Extremophiles (2010) 14:297–304
DOI 10.1007/s00792-010-0308-5

ORIGINAL PAPER

Characterization of *Microaerobacter geothermalis* gen. nov., sp. nov., a novel microaerophilic, nitrate- and nitrite-reducing thermophilic bacterium isolated from a terrestrial hot spring in Tunisia

Nadia Khelifi · Emna Ben Romdhane · Abdeljabbar Hedi · Anne Postec · Marie-Laure Fardeau · Moktar Hamdi · Jean-Luc Tholozan · Bernard Ollivier · Agnès Hirschler-Réa

Received: 13 November 2009 / Accepted: 12 March 2010 / Published online: 1 April 2010

Abstract A novel thermophilic anaerobic and microaerophilic bacterium (optimal growth in the presence of 5–10% O_2), strain Nad S1^T was isolated from the terrestrial hot spring of Hammam Sidi Jdidi, Nabeul, Tunisia. Cells were motile rods having a Gram-positive cell wall structure. Strain Nad S1^T grew optimally at 55°C (range 37–70°C). Optimum pH for growth was 6.5–7.0. It was halotolerant growing with NaCl up to 7% (optimum concentration 1.5–3.0%). It grew chemoorganotrophically on various carbohydrates, organic-acids and amino-acids as energy sources, or chemolithotrophically on H_2 using nitrate, as terminal electron acceptor. Beside oxygen (under microaerobic conditions) and nitrate, nitrite was also used. Nitrate was completely reduced to N_2. No fermentation occurred. The genomic DNA G + C content was 41.8 mol%. Based on 16S rRNA gene sequence analysis, strain Nad S1^T belongs to the *Bacillaceae* family within the class '*Bacilli*'. Because of its phylogenetic and phenotypic characteristics, we propose this isolate to be assigned as a novel genus and a novel species within the domain *Bacteria*, *Microaerobacter geothermalis* gen. nov., sp. nov. The type strain is Nad S1^T (=DSM 22679^T =JCM 16213^T).

Keywords *Microaerobacter geothermalis* · Denitrifying bacterium · Microaerophilic · Thermophilic · Terrestrial hot spring

Communicated by T. Matsunaga.

N. Khelifi · E. Ben Romdhane · A. Hedi · A. Postec · M.-L. Fardeau · J.-L. Tholozan · B. Ollivier · A. Hirschler-Réa (✉)
Laboratoire de Microbiologie et Biotechnologie des Environnements Chauds, UMR_D180, IRD, Universités de Provence et de la Méditerranée, ESIL, case 925, 163 avenue de Luminy, 13288 Marseille Cedex 9, France
e-mail: agnes.hirschler@univmed.fr

N. Khelifi · E. Ben Romdhane · M. Hamdi
Laboratoire d'Ecologie et de Technologie Microbienne, Institut National des Sciences Appliquées et de Technologie, Centre Urbain Nord, BP 676, 1080 Tunis Cedex, Tunisia

Introduction

Over the past years, microbiological studies based on molecular (Hall et al. 2008) and cultural approaches revealed a high phylogenetic and physiological diversity of microorganisms within terrestrial thermal springs. Numerous aerobic (Xiang et al. 2003) and/or anaerobic microorganisms including sulfate-reducing (Zeikus et al. 1983; Ferris et al. 2003; Fishbain et al. 2003; Haouari et al. 2008; Wagner and Wiegel 2008), nitrate-reducing (Lino et al. 2008), iron-reducing (Boone et al. 1995; Kanso et al. 2002), and iron-oxidizing (Johnson et al. 2003) bacteria together with methanoarchaea (Lauerer et al. 1986; Laurinavichyus et al. 1988) have been recovered from these extreme environments. Interestingly, microaerophilic bacteria belonging exclusively to the order *Aquificales* (e.g. *Aquificaceae* and *Hydrogenothermaceae* families) were found dominant within terrestrial hot springs (Blank et al. 2002; Aguiar et al. 2004; Meyer-Dombard et al. 2005) where they are known to contribute significantly in hydrogen oxidation under aerobic/microaerobic conditions (Reysenbach 2001; Aguiar et al. 2004; L'Haridon et al. 2006b). Representatives of this order are hyperthermophiles/thermophiles, capable of chemolithotrophic growth under microaerobic conditions. However, their ability to also use nitrate as possible terminal electron acceptor

within the hot terrestrial ecosystems has been poorly documented. To our knowledge, so far only *Hydrogenobacter acidophilus*, family *Aquificaceae*, originating from a coastal hot spring in Ibusuki (Japan) has been reported to use oxygen and nitrate as electron acceptors.

In this paper, we describe a novel thermophilic, anaerobic and microaerophilic bacterium (strain Nad $S1^T$) isolated from a terrestrial thermal spring in Tunisia, which reduced nitrate up to nitrogen. Besides oxygen and nitrate, strain Nad $S1^T$ also reduced nitrite. It had phenotypic, chemotaxonomic and phylogenetic characteristics that allowed its assignment to a novel genus and a novel species within the *Bacillaceae* family.

Materials and methods

Origin of the strain

Strain Nad $S1^T$ was isolated from the terrestrial thermal spring Hammam Sidi Jdidi of Nabeul located in the northern-east of Tunisia. When collected, water samples had a temperature of 60°C, a pH of 6.8 and a total salinity of 18 g L^{-1}. They were dispatched anaerobically into sterile glass vials, under N_2 atmosphere, and stored at 4°C until used.

Enrichment and isolation

For the enrichment culture, 5 mL of hot spring water was used to inoculate 50 mL of EM medium containing (g L^{-1} unless otherwise indicated): NH_4Cl, 0.3; KH_2PO_4, 0.3; KCl, 0.5; $CaCl_2{\cdot}2H_2O$, 0.1; NaCl, 11; KNO_3, 1.0; yeast extract, 0.1; trace element solution (Balch et al. 1979), 10 mL L^{-1}. Prior to sterilisation, the pH was adjusted to 6.8. The medium was boiled under a stream of O_2-free N_2 gas and cooled at room temperature. The headspace was filled with N_2/CO_2 (80:20) gas mixture, and the vessels were autoclaved for 25 min at 120°C. Before inoculation, $NaHCO_3$, 2.0 g L^{-1}; $MgCl_2{\cdot}6H_2O$, 1.0 g L^{-1} and vitamin solution (Wolin et al. 1963), 10 mL L^{-1} were injected from sterile solutions.

Strain Nad $S1^T$ was isolated from an enrichment culture containing a mixture of unsaturated aliphatic hydrocarbons (dodec-1-ene and hexadec-1-ene) used as substrates [0.1 mL of a hydrocarbon mixture (1:1; v/v), was added to 50 mL of enrichment medium] together with nitrate used as terminal electron acceptor. The enrichment cultures were incubated at 55°C for 2–4 weeks and transferred several times to fresh EM medium. From these enrichment cultures, two bacterial strains were further isolated by a serial tenfold dilution technique using sodium octanoate (0.35 g L^{-1}) as substrate, always in the presence of nitrate. As no colony could be obtained in solidified medium (2% agar noble), the purification procedure by serial dilutions (5) to extinction was performed as reported earlier (Baross 1995). Several isolates growing in the same physico-chemical conditions had similar morphology and reduced nitrate or oxygen under microaerobic conditions. Only strain Nad $S1^T$ was further characterized. During routine cultivation, glucose (3.6 g L^{-1}) or sodium octanoate (0.35 g L^{-1}) was used as substrate, in the presence of 0.2 g L^{-1} yeast extract and nitrate (1 g L^{-1}) as terminal electron acceptor. The incubation temperature was 55°C.

Optimum growth conditions

The pH, temperature and NaCl ranges for growth were determined in EM medium containing yeast extract (0.5 g L^{-1}) and glucose (3.6 g L^{-1}) (Fardeau et al. 2010). The effects of pH and NaCl concentrations were determined at the optimal temperature for growth. Cultures were subcultured at least twice under the optimal conditions before determining growth rates.

Microscopy

Routine examinations were performed with a phase-contrast microscope (Nikon optiphot). The Gram reaction was determined with heat-fixed liquid cultures stained with Sigma kit reagents. The presence of flagella was determined using the SpotTest Flagella Stain (Difco). After coloration, cells were observed with a phase-contrast microscope.

Electron microscopy was performed from exponentially grown cells that were centrifuged and fixed 20 min in v/v glutaraldehyde (10%), HCl-cacodylate buffer (0.1 M). Cells were then washed with a mix v/v of HCl-cacodylate (0.1 M) and sucrose (0.5 M), and fixed with buffer (pH 6.0) containing 0.25 M sucrose and 1% osmium tetroxide. Afterwards, cells were washed again, embedded in 2.5% agarose and stained with 4% uranyl acetate. For thin sections, the agar was cut in small cubes of 1 mm, dehydrated in acetone and embedded in EPON. Finally, the polymerization was done at 60°C for 2 days. Micrographs were taken with a Zeiss model EM 912 electron microscope.

Substrate utilization tests

The substrates tested in the presence of 0.2 g L^{-1} yeast extract as growth factor and nitrate as electron acceptor were formate (10 mM), acetate (10 mM), propionate (10 mM), butyrate (5 mM), valerate (5 mM), octanoate (2 mM), palmitate (2 mM), eicosanoate (2 mM), tetracosanoate (2 mM), pyruvate (10 mM), lactate (10 mM), malate (10 mM), succinate (10 mM), ethanol (10 mM), methanol (10 mM), glucose (10 mM), galactose (5 mM),

arabinose (5 mM), fructose (5 mM), sucrose (2 mM), cellobiose (5 mM), lactose (5 mM), tryptone [0.1% (w/v)], yeast extract (1 g L^{-1}), casamino-acids (2 g L^{-1}). The following amino-acids were tested separately in the presence of 1 g L^{-1} of yeast extract: alanine, arginine, aspartate, cysteine, glutamine, glycine, lysine, methionine, proline, serine, threonine, valine, each at 0.5 M, and asparginine, glutamate, histidine, iso-leucine, leucine, phenylalanine, tryptophane and tyrosine, each at 0.25 M. Hydrogen as electron donor was tested in the presence of yeast extract. In this case, the headspace was filled with a H_2/CO_2 mixture (80:20, 2 bar).

Electron acceptors

The electron acceptors tested were elemental sulfur 1% (w/v), thiosulfate (10 mM), sulfate (20 mM), nitrite (2 mM), amorphous iron(III) oxide (90 mM), iron(III) citrate (20 mM) and fumarate (10 mM). The ability to grow aerobically was determined in 100 mL flasks containing 10 mL of the EM medium lacking nitrate and prepared aerobically (with dissolved O_2 in the liquid medium). The cultures were incubated under shaking (140 rpm). To check for microaerobic growth, various amount of air (0–20% final O_2 in the gas phase) were injected in the headspace of tubes filled with EM medium prepared anaerobically (with no dissolved oxygen in the liquid medium) lacking nitrate; tubes were incubated in a horizontal position and shaked (140 rpm).

Cell heat resistance

Heat resistance of cells was determined as follows: after incubation at 55°C, duplicated cultures were treated at 80°C for 10 min and subcultured with 5% inoculum in fresh EM medium with or without $MnSO_4$ (0.17%). Thereafter, the resulting cultures were incubated at 55°C and observed for possible growth under a phase-contrast microscope.

Analytical techniques

Growth was performed by measuring the optical density at 580 nm (UV-160 A, Shimadzu Corp., Kyoto, Japan). Sulfide contents were determined photometrically by the Cord-Ruwish method (1985). Iron(II) was measured by the colorimetric method (Lovley and Phillips 1986). Nitrate was determined by ion chromatography equipped with a metrosep anion supp 1 column (Metrohm). H_2, CO_2, N_2O and products of metabolism (alcohols, volatile and non-volatile fatty acids) were determined as described previously (Fardeau et al. 1993). N_2 was measured using a GC equipped with an Alltech CTR column at 60°C and at flow rates of 20 mL min^{-1} (the carrier gas was helium).

Chemotaxonomic characterization

Cells for fatty acids analysis were grown in the presence of 1.0 g L^{-1} yeast extract, and 3.6 g L^{-1} glucose, together with nitrate as electron acceptor. Polar lipids and respiratory quinones were extracted from freeze-dried cell material. Cell wall composition was determined with cells of strain Nad $S1^T$ that were hydrolysed (4 N HCl, 100°C, 15 h) and then subjected to thin-layer chromatography on cellulose plates using the solvent system of Rhuland et al. (1955). All these analysis were performed by the Identification Service of the DSMZ (Braunschweig, Germany).

Determination of G + C content

The genomic DNA G + C content was determined at DSMZ as described by Mesbah et al. (1989).

16S rRNA sequence studies

The extraction and purification of total DNA followed by the amplification and sequencing of 16S rRNA gene were previously described by Miranda-Tello et al. (2003). The 16S rRNA gene sequence was then compared with available sequences in the Genbank database (Benson et al. 1999) using the BLAST search (Altschul et al. 1990). A multiple sequence file was generated using the BioEdit program, version 5.0.9 (Hall 1999). Alignments and similarity levels were obtained by the CLUSTAL W method (Thompson et al. 1994). Alignments were manually refined using BioEdit. Positions of sequence with alignment uncertainty were omitted from the analysis. Phylogenetic construction was performed using TREECON with the following algorithms: neighbour-joining method with Kimura 2 Parameters correction (Saitou and Nei 1987), maximum-parsimony and maximum-likelihood methods (Felsenstein 1981). Bootstrap values were determined (Felsenstein 1985).

Nucleotide sequence accession number

The 16S rRNA sequence of strain Nad $S1^T$ has been deposited in the Genbank database under accession number FN552009.

Results

Cell properties

Strain Nad $S1^T$ was an anaerobic and microaerophilic bacterium with no growth occurring under aerobic conditions. Cells were rod-shaped and approximately 0.5 μm wide and 2–10 μm long. They usually occurred singly.

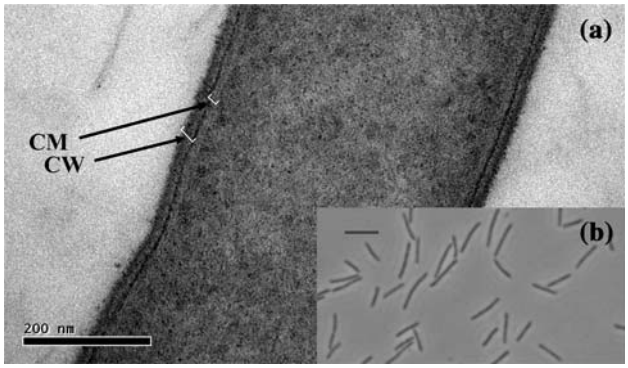

Fig. 1 **a** Thin-section electron micrograph of strain Nad S1^T showing the Gram-positive cell wall (*bar* 0.2 μm), **b** phase-contrast photomicrograph showing cells of strain Nad S1^T (*bar* 10 μm)

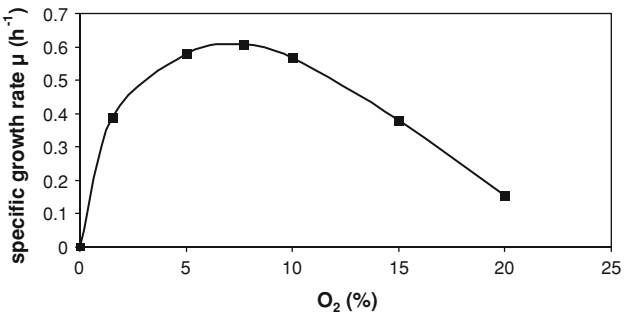

Fig. 2 Effect of oxygen concentration (% in the gas phase of an anoxic medium) on the specific growth rate of strain Nad S1^T

They stained Gram negative, but electron micrographs revealed a Gram-positive type of cell wall (Fig. 1). A polar flagellation was observed. Strain Nad S1^T was negative for catalase reaction and positive for oxydase reaction. No growth was obtained after heat treatment of cultures at 80°C for 10 min, and no endospores were observed.

Growth characteristics

In the presence of nitrate as terminal electron acceptor, growth temperature for strain Nad S1^T ranged from 37 to 70°C, with an optimum at 55°C. The pH range for growth was 5.8–7.4, with an optimum at pH 6.5–7.0. Growth occurred at NaCl concentrations ranging from 0 to 70 g L^{-1}, with an optimum between 15 and 30 g L^{-1}.

Nitrate and nitrite were successfully used as terminal electron acceptors, but not thiosulfate, sulfate, elemental sulfur, iron(III) or fumarate. Nitrate was reduced up to N_2.

No growth was observed under strictly aerobic conditions. However, O_2 was used as electron acceptor when present at low concentrations in the gas phase (optimum between 5 and 10%) with EM medium prepared anaerobically (see above) without nitrate (Fig. 2). Yeast extract was not required for growth.

The substrates used for growth in the presence of nitrate as electron acceptor were yeast extract, tryptone, glucose, sucrose, lactate, pyruvate, valerate, octanoate, palmitate, eicosanoate and tetracosanoate. Only a slight growth was observed in the presence of acetate, propionate, butyrate, succinate and malate. Strain Nad S1^T was also able to grow on amino-acids. In the presence of 1 g L^{-1} of yeast extract, growth was observed on alanine, asparagine, glutamine, glutamate, glycine, iso-leucine, leucine, proline, serine, threonine and valine.

Hydrogen was only oxidized in the presence of yeast extract together with nitrate, but not with oxygen as terminal electron acceptor.

The end products of pyruvate oxidation in the presence of nitrate as electron acceptor were acetate and CO_2 (1 mol acetate and 1 mol CO_2 per mole pyruvate used). Under nitrate-reducing conditions (glucose, yeast extract, 55°C, pH 6.8), the doubling time was 0.71 h.

Neither pyruvate nor glucose was fermented even in presence of yeast extract.

Chemotaxonomic analyses

Strain Nad S1^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan revealing a type A1γ peptidoglycan. The predominant menaquinone was MK-7. Strain Nad S1^T contained linear and branched cellular fatty acids, being dominated by branched saturated fatty acids including iso-$C_{15:0}$ (42.8%), anteiso-$C_{15:0}$ (15.5%), iso-$C_{16:0}$ (11.9%) and iso-$C_{17:0}$ (14.2%) (Table 2). The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two unidentified phospholipids and an unidentified aminophospholipid.

DNA composition and 16S rDNA sequence analysis

The G + C content of genomic DNA of strain Nad S1^T was 41.8 mol%. The obtained 16S rRNA sequence contained 1440 nucleotides. The sequence was deposited in the GenBank database under accession number NF552009. A phylogenetic tree based on neighbour-joining method was constructed (Fig. 3). Strain Nad S1^T belongs to the class of '*Bacilli*' and was phylogenetically affiliated to the members of *Bacillaceae* family. Among the closest phylogenetic relatives of strain Nad S1^T, *Vulcanibacillus modesticaldus* BR^T (L'Haridon et al. 2006a), *Bacillus horti* DSM 12751^T (Yumoto et al. 1998), *Virgibacillus halodenitrificans* DSM 10037^T (Yoon et al. 2004) and *Caldalkalibacillus uzonensis* (Zhao et al. 2008) shared 91.0, 90.8, 90.5 and 90.4% 16S rRNA gene sequence identity, respectively.

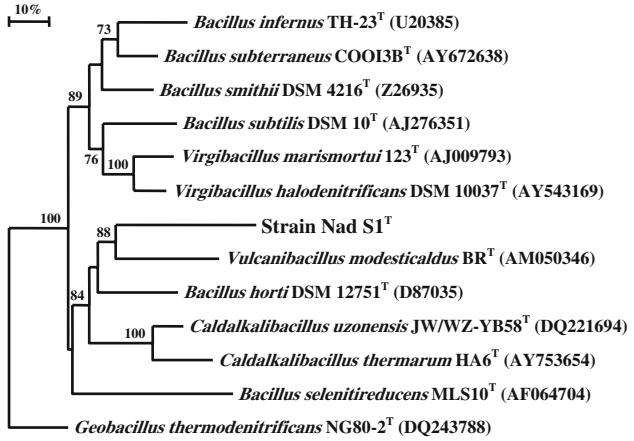

Fig. 3 Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain Nad S1^T among members the *Bacillaceae*. A total of 1159 sites were used for the phylogenetic analysis. Accession numbers are indicated in *brackets*. The topology corresponds to an unrooted tree obtained by the Neighbour-Joining method. *Numbers at nodes* indicate bootstrap values from 500 replications. The *scale bar* indicates 10% substitution per nucleotide position

Discussion

Strain Nad S1^T, isolated from a Tunisian terrestrial hot spring represents the first thermophilic, anaerobic and microaerophilic bacterium belonging to the family of *Bacillaceae,* order *Bacillales*. In this respect, this strain is phylogenetically distant from all the other microaerophilic microorganisms originating from terrestrial hot springs which belonged to the *Aquificales* so far.

The *Bacillaceae* contains 38 genera (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi). The phylogenetic analyses based on 16S rRNA gene sequences indicated that this family is paraphyletic and under reevaluation. Indeed, this family includes different genera misassigned to it as well as species misassigned to the genus *Bacillus* (Logan et al. 2009; Ludwig et al. 2009). After several reorganizations, the *Bacillaceae* now covers aerobic, aerotolerant, facultative anaerobic and strictly anaerobic strains, growing under psychrophilic, mesophilic or thermophilic conditions. Members of this family generally comprised endospore-forming microorganisms, but this phenotypic concept has been recently revised with isolation of non-spore-formers belonging to the genera *Marinococcus* and *Saccharococcus* (Logan et al. 2009).

Within this family, strain Nad S1^T was phylogenetically distinct from all genera currently recognised. It has *Vulcanibacillus modesticaldus*, *Bacillus horti*, *Virgibacillus halodenitrificans*, and *Caldalkalibacillus uzonensis* as its closest relatives. Besides phylogenetic differences, there are marked phenotypic characteristics which clearly do not allow us to assign strain Nad S1^T to any of these genera.

For example, nitrate could be used as electron acceptor by strain Nad S1^T under anaerobic conditions, nitrate being completely reduced up to N_2. This is not the case for *C. uzonensis* and *B. horti* which are known as strict aerobes. In addition, *B. horti* grows in a mesophilic range of temperature (Table 1). Despite *Vulcanibacillus modesticaldus* and *Virgibacillus halodenitrificans* are considered as nitrate reducers, nitrate was only reduced by these species to nitrite. Furthermore, in contrast to strain Nad S1^T, all these species form endospores and none of them is reported to oxidize hydrogen or to be microaerophilic. Many other differentiating characteristics between strain Nad S1^T and the bacterial species mentioned above are summarized in Table 1. They include in particular the temperature or the pH range for growth, and the cellular fatty acids profile (Tables 1, 2). In strain Nad S1^T, this profile was dominated by iso- and anteiso-fatty acids (Table 2), and the absence of hydroxyl fatty acids was observed thus confirming that strain Nad S1^T could be associated with "*Bacillus* and relatives" (L'Haridon et al. 2006a). While the major fatty acids of Nad S1^T were iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$ and iso-$C_{16:0}$, those of its closest relatives, *Vulcanibacillus modesticaldus* and *Bacillus horti* consisted mainly of iso- and anteiso-$C_{15:0}$, whereas those of *Virgibacillus halodenitrificans* were dominated by anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. Therefore chemotaxonomical considerations are also in agreement with strain Nad S1^T having an original taxonomical status within the *Bacillaceae*.

Besides oxygen, strain Nad S1^T uses nitrate and nitrite as terminal electron acceptors thus conferring to this bacterium a great adaptability. Moreover, its ability to denitrify makes it a good candidate to actively participate in the global nitrogen cycle within terrestrial hot springs which has been poorly studied so far.

Finally, our results demonstrate that thermophilic microaerophilic and hydrogenotrophic microorganisms inhabiting terrestrial hot springs do not pertain only to the *Aquificales* lineage. Strain Nad S1^T represents the first microorganism of the *Bacillaceae* family having these peculiar physiological and metabolical properties which might be relevant ecologically for microbes to adapt to hot terrestrial ecosystems.

Based on morphological, physiological, chemotaxonomic and genetic characteristics, we propose to assign it within this family as a novel genus and a novel species, *Microaerobacter geothermalis* sp. nov., with Nad S1^T as the type strain.

Description of *Microaerobacter* gen. nov.

Microaerobacter (Mi.cro.a.e.ro.bac'ter. Gr. adj. mikros, small, little; Gr. n. aer aeros, air; N. L. masc. n. bacter, rod;

Table 1 Differentiating characteristics of strain Nad S1^T from phylogenetically related strains of the *Bacillaceae* family

Character	1	2	3	4	5
Motility	+	−	+	+	+
Sporulation	−	+	+	+	+
Oxydase	+	−	+	+	+
Catalase	−	−	+	−	+
Temperature range (°C)	37–70	37–60	10–45	42–64	15–40
Optimum temperature (°C)	55	55	35–40	50–52	ND
pH range	5.8–7.4	6–8.5	5.8–9.6	6.4–9.7	ND
Optimum pH	6.5–7	7	7.4–7.5	8.2–8.4	8–10
NaCl range (%)	0–7.0	10–40	2.0–23	0–6.0	3.0–11
Optimum NaCl (%)	1.5–3.0	20–30	3.0–7.0	ND	ND
Anaerobic growth	+	+	+	−	−
Nitrate respiration	+	+	+	−	−
Nitrite respiration	+	−	ND	−	ND
Denitrification	+	−	−	−	−
Aerobic growth	−	−	−	+	+
Microaerophilic growth	+	−	−	−	−
Hydrogenotrophy	+	−	ND	−	ND
G + C content (mol%)	41.8	34.5	38–39	45	40.9
Major cellular fatty acids	i-$C_{15:0}$, ai-$C_{15:0}$, i-$C_{16:0}$, i-$C_{17:0}$	i-$C_{15:0}$, ai-$C_{15:0}$	ai-$C_{15:0}$, ai-$C_{17:0}$	i-$C_{15:0}$, ai-$C_{15:0}$,, i-$C_{16:0}$, i-$C_{17:0}$, ai-$C_{17:0}$	i-$C_{15:0}$, ai-$C_{15:0}$

1, Strain Nad S1^T; *2*, *Vulcanibacillus modesticaldus* BR^T (L'Haridon et al. 2006a); *3*, *Virgibacillus halodenitrificans* DSM 10037^T (Yoon et al. 2004); *4*, *Caldalkalibacillus uzonensis* JW/WZ-YB58^T (Zhao et al. 2008); *5*, *Bacillus horti* K13^T (Yumoto et al. 1998); +, positive; −, negative; *ND*, not determined

Table 2 Cellular fatty acid composition (%) of strain Nad S1^T and phylogenetically related species

Fatty acids	1	2	3[a]	4	5
ai-$C_{14:0}$					2.4
i-$C_{14:0}$	3.42			1.0	
$C_{14:0}$	0.70	1.15–1.13		1.2	
ai-$C_{15:0}$	15.55	8.10–8.11	54.1	18.3	30.2
i-$C_{15:0}$	42.83	72.43–69.10		24.5	38.4
$C_{15:0}$	1.52	1.21–1.19		5.1	
ai-$C_{16:0}$		1.71–1.77			4.4
i-$C_{16:0}$	11.87			9.9	4.4
$C_{16:0}$	5.15	4.82–4.84		7.6	1.1
$C_{16:1}$				2.1	5.3
ai-$C_{17:0}$	3.12	1.44–1.76	32	9.7	
i-$C_{17:0}$	14.16	6.10–6.99		17.5	1.4
$C_{18:0}$	0.87	1.97–2.61			
$C_{18:1}$ w9c	0.81	1.05–1.65			

1, Strain Nad S1^T; *2*, *Vulcanibacillus modesticaldus* BR^T (L'Haridon et al. 2006a); *3*, *Virgibacillus halodenitrificans* DSM 10037^T (Yoon et al. 2004); *4*, *Caldalkalibacillus uzonensis* JW/WZ-YB58^T (Zhao et al. 2008); *5*, *Bacillus horti* K13^T (Yumoto et al. 1998); i, iso; ai, anteiso

[a] Incomplete data

N. L. masc. n. *microaerobacter*, able to live in the presence of small quantities of O_2).

Cells are motile rods, appearing mostly singly. The cell-wall structure is Gram-positive. Endospores not observed. Anaerobic and microaerophilic, thermophilic, neutrophilic and halotolerant. Chemoorganotrophic. Lithotrophic (hydrogen oxidation in the presence of yeast extract) only under anaerobic conditions. Besides oxygen, only used under microaerobic conditions, nitrate and nitrite can be also used as terminal electron acceptors. Nitrate is reduced to N_2. Do not ferment. Oxidase positive and catalase negative. The predominant fatty acids are iso- and anteiso-$C_{15:0}$, iso-$C_{16:0}$ and iso-$C_{17:0}$.

The type species is *Microaerobacter geothermalis.*

Description of *Microaerobacter geothermalis* sp. nov

Microaerobacter geothermalis [ge.o.ther'ma.lis. N. L. masc. adj. geothermalis (from Gr. n. gê, earth; Gr. n. therme, heat; L. masc. suff. -alis, suffix used with the sense of pertaining to) geothermal, referring to hot terrestrial spring, the spring where the bacterium was isolated].

This strain has the same characteristics given in the genus description. In addition, cells are rods (0.5 μm × 2–10 μm).

The temperature range for growth is 37–70°C, with an optimum at 55°C. The optimum pH for growth is 6.5–7.0. Halotolerant, growing at 0–7.0% NaCl (optimum at 1.5–3.0%). Yeast extract and vitamins are not required for growth. Yeast extract, tryptone, amino-acids, glucose, sucrose, lactate, pyruvate, valerate, octanoate, palmitate, eicosanoate, tetracosanoate and H_2 are used as electron donors under nitrate-reducing conditions. The genomic DNA G + C content is 41.8 mol%. The type strain, Nad S1^T(=DSM 22679^T =JCM 16213^T) was isolated from the terrestrial hot spring of Hammam Sidi Jdidi, Nabeul, Tunisia.

Acknowledgments We thank Manon Joseph for doing electron microscopy, Professor Jean Paul Euzéby for assistance with the naming of the organism and Dr. Pierre Roger for revising the manuscript. This work was supported, in part, by a French CNRS-INSU grant (BIOHYDEX EC2CO-MicrobiEn project).

References

Aguiar P, Beveridge TJ, Reysenbach AL (2004) *Sulfurihydrogenibium azorense*, sp. nov., a thermophilic hydrogen-oxidizing microaerophile from terrestrial hot springs in the Azores. Int J Syst Evol Microbiol 54:33–39

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Balch WE, Fox GE, Magrum LJ, Woese CR, Wolfe RS (1979) Methanogens: reevaluation of a unique biological group. Microbiol Rev 43:260–296

Baross JA (1995) Isolation, growth and maintenance of hyperthermophiles. In: Robb FT, Place AR (eds) *Archaea*. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 15–23

Benson DA, Boguski MS, Lipman DJ, Oullette BFF, Rapp BA, Wheeler DL (1999) GenBank. Nucleic Acids Res 27:12–17

Blank CE, Cady SL, Pace NR (2002) Microbial composition of near-boiling silica-depositing thermal springs throughout Yellowstone National Park. Appl Environ Microbiol 68:5123–5135

Boone DR, Liu Y, Zhao ZJ, Balkwill DL, Drake GR, Stevens TO, Aldrich HC (1995) *Bacillus infernus* sp. nov., an Fe(III)- and Mn(IV)-reducing anaerobe from the deep terrestrial subsurface. Int J Syst Bacteriol 45:441–448

Cord-Ruwish R (1985) A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. J Microbiol Methods 4:33–36

Fardeau ML, Cayol JL, Magot M, Ollivier B (1993) H_2 oxidation in the presence of thiosulfate, by a *Thermoanaerobacter* strain isolated from an oil-producing well. FEMS Microbiol Lett 113:327–332

Fardeau ML, Barsotti V, Cayol JL, Guasco S, Michotey V, Joseph M, Bonin P, Ollivier B (2010) *Caldinitratiruptor microaerophilus*, gen. nov., sp. nov. isolated from a French hot spring (Chaudes-Aigues, Massif Central): a novel cultivated facultative microaerophilic anaerobic thermophile pertaining to the *Symbiobacterium* branch within the *Firmicutes*. Extremophiles. doi: 10.1007/s00792-010-0302-y

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Ferris MJ, Magnuson TS, Fagg JA, Thar R, Kühl M, Sheehan KB, Henson JM (2003) Microbially mediated sulphide production in a thermal, acidic algal mat community in Yellowstone National Park. Environ Microbiol 5:954–960

Fishbain S, Dillon JG, Gough HL, Stahl DA (2003) Linkage of high rates of sulfate reduction in Yellowstone hot springs to unique sequence types in the dissimilatory sulfate respiration pathway. Appl Environ Microbiol 69:3663–3667

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hall JR, Mitchell KR, Jackson-Weaver O, Kooser AS, Cron BR, Crossey LJ, Takacs-Vesbach CD (2008) Molecular characterization of the diversity and distribution of a thermal spring microbial community by using rRNA and metabolic genes. Appl Environ Microbiol 74:4910–4922

Haouari O, Fardeau ML, Cayol JL, Casiot C, Elbaz-Poulichet F, Hamdi M, Joseph M, Ollivier B (2008) *Desulfotomaculum hydrothermale* sp. nov., a thermophilic sulfate-reducing bacterium isolated from a terrestrial Tunisian hot spring. Int J Syst Evol Microbiol 58:2529–2535

Johnson DB, Okibe N, Roberto FF (2003) Novel thermo-acidophilic bacteria isolated from geothermal sites in Yellowstone National Park: physiological and phylogenetic characteristics. Arch Microbiol 180:60–68

Kanso S, Greene AC, Patel BKC (2002) *Bacillus subterraneus* sp. nov., an iron- and manganese-reducing bacterium from a deep subsurface Australian thermal aquifer. Int J Syst Evol Microbiol 52:869–874

L'Haridon S, Miroshnichenko ML, Kostrikina NA, Tindall BJ, Spring S, Schumann P, Stackebrandt E, Bonch-Osmolovskaya EA, Jeanthon C (2006a) *Vulcanibacillus modesticaldus* gen. nov., sp. nov., a strictly anaerobic, nitrate-reducing bacterium from deep-sea hydrothermal vents. Int J Syst Evol Microbiol 56:1047–1053

L'Haridon S, Reysenbach AL, Tindall BJ, Schönheit P, Banta A, Johnsen U, Schumann P, Gambacorta A, Stackebrandt E, Jeanthon C (2006b) *Desulfurobacterium atlanticum* sp. nov., *Desulfurobacterium pacificum* sp. nov. and *Thermovibrio guaymasensis* sp. nov., three thermophilic members of the *Desulfurobacteriaceae* fam. nov., a deep branching lineage within the *Bacteria*. Int J Syst Evol Microbiol 56:2843–2852

Lauerer G, Kristjansson JK, Langworthy TA, Konig H, Stetter KO (1986) *Methanothermus sociabilis* sp. nov., a second species within the *Methanothermaceae* growing at 97°C. Syst Appl Microbiol 8:100–105

Laurinavichyus KS, Kotelnikova SV, Obraztsova AY (1988) New species of thermophilic methane-producing bacteria *Methanobacterium thermophilum*. Microbiology 57:832–838

Lino T, Nakagawa T, Mori K, Harayama S, Suzuki K (2008) *Calditerrivibrio nitroreducens* gen. nov., sp. nov., a thermophilic, nitrate-reducing bacterium isolated from a terrestrial hot spring in Japan. Int J Syst Evol Microbiol 58:1675–1679

Logan NA, Berge O, Bishop AH, Busse HJ, De Vos P, Fritze D, Heyndrickx M, Kämpfer P, Rabinovitch L, Salkinoja-Salonen MS, Seldin L, Ventosa A (2009) Porposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria. Int J Syst Evol Microbiol 59:2114–2121

Lovley DR, Phillips EJP (1986) Organic matter mineralization with reduction of ferric iron in anaerobic sediments. Appl Environ Microbiol 51:683–689

Ludwig W, Schleifer KH, Whitman WB (2009) Revised road map to the phylum *Firmicutes*. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB (eds) Bergey's manual of systematic bacteriology. The Firmicutes, vol 3. Springer, New York, pp 1–13

Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G + C content of deoxyribonucleic acid

by high-performance liquid chromatography. Int J Syst Evol Microbiol 39:159–167

Meyer-Dombard DR, Shock EL, Amend JP (2005) Archaeal and bacterial communities in geochemically diverse hot springs of Yellowstone National Park, USA. Geobiology 3:211–227

Miranda-Tello E, Fardeau ML, Fernández L, Ramírez F, Cayol JL, Thomas P, Garcia JL, Ollivier B (2003) *Desulfovibrio capillatus* sp. nov., a novel sulfate-reducing bacterium isolated from an oil field separator located in the Gulf of Mexico. Anaerobe 9:97–103

Reysenbach AL (2001) Phylum BI *Aquificaceae* phy. nov. In: Boone DR, Castenholz RW, Garrity GM, Staley JT, Brenner DJ, Goodfellow M, Krieg NR, Rainey FA, Schleifer KH (eds) Bergey's manual of systematic bacteriology. The *Archaea* and the deeply branching and phototrophic *Bacteria*, vol 1. Springer, New York, pp 359–367

Rhuland LE, Work E, Denman RF, Hoare DS (1955) The behaviour of the isomers of α,ε-diaminopimelic acid on paper chromatograms. J Am Chem Soc 77:4844–4846

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Wagner ID, Wiegel J (2008) Diversity of thermophilic anaerobes. Ann N Y Acad Sci 1125:1–43

Wolin EA, Wolin MJ, Wolfe RS (1963) Formation of methane by bacterial extracts. J Biol Chem 238:2882–2886

Xiang X, Dong X, Huang L (2003) *Sulfolobus tengchongensis* sp. nov., a novel thermoacidophilic archaeon isolated from a hot spring in Tengchong, China. Extremophiles 7:493–498

Yoon JH, Oh TK, Park YH (2004) Transfer of *Bacillus halodenitrificans* Denariaz et al. 1989 to the genus *Virgibacillus* as *Virgibacillus halodenitrificans* comb. nov. Int J Syst Evol Microbiol 54:2163–2167

Yumoto I, Yamazaki K, Sawabe T, Nakano K, Kawasaki K, Ezura Y, Shinano H (1998) *Bacillus horti* sp. nov., a new Gram-negative alkaliphilic *bacillus*. Int J Syst Bacteriol 48:565–571

Zeikus JG, Dawson MA, Thompson TE, Ingvorsen K, Hatchikian EC (1983) Microbial ecology of volcanic sulfidogenesis: isolation and characterization of *Thermodesulfotobacterium commune* gen. nov. and sp. nov. J Gen Microbiol 129:1159–1169

Zhao W, Zhang CL, Romanek CS, Wiegel J (2008) Description of *Caldalkalibacillus uzonensis* sp. nov. and amended description of the genus *Caldalkalibacillus*. Int J Syst Evol Microbiol 58:1106–1108