Hydrotalea sandarakina sp. nov., isolated from a hot spring runoff, and emended descriptions of the genus *Hydrotalea* and the species *Hydrotalea flava*

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Two bacterial isolates, designated AF-51^T and AF-50, with an optimum growth temperature of about 45 °C and an optimum pH for growth between 6.0 and 6.5, were recovered from a hot spring in the Furnas, Área da Fonte 1825, on the Island of São Miguel in the Azores. Based on 16S rRNA gene sequence analysis, these strains were related most closely to the type strain of *Hydrotalea flava* at a pairwise similarity of 95.7 %. The two strains were orange-pigmented and formed non-motile, rod-shaped cells that stained Gram-negative and were aerobic and oxidase- and catalase-positive. The major fatty acids were iso-C_{15:0}, iso-C_{17:0} 3-OH and iso-C_{16:0}. The major respiratory quinone was menaquinone 7. Based on phylogenetic, physiological and biochemical characteristics, these strains from the Azores are considered to represent a single novel species of the genus *Hydrotalea*, for which the name *Hydrotalea sandarakina* sp. nov. is proposed. The type strain is AF-51^T (=DSM 23241^T=LMG 25526^T). We provide emended descriptions of the genus *Hydrotalea* and of *H. flava* to reflect new results obtained in this study.

The phylum *Bacteroidetes* comprises very few known thermophilic or slightly thermophilic organisms. Some of these organisms have slightly elevated growth temperatures in the range 40–45 °C, namely *Pseudozobellia thermophila* (Nedashkovskaya *et al.*, 2009) and *Lutaonella thermophila* (Arun *et al.*, 2009), or are slightly thermophilic, such as *Anaerophaga thermohalophila* (Denger *et al.*, 2002) and *Schleiferia thermophila* (Albuquerque *et al.*, 2011), with optimum growth temperatures around 50 °C. *Thermonema lapsum*, *Thermonema rossianum* and two species of the genus *Rhodothermus*, *Rhodothermus marinus* and *Rhodothermus profundus*, are genuine thermophiles with optimum growth temperatures around 60 °C or higher (Alfredsson *et al.*, 1987; Hudson *et al.*, 1989; Marteinsson *et al.*, 2010; Nunes *et al.*, 1995; Silva *et al.*, 2000; Tenreiro *et al.*, 1997).

We recently isolated several slightly thermophilic organisms from the Furnas area on the Island of São Miguel in the Azores, such as *S. thermophila* (Albuquerque *et al.*, 2011), *Meiothermus granaticus* (Albuquerque *et al.*, 2010), *Eliaera tepidiphila* (Albuquerque *et al.*, 2008),

Tepidicella xavieri (França *et al.*, 2006), *Truepera radiovictrix* (Albuquerque *et al.*, 2005) and *Meiothermus timidus* (Pires *et al.*, 2005). We also isolated two additional strains (AF-51^T and AF-50) with an optimum growth temperature around 45 °C and a maximum growth temperature of 52.5 °C from this area, which are closely related to the recently described *Hydrotalea flava*, which has an optimum growth temperature around 30 °C (Kämpfer *et al.*, 2011). Based on phylogenetic, physiological and biochemical parameters, we consider that these two strains represent a single novel species of the genus *Hydrotalea*.

Strains AF-51^T and AF-50 were isolated from a hot spring of the Furnas area, known as Área da Fonte 1825. Water samples were maintained without temperature control for 6 days, and 10–100 ml was 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 solidified *Thermus* medium (Williams & da Costa, 1992), the plates were wrapped in plastic to prevent evaporation and incubated at 50 °C for up to 4 days. Cultures were purified by subculturing and the isolates were stored at –70 °C in *Thermus* medium with 15 % (w/v) glycerol. *H. flava* CCUG 51397^T and *Escherichia coli* DSM 30083^T were used for comparative purposes.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AF-51^T is JF739858.

A supplementary figure is available with the online version of this paper.

Unless stated otherwise, all biochemical and tolerance tests were performed as described previously (Albuquerque *et al.*, 2011; Smibert & Krieg, 1981), in *Thermus* medium or on *Thermus* agar (Williams & da Costa, 1992) at 45 °C for up to 6 days. Cell morphology and motility were examined by phase-contrast microscopy during the exponential-growth phase. The presence of flexirubin-type pigments was determined by flooding a small mass of bacterial cells with 20 % KOH according to Bernardet *et al.* (2002). The growth temperature range of the strains was examined between 20 and 55 °C (5 °C increments) by measuring the turbidity (610 nm) of cultures incubated in 300 ml metal-capped Erlenmeyer flasks, containing 100 ml medium in a rotary water-bath shaker at 150 r.p.m. The pH range for growth was examined in liquid medium by using 50 mM citrate-phosphate buffer, MES, HEPES, TAPS and CAPSO over a pH range from 4.0 to 9.0 in a rotary water-bath shaker. The salt tolerance of the organisms was determined in liquid medium, with NaCl ranging between 0.0 and 2.0 %. Catalase, oxidase and DNase activities were examined as described previously (Albuquerque *et al.*, 2011; Smibert & Krieg, 1981). Additional enzyme activities were determined by using the API ZYM system (bioMérieux) at 45 °C. Anaerobic growth was assessed in cultures in the same medium, containing KNO₃ (1.0 g l⁻¹), incubated in anaerobic chambers (GENbox anaer; bioMérieux). Single carbon source assimilation tests were performed in a medium composed of *Thermus* basal salts (Williams & da Costa, 1992) to which filter-sterilized ammonium sulfate (0.5 g l⁻¹), yeast extract (0.2 g l⁻¹) and the carbon source (2.0 g l⁻¹) were added. Growth of the strains on single carbon sources was examined by measuring the turbidity of cultures incubated at 45 °C in 20 ml screw-capped tubes containing 10 ml of medium for up to 6 days. Acid production from carbohydrates was determined by using the API 50 CH system (bioMérieux) according to the manufacturer's instructions, with API 50 CHB/E medium. Results were recorded after 48 h and 6 days incubation at 45 °C.

Cultures for polar lipid analysis of *H. flava* CCUG 51397^T and strains AF-51^T and AF-50 were grown in 1 l Erlenmeyer flasks containing 250 ml medium at the optimum growth temperature in a rotary water-bath shaker until the late-exponential phase of growth. Harvesting of the cultures and extraction of lipids were performed as described previously (da Costa *et al.*, 2006). Individual polar lipids were separated by two-dimensional TLC and visualized as described previously (da Costa *et al.*, 2006). Lipoquinones were extracted from freeze-dried cells and purified by TLC as described previously (da Costa *et al.*, 2006). The lipoquinones were separated by HPLC with a Gilson HPLC system by using a reversed-phase Hichrom 5 C18 column and methanol/heptane (10:2, v/v) as the mobile phase and were detected at 269 nm. Cultures for fatty acid analysis were grown on solidified R2A medium (Reasoner & Geldreich, 1985), in sealed plastic bags submerged in a water bath at 28 and 45 °C for 24 h. Fatty acid methyl esters were obtained from fresh wet biomass and separated, identified and quantified with the standard MIS Library Generation Software (Microbial ID) as described previously (da Costa *et al.*, 2006).

DNA for determination of the G + C content was isolated as described by Nielsen *et al.* (1995). The G + C content of the DNA was determined by HPLC as described by Mesbah *et al.* (1989).

16S rRNA gene sequences were determined as described previously (Rainey *et al.*, 1996). A phylogenetic dendrogram (Fig. 1) showing the relationships between strain AF-51^T and related taxa was constructed by the neighbour-joining method with the MEGA 4.0 software package (Tamura *et al.*, 2007). Comparative analysis of the 16S rRNA gene sequences of strains AF-51^T and AF-50 showed that they were affiliated with taxa of the family *Chitinophagaceae*, order *Sphingobacteriales*, phylum *Bacteroidetes*. The sequences of the two strains shared 100 % pairwise similarity and showed highest similarity to the type strains of *Hydrotalea flava*

Fig. 1. Neighbour-joining phylogenetic dendrogram based on 16S rRNA gene sequences showing the relationship between strain AF-51^T and related taxa of the family *Chitinophagaceae*. Bootstrap values percentages of 1000 replications are shown at branch points. Bar, 2 inferred changes per 100 nt.

(95.7 %) (Kämpfer *et al.*, 2011) and *Sediminibacterium salmoneum* (91.7 %) (Qu & Yuan, 2008).

Strains AF-51^T and AF-50 formed short, Gram-negative, rod-shaped cells (0.3–0.5 µm wide and 1.0–3.0 µm long) that lacked flagella. Colonies were orange-pigmented on *Thermus* medium, but flexirubin-type pigments were not detected. The organisms had an optimum growth temperature of about 45 °C but did not grow at 20 or 55 °C, in contrast to the type strain of *H. flava*, which has a growth temperature range of 20–37 °C and an optimum growth temperature of 25–30 °C (Table 1). The strains were oxidase- and catalase-positive and hydrolysed hippurate, aesculin, arbutin, starch, gelatin and casein. Xylan was not hydrolysed. Other enzyme activities are listed in the species description below. A small amount of yeast extract (0.2 g l⁻¹) was added to the minimal medium to assess assimilation of single carbon sources. Strains AF-51^T and AF-50 produced acid from several carbohydrates. Nitrate was not reduced to nitrite. Anaerobic growth with nitrate as electron acceptor was not observed.

The polar lipid pattern on thin-layer chromatograms revealed the presence of phosphatidylethanolamine, two aminophospholipids, two aminolipids and four unidentified lipids with migration identical to those of the type strain of *H. flava* (Fig. S1 in IJSEM Online). The major respiratory lipoquinone was menaquinone 7, as in all members of the family *Chitinophagaceae* examined. The fatty acid profile of strains AF-51^T and AF-50 was dominated by iso-C_{15:0}, iso-C_{17:0} 3-OH and iso-C_{16:0} (Table 2). The type strain of *H. flava* as well as strains AF-51^T and AF-50 possessed similar fatty acids, but there were noticeable differences in the levels of several

components, namely iso-C_{17:0} 3-OH and iso-C_{16:0}, among the strains when grown at 28 °C. The G+C content of the DNA of strains AF-51^T and AF-50 was 35.2 ± 0.5 and 35.3 ± 0.5 mol%, respectively (mean ± SD of 3 determinations).

We thus propose that strains AF-51^T and AF-50 represent a novel species of the genus *Hydrotalea*. These strains can be differentiated from the type and only species of the genus *Hydrotalea*, *H. flava*, based on both phylogenetic and phenotypic characteristics.

To carry out a full comparative study, we obtained *H. flava* CCUG 51397^T from the Culture Collection of the University of Göteborg, Sweden (CCUG). The DNA G+C content of strain AF-51^T (35.2 ± 0.5 mol%, Table 2) differed from that of *H. flava* CCUG 51397^T (42 mol%, Kämpfer *et al.*, 2011) by approx. 7 mol%. To confirm this, the DNA G+C content of *H. flava* CCUG 51397^T was determined in the present study, giving a value of 37.0 ± 0.3 mol%. This discrepancy may be due to the use of a different method of determination, although the method used in the original paper (Kämpfer *et al.*, 2011) was not specified. We also used genomic DNA of *E. coli* DSM 30083^T, which had a G+C content of 50.7 ± 0.6 mol%, as a control. Comparison of *H. flava* CCUG 51397^T and strain AF-51^T in carbon-source assimilation and acid-production studies revealed some differences between the results that we obtained and those reported by Kämpfer *et al.* (2011). These differences are indicated in Table 1 and in the emended description of *H. flava*. These differences could result from the use of different basal media and/or the different methods used in the original study of Kämpfer *et al.* (2011), but with the two strains

Table 1. Differential characteristics between strains AF-51^T and AF-50 and *Hydrotalea flava* CCUG 51397^T

Data are from this study except where indicated otherwise.

Characteristic	Strains AF-51 ^T /AF-50	<i>H. flava</i> CCUG 51397 ^T
Pigmentation	Orange	Orange–yellow
Growth temperature range (°C)	25–52.5	20–37*
Optimum growth temperature (°C)	45	25–30*
Assimilation of:		
L-Rhamnose	–	+
Glycerol	+	–
DL-Lactate	+	–
D-Gluconate	+	–
Proline	+	–
Acid production from:		
D-Ribose	+	–
D-Galactose	–	+
L-Rhamnose	–	+
Potassium 5-ketogluconate	+	–
Major fatty acids	iso-C _{15:0} , iso-C _{17:0} 3-OH, iso-C _{16:0}	iso-C _{15:0} , iso-C _{17:0} 3-OH
DNA G+C content (mol%, mean ± SD)	35.2 ± 0.5/35.3 ± 0.5	37.0 ± 0.3

*Data from Kämpfer *et al.* (2011).

Table 2. Fatty acid profiles of strains AF-51^T and AF-50 grown at 28 and 45 °C, and of *Hydrotalea flava* CCUG 51397^T grown at 28 °C

Results are percentages of the total fatty acids (mean $\pm$ SD of two to four analyses); tr, trace (<0.5%); –, not detected.

Fatty acid*	AF-51 ^T		AF-50		<i>H. flava</i> CCUG 51397 ^T
	28 °C	45 °C	28 °C	45 °C	28 °C
Unknown ECL 12.560	1.6 $\pm$ 0.1	tr	1.5 $\pm$ 0.1	tr	1.1 $\pm$ 0.2
iso-C _{13:0}	2.0 $\pm$ 0.1	2.5 $\pm$ 0.1	2.1 $\pm$ 0.1	2.3 $\pm$ 0.2	3.8 $\pm$ 0.3
Unknown ECL 13.565	4.9 $\pm$ 0.3	2.0 $\pm$ 0.1	4.8 $\pm$ 0.3	1.2 $\pm$ 0.1	3.0 $\pm$ 0.4
iso-C _{14:0}	1.8 $\pm$ 0.1	2.0 $\pm$ 0.2	1.8 $\pm$ 0.1	1.9 $\pm$ 0.2	0.7 $\pm$ 0.1
C _{14:0}	tr	0.7 $\pm$ 0.1	tr	0.8 $\pm$ 0.2	–
iso-C _{15:1} G	–	tr	–	tr	2.5 $\pm$ 0.3
iso-C _{15:0}	26.2 $\pm$ 0.2	41.2 $\pm$ 1.4	27.2 $\pm$ 0.3	42.6 $\pm$ 0.2	24.9 $\pm$ 0.5
anteiso-C _{15:0}	5.2 $\pm$ 0.1	0.6 $\pm$ 0.1	5.6 $\pm$ 0.1	0.7 $\pm$ 0.1	2.8 $\pm$ 0.3
C _{15:0}	0.7 $\pm$ 0.1	0.6 $\pm$ 0.1	1.0 $\pm$ 0.1	1.3 $\pm$ 0.3	1.7 $\pm$ 0.2
iso-C _{16:1} H	1.7 $\pm$ 0.1	–	1.7 $\pm$ 0.1	–	0.7 $\pm$ 0.1
iso-C _{16:0}	8.4 $\pm$ 0.4	10.2 $\pm$ 0.5	8.0 $\pm$ 0.2	11.1 $\pm$ 0.3	4.3 $\pm$ 0.4
Summed feature 3	6.9 $\pm$ 0.2	2.6 $\pm$ 0.2	7.1 $\pm$ 0.2	1.8 $\pm$ 0.2	3.2 $\pm$ 0.3
C _{16:0}	2.4 $\pm$ 0.1	4.4 $\pm$ 0.3	2.3 $\pm$ 0.1	6.4 $\pm$ 0.6	3.1 $\pm$ 0.2
iso-C _{15:0} 3-OH	2.8 $\pm$ 0.1	3.8 $\pm$ 0.2	2.8 $\pm$ 0.1	3.0 $\pm$ 0.2	3.8 $\pm$ 0.4
C _{15:0} 2-OH	0.6 $\pm$ 0.1	–	0.6 $\pm$ 0.1	–	0.6 $\pm$ 0.1
Summed feature 9	6.6 $\pm$ 0.3	tr	6.0 $\pm$ 0.2	tr	3.0 $\pm$ 0.2
Summed feature 4	0.8 $\pm$ 0.2	–	0.8 $\pm$ 0.2	–	–
Unknown ECL 16.582	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	1.3 $\pm$ 0.2
iso-C _{17:0}	3.1 $\pm$ 0.2	4.4 $\pm$ 0.2	2.7 $\pm$ 0.2	3.8 $\pm$ 0.2	5.6 $\pm$ 0.4
C _{17:1} ω 6c	1.6 $\pm$ 0.1	1.1 $\pm$ 0.1	1.5 $\pm$ 0.1	0.9 $\pm$ 0.1	tr
iso-C _{16:0} 3-OH	4.2 $\pm$ 0.2	5.5 $\pm$ 0.3	4.3 $\pm$ 0.2	4.4 $\pm$ 0.4	5.3 $\pm$ 0.5
C _{16:0} 3-OH	1.5 $\pm$ 0.1	1.7 $\pm$ 0.2	1.5 $\pm$ 0.1	2.1 $\pm$ 0.2	2.5 $\pm$ 0.3
iso-C _{17:0} 3-OH	10.5 $\pm$ 0.4	14.2 $\pm$ 0.6	9.8 $\pm$ 0.3	13.5 $\pm$ 0.8	20.9 $\pm$ 1.0
C _{17:0} 2-OH	1.3 $\pm$ 0.1	tr	1.3 $\pm$ 0.1	tr	1.7 $\pm$ 0.4
C _{17:0} 3-OH	tr	–	tr	–	0.6 $\pm$ 0.1

*ECL, Equivalent chain-length. Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI System: summed feature 3 comprises C_{16:1} ω 7c and/or C_{16:1} ω 6c; summed feature 4 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B; summed feature 9 comprises iso-C_{17:1} ω 9c and/or C_{16:0} 10-methyl.

compared side by side, the data that we obtained on *Thermus* basal salts medium still show taxonomically significant differences between the new isolate and *H. flava*. Given the differences that we observed when testing the type strain of *H. flava* in our laboratory and to verify the identity of the strain that we obtained from CCUG, we determined the full 16S rRNA gene sequence of *H. flava* CCUG 51397^T and found it to be identical to that deposited in GenBank under accession no. FN665659. The emended descriptions of the genus *Hydrotalea* and of *H. flava* provided below reflect the additional data obtained in this study. The two novel strains differ from *H. flava* CCUG 51397^T by >3% in 16S rRNA gene sequence; the optimal temperature for growth differs by 15–20 °C; there are also several differences in assimilation of single carbon sources and they can be easily distinguished from *H. flava* based on their fatty acid composition. On the basis of these results, we suggest that strains AF-51^T and AF-50 represent a single novel species of the

genus *Hydrotalea*, for which we propose the name *Hydrotalea sandarakina* sp. nov.

Emended description of *Hydrotalea* Kämpfer *et al.* 2011

Characteristics are as given for the description of the genus by Kämpfer *et al.* (2011) with the following amendments. Some species of the genus are slightly thermophilic. The major fatty acids are iso-C_{15:0} and iso-C_{17:0} 3-OH.

Emended description of *Hydrotalea flava* Kämpfer *et al.* 2011

Characteristics are as given for the description of the species by Kämpfer *et al.* (2011) with the following amendments. Grows in media with up to 1% NaCl. The following carbon sources are assimilated using *Thermus* basal salts medium: D-glucose, D-fructose, D-galactose,

D-mannose, L-rhamnose, D-xylose, sucrose, maltose, cellobiose, trehalose, aspartate and L-ornithine; glycerol, sorbitol, DL-lactate, pyruvate, D-gluconate, L-alanine, proline and L-serine are not assimilated. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase, but negative for lipase (C14). Acid is produced from D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin ferric citrate, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, starch, glycogen, gentiobiose and turanose (API 50 CH). The DNA G+C content of strain CCUG 51397^T is 37.0 ± 0.3 mol% (HPLC).

Description of *Hydrotalea sandarakina* sp. nov.

Hydrotalea sandarakina (san.da.ra'ki.na. N.L. fem. adj. *sandarakina* from Gr. fem. adj. *sandarakinê* of orange colour).

Cells are non-motile rods, 1.0–3.0 μ m long and 0.3–0.5 μ m wide. Gram-stain-negative. Endospores are not formed. Colonies on *Thermus* medium are orange-pigmented. Aerobic, organotrophic, oxidase- and catalase-positive. Growth occurs at 25–52.5 °C. The optimum growth temperature is about 45 °C; growth does not occur at 20 or 55 °C. The optimum pH for growth is 6.0–6.5; growth does not occur at pH 4.0 or 9.0. Optimum growth occurs in the absence of NaCl, but grows in media with up to 1 % NaCl. Yeast extract is required for growth. Nitrate is not reduced to nitrite. Flexirubin-type pigments are absent. The polar lipid profile is composed of phosphatidylethanolamine, two aminophospholipids, two aminolipids and four unidentified lipids; the major fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH and iso-C_{16:0}. Menaquinone 7 is the major respiratory quinone. Gelatin, casein, starch, aesculin, arbutin and hippurate are degraded but xylan is not. DNase-positive. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase, but negative for lipase (C14). Assimilates D-glucose, D-fructose, D-galactose, D-mannose, D-xylose, sucrose, maltose, lactose, cellobiose, trehalose, raffinose, melezitose, glycerol, DL-lactate, D-gluconate, D-glucuronate, aspartate, glutamate, proline and L-ornithine, but not L-rhamnose, L-fucose, L-sorbose, D-ribose, D-arabinose, L-arabinose, ribitol, xylitol, sorbitol, mannitol, *myo*-inositol, erythritol, D-arabitol, L-arabitol, α -ketoglutarate, acetate, pyruvate,

succinate, malate, citrate, benzoate, fumarate, formate, L-alanine, L-asparagine, glycine, L-histidine, L-lysine, L-glutamine, L-arginine, L-serine, valine, L-phenylalanine, L-leucine, L-isoleucine, methionine or threonine. Acid is produced from D-ribose, D-xylose, D-glucose, D-fructose, D-mannose, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin ferric citrate, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, starch, glycogen, gentiobiose, turanose and potassium 5-ketoglucuronate (API 50 CH). The DNA G+C content of the type strain is 35.2 ± 0.5 mol% (HPLC).

The type strain, AF-51^T (=DSM 23241^T=LMG 25526^T), was isolated from runoff of the hot spring known as Área da Fonte 1825, Furnas, on the Island of São Miguel in the Azores. AF-50 (=DSM 23242=LMG 25527) is an additional strain of the species.

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