temperature range for growth compared to many species of this subclass, it resembles, in many respects, other mesophilic species of the β -*Proteobacteria*. The fatty acid composition, the lipokinones and the polar lipid pattern are typical of the β -*Proteobacteria*. This organism does not grow on any of the sugars or polyols tested and does not appear to have a functional glycolytic pathway for the breakdown of sugars into pyruvate. The inability to grow on carbohydrates is known in the β -*Proteobacteria*, such as in species of the genera *Delftia* and *Thiomonas* (Moreira & Amils, 1997); however, the reason for the inability to utilize carbohydrates is, to our knowledge, not known. Like many other β -*Proteobacteria*, strain SPS-1037^T is able to obtain utilizable energy from the oxidation of sulfur compounds in the presence of utilizable organic carbon sources, but the organism does not appear to be chemolithoautotrophic under the conditions examined. The abundance of species of β -*Proteobacteria* that carry out sulfur oxidation of one type or another has led to the hypothesis that it represents an ancient property of these bacteria (Moreira & Amils, 1997).

On the basis of phenotypic characteristics and 16S rRNA gene sequence analysis we propose that strain SPS-1037^T represents a new species of a new genus for which the name *Tepidimonas ignava* is proposed.

Description of *Tepidimonas* gen. nov.

Tepidimonas (Te.pi.di.mo'nas. L. adj. *tepidus* warm; Gr. n. *monas* unit, monad; M.L. fem. n. *tepidimonas* warm monad).

Forms rod-shaped cells that stain Gram-negative. Endospores are not formed. Slightly thermophilic. Strictly aerobic; oxidase- and catalase-positive. Fatty acids are straight-chained; major phospholipids are phosphatidylethanolamine and phosphatidylglycerol; ubiquinone 8 is the major respiratory quinone. Reduced sulfur compounds are oxidized to sulfate. Chemolithoheterotrophic. Organic acids and amino acids are used as carbon and energy sources, but sugars and polyols are not assimilated. The type species is *Tepidimonas ignava*.

Description of *Tepidimonas ignava* sp. nov.

Tepidimonas ignava (ig.na'va. L. adj. *ignavus* lazy, pertaining to the organism's trait not to use sugars for growth).

Forms short rod-shaped cells 1.0–2.0 μ m in length and 0.5–1.0 μ m in diameter. Gram stain is negative. The cells are motile by one polar flagellum. Colonies on

Degryse 162 medium are not pigmented and are 1–2 mm in diameter after 60 h growth. Growth occurs above 35 °C and below 65 °C; the optimum growth temperature for strain SPS-1037^T is 50–55 °C. The optimum pH is between pH 7.5 and 8.5; growth does not occur at pH 6.0 or pH 10.0. The major fatty acids are C16:0 and C17:0; unsaturated fatty acids are also present in large amounts. Ubiquinone 8 is the major respiratory quinone. Strain SPS-1037^T is strictly aerobic, and positive for cytochrome oxidase and catalase. Yeast extract or growth factors are required for growth. Strain SPS-1037^T does not reduce nitrate to nitrite. Thiosulfate is oxidized to sulfate and serves as an energy source coupled to the assimilation of organic substrates. Strain SPS-1037^T is not chemoautotrophic on reduced sulfur compounds. Urease is positive; methyl red, Voges–Proskauer and indole are negative. Hide powder is degraded, but xylan, starch, casein, elastin and fibrin are not. Several amino acids and organic acids are utilized for growth but strain SPS-1037^T does not utilize hexoses, disaccharides, pentoses or polyols. The DNA of strain SPS-1037^T has a G + C content of 69.7 mol%. This bacterium was isolated from the hot spring at São Pedro do Sul in central Portugal. Strain SPS-1037^T has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, as strain DSM 12034^T.

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REFERENCES

- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci USA* **75**, 4801–4805.
- Caldwell, D. E., Caldwell, S. J. & Laycock, J. P. (1976). *Thermothrix thioparus* gen. et sp. nov., a facultatively anaerobic facultative chemolithoautotroph living at neutral pH and high temperature. *Can J Microbiol* **22**, 1509–1517.
- Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Das, S. K., Mishra, A. K., Tindall, B. J., Rainey, F. A. & Stackebrandt, E. (1996). Oxidation of thiosulfate by a new bacterium, *Bosea thiooxidans* (strain BI-42) gen. nov., sp. nov.: analysis of phylogeny based on chemotaxonomy and 16S ribosomal DNA sequencing. *Int J Syst Bacteriol* **46**, 981–987.
- Degryse, E., Glansdorff, N. & Pierard, A. (1978). A comparative analysis of extreme thermophilic bacteria belonging to the genus *Thermus*. *Arch Microbiol* **117**, 189–196.
- Deutsche Sammlung von Mikroorganismen und Zellkulturen (1993). DSM Catalogue of Strains. Braunschweig, Germany: DSMZ.
- Felsenstein, J. (1993). PHYLIP (phylogenetic inference package) version 3.5.1. Seattle: Department of Genetics, University of Washington.
- Ferreira, A. C., Nobre, M. F., Rainey, F. A., Silva, M. T., Wait, R., Burghardt, J., Chung, A. P. & da Costa, M. S. (1997). *Deinococcus*

- geothermalis* sp. nov. and *Deinococcus murrayi* sp. nov., two extremely radiation-resistant and slightly thermophilic species from hot springs. *Int J Syst Bacteriol* **47**, 939–947.
- Ferreira, A. C., Nobre, M. F., Moore, E., Rainey, F. A., Battista, J. R. & da Costa, M. S. (1999). Characterization and radiation resistance of new isolates of *Rubrobacter radiotolerans* and *Rubrobacter xylanophilus*. *Extremophiles* **3**, 235–238.
- Heimbrook, M. E., Wang, W. L. & Campbell, G. (1989). Staining bacterial flagella easily. *J Clin Microbiol* **27**, 2612–2615.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.
- Maidak, B. L., Cole, J. R., Parker, C. T., Jr & 11 other authors (1999). A new version of the RDP (Ribosomal Database Project). *Nucleic Acids Res* **27**, 171–173.
- Mesbashi, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Moreira, D. & Amils, R. (1997). Phylogeny of *Thiobacillus cuprinus* and other mixotrophic Thiobacilli: proposal for *Thiomonas* gen. nov. *Int J Syst Bacteriol* **47**, 522–528.
- Nobre, M. F., Carreto, L., Wait, R., Tenreiro, S., Fernandes, O., Sharp, R. J. & da Costa, M. S. (1996). Fatty acid composition of the species of the genera *Thermus* and *Meiothermus*. *Syst Appl Microbiol* **19**, 303–311.
- Nold, S. C., Kopczynski, E. D. & Ward, D. M. (1996). Cultivation of aerobic chemoorganotrophic proteobacteria and gram-positive bacteria from a hot spring microbial mat. *Appl Environ Microbiol* **62**, 3917–3921.
- Nunes, O. C., Donato, M. M. & da Costa, M. S. (1992). Isolation and characterization of *Rhodothermus* strains from S. Miguel, Azores. *Syst Appl Microbiol* **15**, 92–97.
- Odintsova, E. V., Jannasch, H. W., Mamone, J. A. & Langworthy, A. (1996). *Thermotrix azorensis* sp. nov., an obligately chemolithoautotrophic, sulfur-oxidizing, thermophilic bacterium. *Int J Syst Bacteriol* **46**, 422–428.
- Prado, A., da Costa, M. S. & Madeira, V. M. C. (1988). Effect of growth temperature on the lipid composition of two strains of *Thermus* sp. *J Gen Microbiol* **134**, 1653–1660.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Santos, M. A., Williams, R. A. D. & da Costa, M. S. (1989). Numerical taxonomy of *Thermus* isolates from hot springs in Portugal. *Syst Appl Microbiol* **12**, 310–315.
- Sharp, R. J. & Williams, R. A. D. (1988). Properties of *Thermus ruber* strains isolated from Icelandic hot springs and DNA-DNA homology of *Thermus ruber* and *Thermus aquaticus*. *Appl Environ Microbiol* **54**, 2049–2053.
- Shooner, F., Bousquet, J. & Tyagi, R. (1996). Isolation, phenotypic characterization, and phylogenetic position of a novel, facultatively autotrophic, moderately thermophilic bacterium, *Thiobacillus thermosulfatus* sp. nov. *Int J Syst Bacteriol* **46**, 409–415.
- Silva, M. T. & Macedo, P. M. (1983). The interpretation of the ultrastructure of mycobacterial cells in transmission electron microscopy of ultrathin sections. *Int J Lepr Other Mycobact Dis* **51**, 225–234.
- Silva, M. T. & Macedo, P. M. (1987). Improved Thiéry staining for the ultrastructural detection of polysaccharides. *J Submicrosc Cytol* **19**, 677–681.
- Sörbo, B. (1987). Sulfate: turbidimetric and nephelometric methods. *Methods Enzymol* **143**, 3–6.
- Tindall, B. J. (1989). Fully saturated menaquinones in the archaeobacterium *Pyrobaculum islandicum*. *FEMS Microbiol Lett* **60**, 251–254.
- Westley, J. (1987). Thiocyanate and thiosulfate. *Methods Enzymol* **143**, 23–25.
- Williams, R. A. D. & da Costa, M. S. (1992). The genus *Thermus* and related microorganisms. In *The Prokaryotes*, 2nd edn, pp. 3745–3753. Edited by A. Balows and others. New York: Springer.