International Journal of Systematic and Evolutionary Microbiology (2007), **57**, 2618–2622 **DOI** 10.1099/ijs.0.65163-0

Herminiimonas saxobsidens sp. nov., isolated from a lichen-colonized rock

Elke Lang,[1] Jolantha Swiderski,[1] Erko Stackebrandt,[1] P. Schumann,[1] Cathrin Spröer[1] and Nurettin Sahin[2]

Correspondence
Elke Lang
ela@dsmz.de

[1]DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7b, 30124 Braunschweig, Germany

[2]Mugla University, Egitim Fakültesi, TR-48170 Kötekli, Mugla, Turkey

A Gram-negative, rod-shaped, non-spore-forming bacterium (strain NS11^T) was isolated from a lichen-colonized rock surface. On the basis of 16S rRNA gene sequence similarity, strain NS11^T was shown to belong to the *Betaproteobacteria*, and was most closely related to *Herminiimonas arsenicoxydans* ULPAs1^T (98.8 %), *Herminiimonas aquatilis* CCUG 36956^T (98.0 %) and *Herminiimonas fonticola* S-94^T (98.0 %). Major whole-cell fatty acids were $C_{16:0}$, $C_{17:0}$ cyclo and $C_{16:1}\omega7c$. Strain NS11^T also contained high proportions of $C_{10:0}$ 3-OH and $C_{18:1}\omega7c$. This pattern is typical for members of the genus *Herminiimonas*. The results of DNA–DNA hybridization experiments and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain NS11^T from the three recognized *Herminiimonas* species. It is therefore concluded that strain NS11^T represents a novel species of the genus *Herminiimonas*, for which the name *Herminiimonas saxobsidens* sp. nov. is proposed. The type strain is NS11^T (=DSM 18748^T=CCM 7436^T).

The genus *Herminiimonas* was established in the family *Oxalobacteraceae*, order *Burkholderiales*, class *Betaproteobacteria*, to accommodate an organism isolated from a borehole source of bottled drinking water (*Herminiimonas fonticola*; Fernandes *et al.*, 2005). Two additional species in the genus, *Herminiimonas aquatilis* isolated from drinking water (Kämpfer *et al.*, 2006) and *Herminiimonas arsenicoxydans* isolated from arsenic-contaminated activated sludge (Muller *et al.*, 2006), were described shortly thereafter. In contrast to the aqueous habitat of these three species, strain NS11^T, determined herein to represent a novel species of the genus *Herminiimonas*, was isolated from the lichen–rock interface of a limestone bedrock colonized by lichen at Mugla, Turkey, from enrichment cultures with potassium oxalate as the sole source of carbon and energy (Sahin *et al.*, 2002). The lichen–rock interface contains a zone with high oxalate content. Oxalic acid produced by the lichen communities accelerates weathering of the rock by solubilizing the cement between the rock grains (Johnston & Vestal, 1993).

The following DSMZ strains were used as reference: *H. aquatilis* DSM 18803^T, *H. arsenicoxydans* DSM 17148^T and *H. fonticola* DSM 18555^T. Strain NS11^T was grown routinely on nutrient agar (per litre: 5 g peptone, 3 g beef extract, 15 g agar; Difco), and the type strains of the three recognized *Herminiimonas* species on R2A medium (Difco; Reasoner & Geldreich, 1985) at 28 °C.

From enrichment cultures with 4 g potassium oxalate l^{-1} as the sole source of carbon and energy in mineral medium (Aragno & Schlegel, 1992), a Gram-negative, rod-shaped bacterium, designated strain NS11^T, was isolated (Sahin *et al.*, 2002). Cells were motile, non-sporulating and strictly aerobic. Colonies of strain NS11^T were cream coloured and convex. Addition of oxalate (2 g l^{-1}) to nutrient agar did not enhance growth.

16S rRNA gene sequences were produced and aligned as described by Somvanshi *et al.* (2006). Phylogenetic dendrograms were constructed by using the neighbour-joining algorithm (De Soete, 1983). Analysis of the almost-complete 16S rRNA gene sequence of strain NS11^T grouped it within the family *Oxalobacteraceae*. Highest sequence similarities were found with members of the genus *Herminiimonas* (Fig. 1), namely with *H. arsenicoxydans* ULPAs1^T (98.8 %), *H. aquatilis* CCUG 36956^T (98.0 %) and *H. fonticola* S-94^T (98.0 %).

Ribotyping of strains was performed with the automated RiboPrinter microbial characterization system (Qualicon; DuPont). Riboprint analyses, using restriction enzyme *Eco*RI, were performed according to the methods described by Allerberger & Fritschel (1999). The fragment patterns of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NS11^T is AM493906.

A figure showing the diversity of normalized ribotype patterns and a dendrogram showing the fatty-acid relationships among the type strains of species of the genus *Herminiimonas* are available as supplementary material with the online version of this paper.

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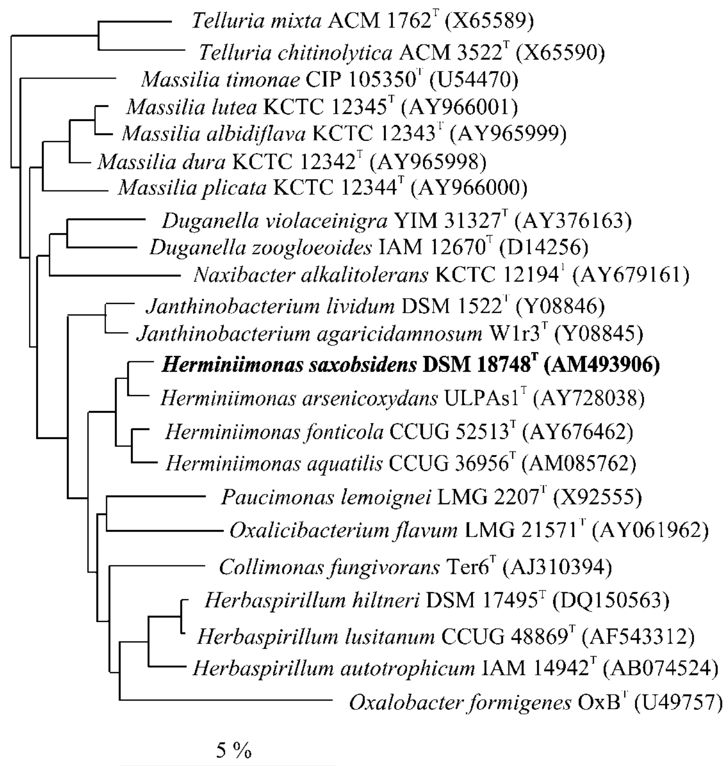

Fig. 1. Phylogenetic dendrogram (De Soete, 1983), based on 16S rRNA gene sequences, showing the nearest neighbours of strain NS11^{T} within the genus *Herminiimonas*, class *Betaproteobacteria*. Bar, 5 % difference in nucleotide sequences, as determined by measuring the lengths of the horizontal lines connecting any two organism positions.

strain NS11^{T} and of the type strains of the three recognized *Herminiimonas* species varied, resulting in a unique pattern for each of the strains (see Supplementary Fig. S1 in IJSEM Online). This dissimilarity between the ribopatterns for strain NS11^{T} and reference *Herminiimonas* type strains excludes the possibility that strain NS11^{T} is affiliated to any of the three recognized *Herminiimonas* species.

For DNA–DNA hybridization experiments, DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). Hybridization was carried out in SSC buffer at 69 °C as described by De Ley *et? al.* (1970), with the modifications given by Huß *et al.* (1983), by using a model Cary 100 Bio Uv/vIS spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian). The level of DNA–DNA relatedness between strain NS11^{T} and *H. arsenicoxydans* DSM 17148^{T}, sharing a 16S rRNA gene sequence similarity of 98.8 %, was 22.4 %, which confirms that strain NS11^{T} does not belong to the genospecies *H. arsenicoxydans.* The level of DNA–DNA relatedness between *H. aquatilis* CCUG 36956^{T} and *H. fonticola* S-94^{T}, sharing a 16S rRNA gene sequence similarity value of 99.3 %, was similarly low (25.7 %) (Kämpfer *et al.*, 2006). These low hybridization values support a previous report of the lack of high levels of DNA–DNA reassociation even at 16S rRNA gene sequence similarities as high as around 99 % (Stackebrandt & Ebers, 2006).

For analysis of fatty acids, cells were grown on R2A agar for 48 h at 28 °C. This growth medium, rather than the trypticase soy agar recommended for analysis according to the MIDI system, was used as strain NS11^{T} and the *Herminiimonas* reference strains did not grow well on the latter medium. Fatty acid methyl esters were obtained by saponification, methylation and extraction as described by Kämpfer & Kroppenstedt (1996) and separated by GC (model 5898A; Hewlett Packard). Peaks were automatically integrated and fatty-acid components and their proportions were determined by using the Microbial Identification standard software package MIDI (Sasser, 1990). Fatty acids of strain NS11^{T} were dominated by $C_{16:0}$ (36.4 %), $C_{17:0}$ cyclo (22.3 %) and $C_{16:1}\omega7c$ (18.7 %) (Table 1). The presence of $C_{10:0}$ 3-OH as a significant component was also characteristic. The fatty acid patterns generated in the present study only partially agree with those provided in the species descriptions previously given for *H. aquatilis*, *H. fonticola* and *H. arsenicoxydans.* Although some components differed slightly only in quantity, a significant deviation was the absence of $C_{17:1}\omega6c$ in *H. aquatilis* DSM 18803^{T}, which is described to be present in *H. aquatilis* CCUG 36956^{T} by Kämpfer *et al.* (2006). $C_{17:1}\omega6c$ is absent in four *H. fonticola* strains (Fernandes *et al.*, 2005) and in *H. arsenicoxydans* ULPAs1^{T} (Muller *et al.*, 2006). Possibly, the choice of the growth medium and age of cells used has influenced the fatty-acid composition. A dendrogram of Euclidian distances depicts the separate position of strain NS11^{T}, confirming the result of 16S rRNA gene sequence analysis and riboprinting, i.e.

Table 1. Whole-cell fatty acid composition of strain NS11^T and recognized *Herminiimonas* species

Strains: 1, NS11^T; 2, *H. arsenicoxydans* DSM 17148^T; 3, *H. aquatilis* DSM 18803^T; 4, *H. fonticola* DSM 18555^T. Cultivation was on R2A medium for 2 days at 28 °C. Data are percentages of total fatty acids. Data in parentheses were taken from Muller *et al.* (2006).

Fatty acid	1	2	3	4
$C_{10:0}$	0.3	0.3 (−)	0.4 (−)	0.4 (0.5)
$C_{10:0}$ 3-OH	7.3	6.0 (8.0)	5.6 (5.2)	6.3 (6.0)
$C_{14:0}$	−	4.2 (5.0)	0.3 (−)	4.4 (4.8)
$C_{15:1}\omega6c$	−	− (−)	0.4 (3.6)	− (−)
$C_{15:0}$	−	− (−)	1.3 (2.8)	− (−)
$C_{16:1}\omega7c$	18.7	32.6 (52.7)	44.4 (47.7)	45.5 (45.6)
$C_{16:0}$	36.4	29.5 (20.4)	25.9 (11.9)	26.4 (26.1)
$C_{17:0}$ cyclo	22.3	16.1 (3.3)	8.4 (−)	8.3 (7.9)
$C_{17:1}\omega6c$	−	− (−)	− (16.0)	− (−)
$C_{18:1}\omega7c$	8.1	4.2 (6.6)	10.3 (9.1)	7.8 (7.3)
$C_{18:0}$	0.8	0.8 (−)	1.0 (−)	0.4 (0.3)
$C_{18:1}$ 11-methyl $\omega7c$	0.6	3.7 (−)	− (−)	− (−)
$C_{19:0}$ cyclo $\omega8c$	6.2	1.7 (−)	0.5 (−)	0.5 (0.4)

the distinct position of strain NS11^T among the type strains of recognized *Herminiimonas* species (see Supplementary Fig. S2 in IJSEM Online).

Physiological and biochemical tests were performed at 28 °C. Conventional biochemical tests were performed according to standard methods (Smibert & Krieg, 1994). API 20NE, API ZYM and API 50 CH strips (bioMérieux) were used according to the manufacturer's instructions, except that nitrate reduction and indole production from tryptophan were read after 2 days, whereas other reactions of the API 20NE and API 50 CH strips were observed after 7 days. Utilization and assimilation of carbohydrates was determined on API 50 CH strips with modified AUX medium in which growth factors and amino acids were replaced by 0.1 g yeast extract l^{-1}. Biolog GN plates (AES) were incubated for 48 h before being read. Cavities showing a photometric value above 20 or 100 were scored as weak or positive, respectively.

Physiologically, strain NS11^T was characterized by poor reactivity. Several organic acids but no carbohydrates or sugar alcohols were metabolized in Biolog GN plates (Table 2). None of the carbohydrates offered in the API 20NE or API 50 CH strips was utilized. The enzyme reactions found for strain NS11^T with API ZYM strips as given in the species description below are in good agreement with those reported for strains of *H. fonticola* (Muller *et al.*, 2006). These characteristics of strain NS11^T are generally in accordance with those described for the three recognized *Herminiimonas* species and differentiate these species from members of the closely related genus *Janthinobacterium*, which utilize carbohydrates (Lincoln *et al.*, 1999). Nevertheless, strain NS11^T was more versatile than the type strains of the three recognized *Herminiimonas* species in the Biolog GN plates. Under the given conditions, strain NS11^T was able to assimilate, among others, acetate, DL-malate, propionate and succinate. Utilization of these organic acids by strain NS11^T as the sole source of carbon and energy was confirmed in mineral medium (Table 2).

Interestingly, some substrates which did not result in tetrazolium staining in Biolog GN plates promoted growth when offered as the sole source of carbon. These included acetic acid, propionic acid and succinic acid for *H. arsenicoxydans* DSM 17148^T. One of the possible reasons for this was the difference in incubation times, which were, respectively, 48 h and 10 days in our study. No such discrepancy between the results obtained with the Biolog GN or growth tests was noted for strain NS11^T. Comparison of our test results for *H. fonticola* DSM 18555^T and *H. aquatilis* DSM 18803^T and the results given by Fernandes *et al.* (2005) and Kämpfer *et al.* (2006) for the respective species reveals that results are reproducible from

Table 2. Differential characteristics between strain NS11^T and the type strains of recognized *Herminiimonas* species

Strains: 1, NS11^T; 2, *H. arsenicoxydans* DSM 17148^T; 3, *H. fonticola* DSM 18555^T; 4, *H. aquatilis* DSM 18803^T. Biolog GN and API 20NE reactions were read after 2 days at 28 °C. All strains are positive for oxidase and (Biolog plates) DL-lactic acid and pyruvic acid methyl ester, weak for L-arabinose but negative for the other panel substrates or reactions not detailed. Biolog GN cavities showing a photometric value above 20 or 100 were scored weak (w) or positive (+), respectively. Reactions with different results in a second independent experiment are separated by a slash. All strains are positive for growth in mineral medium with acetate, oxalate and succinate as the substrate within 10 days of incubation.

Reaction	1	2	3	4
Biolog GN				
Acetic acid	+	w/−	−	w/−
Bromosuccinic acid	+	−	−	−
Succinamic acid	+	−	−	−
Formic acid	+	+/w	w/−	+/w
D-Alanine	+	w	−	w/−
Propionic acid	+/w	−	−	−
α-Hydroxybutyric acid	+	w/−	−	−
Succinic acid	+/w	−	−	−
L-Arabinose	w/−	w	w	w/−
API 20NE				
Nitrate reduction, nitrite production	+	+	−	+
Malate utilization	+	−	+	−
Citrate utilization	−	−	+	−
Growth in mineral medium				
Propionate	+	+	+	−
DL-Malate	+	−	+	−
Citrate	−	−	+	−

laboratory to laboratory if the same method (microplates or mineral medium in tubes) is applied.

The specific growth rate (μ) of strain NS11^T with oxalate as the sole source of carbon and energy was 0.112 h^{-1} (t_d=6.2 h) at 4 g potassium oxalate l^{-1} and 30 °C. No growth was observed in the presence of 20 g potassium oxalate l^{-1}. Resistance to antibiotics as well as resistance to heavy metal ions were determined as described by Sahin *et al.* (2002). The results are given in the species description below. Both strain NS11^T and *H. arsenicoxydans* were tolerant to heavy metals, although different metal elements were tested for the two taxa (Sahin *et al.*, 2002; Muller *et al.*, 2006).

The inability to utilize carbohydrates, the utilization of short-chain organic acids and the fatty-acid profile were in agreement with the placement of strain NS11^T within the genus *Herminiimonas* as suggested by the phylogenetic analysis. On the other hand, some traits distinguished strain NS11^T from the type strains of the three recognized species in the genus *Herminiimonas* (assimilation reactions with acetic acid, propionic acid, α-hydroxybutyric acid, succinic acid, bromosuccinic acid, succinamic acid; utilization of malate but not of citrate; and lack of $C_{14:0}$ fatty acid). Therefore, strain NS11^T is considered to represent a novel species of the genus *Herminiimonas*, for which the name *Herminiimonas saxobsidens* sp. nov. is proposed.

Description of *Herminiimonas saxobsidens* sp. nov.

Herminiimonas saxobsidens (sax.ob′si.dens. L. n. *saxum* rock; L. v. *obsideo* to occupy; N.L. part. adj. *saxobsidens* rock-occupying).

Cells are Gram-negative, small ovoid rods 0.8 × 0.4 μm, motile by means of polar flagella. Cells occur singly or in pairs. No spores are found. Non-pigmented. Forms round, translucent, cream-coloured, convex colonies with flat margins, reaching 1.5 mm in diameter on nutrient agar after 3 days incubation. Growth occurs at 4–37 °C. Optimum growth occurs at pH 7.0–7.5. Weak growth occurs in media containing 2 % NaCl. No acid is produced from glucose. Oxidase- and catalase-positive. Nitrate is reduced to nitrite but not further to dinitrogen. Alkaline phosphatase, C_4-esterase, C_8-esterase lipase, leucine arylamidase, trypsin and phosphohydrolase are produced. Negative for indole production, arginine dihydrolase, urease, aesculin, casein and gelatin hydrolysis, and β-galactosidase. Has a very limited substrate spectrum. Does not utilize carbohydrates or polyols. Utilizes acetate, propionate, oxalate, succinate and malate. Does not utilize adipate, citrate, gluconate, caprate, malonate or ethanol. Predominant fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo and $C_{16:1}\omega7c$; $C_{18:1}\omega7c$ and $C_{10:0}$ 3-OH are present in smaller amounts. Does not contain $C_{14:0}$. Resistant to ampicillin, bacitracin and streptomycin (10 μg per disc each), but susceptible to erythromycin (15 μg), chloramphenicol (30 μg) and gentamicin (10 μg). Sensitive to $HgCl_2$ (2.5 μg per disc), but resistant to $ZnSO_4 . 7H_2O$, $NiCl_2 . 6H_2O$, $CoCl_2 . 6H_2O$, $CuSO_4 . 5H_2O$, lead acetate and K_2CrO_7 (2.5 μg per disc each).

The type strain, NS11^T (=DSM 18748^T=CCM 7436^T), was isolated from limestone covered by lichen after enrichment with oxalate in mineral medium.

Acknowledgements

We wish to thank R. Kroppenstedt for providing fatty acid analyses, and M. Kopitz, P. Aumann, S. Welnitz, I. Kramer, B. Sträubler and J. Gregor for excellent technical assistance.

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