Azorhizobium oxalatiphilum sp. nov., and emended description of the genus *Azorhizobium*

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A Gram-negative, motile, non-spore-forming rod, designated NS12^T, was isolated from macerated petioles of *Rumex* sp. after enrichment with oxalate. On the basis of 16S rRNA gene sequence similarity, strain NS12^T was phylogenetically related to the genera *Azorhizobium* and *Xanthobacter* in the class *Alphaproteobacteria*. Strain NS12^T was most closely related to *Azorhizobium doebereinerae* BR 5401^T and *Azorhizobium caulinodans* ORS 571^T (98.3 and 97.3 % 16S rRNA gene sequence similarity, respectively). Membership of the genus *Xanthobacter* was excluded by phenotypic characterization. The whole-cell fatty acid compositions of the isolate was typical of members of the genus *Azorhizobium* with $C_{18:1}\omega7c$, cyclo-$C_{19:0}\omega8c$, 11-methyl-$C_{18:1}\omega7c$ and $C_{16:0}$ as the main components. The results of DNA–DNA hybridization and physiological tests allowed the genotypic and phenotypic differentiation of strain NS12^T from the two members of the genus *Azorhizobium*. Therefore it is concluded that the isolate represents a novel species of the genus *Azorhizobium*, for which the name *Azorhizobium oxalatiphilum* sp. nov. is proposed. The type strain is NS12^T (=DSM 18749^T=CCM 7897^T). The description of the genus *Azorhizobium* is also emended.

Some diazotrophic strains of the genera *Xanthobacter*, *Mesorhizobium*, *Burkholderia* and *Beijerinckia* have been reported as being able to use oxalate as a sole carbon source (Sahin, 2003). In addition, a nitrogen-fixing, oxalate-oxidizing member of the genus *Azorhizobium* has been isolated from soil litter close to sour grass (*Oxalis pes-caprae*) by enrichment with oxalate on N-free calcium-oxalate agar (Sahin, 2005). While the genera *Azorhizobium* and *Xanthobacter* were grouped in the family *Hyphomicrobiaceae* until 2005 (Garrity *et al.*, 2005), they were assigned to the newly created family *Xanthobacteraceae* by Lee *et al.* (2005). The genus *Azorhizobium* currently comprises two species: *Azorhizobium caulinodans*, which is represented by strains isolated from stem nodules of *Sesbania rostrata* (Africa) (Dreyfus *et al.*, 1988), and *Azorhizobium doebereinerae*, which is represented by strains from root nodules of *Sesbania virgata* (Brazil) (de Souza Moreira *et al.*, 2006). *Azorhizobium* species may appear intermixed with *Xanthobacter* species in phylogenetic trees based on 16S rRNA gene sequences depending on the conditions chosen (Lang *et al.*, 2008; Lee *et al.*, 2005; Yarza *et al.*, 2008).

Among others, a Gram-negative, motile, non-sporulating and strictly aerobic rod, designated NS12^T, was isolated from a macerated *Rumex* sp. petiole after enrichment with 4 g potassium oxalate l^{-1} as the sole source of carbon and energy (Sahin *et al.*, 2002). The isolate was motile by peritrichous flagella. No inclusions in the cells were detected by light microscopy. Colonies were translucent, cream and convex. No yellow pigment, as described for members of the genus *Xanthobacter*, was detected when tested on nine different media for heterotrophic bacteria. Slime production depended on the medium used. On nutrient agar (per litre: 5 g peptone, 3 g beef extract, 15 g agar agar; Difco), slime was produced; whereas on tryptone soya agar (TSA; DSMZ medium 535), no slime production was observed. The following strains were used as references: *A. caulinodans* DSM 5975^T, *A. doebereinerae* DSM 18977^T, *Xanthobacter flavus* DSM 338^T, *X. autotrophicus* DSM 432^T, *X. aminoxidans* DSM 15009^T, *X. agilis* DSM 3770 ^{T}, *X. tagetidis* DSM 11105 ^{T} and *X. viscosus* DSM 21355 ^{T}. All strains were grown routinely on nutrient agar at 28 °C.

16S rRNA gene sequences were generated and aligned as described by Somvanshi *et al.* (2006). Phylogenetic

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DSM 18749^T is FR799325.

A supplementary figure and a supplementary table are available with the online version of this paper.

dendrograms were constructed using the algorithms of De Soete (1983) and Saitou & Nei (1987). Analysis of the 16S rRNA gene sequence grouped the isolate within the family *Xanthobacteraceae*. Strain NS12^T shared the highest 16S rRNA gene sequence similarity with *A. doebereinerae* BR 5401^T (98.3 %) and *A. caulinodans* ORS 571^T [97.3 % when compared with the sequence entry D11342 and 98.03 % when compared with the shotgun genome sequence AP009384 by the Ezbiocloud programme (Kim *et al.*, 2012)].

Phylogenetic analysis using different distance matrix algorithms clearly assigned strain NS12^T to the genus *Azorhizobium* (Fig. 1). As long as the relationship between the two genera *Xanthobacter* and *Azorhizobium* remains not fully explored, the closer phylogenetic and phenotypic similarity of strain NS12^T to the two members of the genus *Azorhizobium* prompted us to consider it a member of the genus *Azorhizobium*. The characters in favour of this decision are listed in Table 1.

For DNA–DNA hybridization, cells were disrupted using a French pressure cell (Thermo Spectronic) and DNA in the crude lysates was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). Hybridization was carried out in SSC buffer with 10 % formamide at 70 °C as described by De Ley *et al.* (1970), with the modifications described by Huss *et al.* (1983), 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). DNA–DNA relatedness between strain NS12^T and *A. caulinodans* DSM 5975^T and *A. doebereinerae* DSM 18977^T was 14 and 31 %, respectively. DNA–DNA relatedness between strain NS12^T and *X. flavus* DSM 338^T, which share >97 % 16S rRNA gene sequence similarity, was 9 %. According to the widely accepted species delineation value of 70 % (Wayne *et al.*, 1987), these results confirm that strain NS12^T does not belong to any of these three genospecies.

Table 1. Characteristics differentiating strain NS12^T from the genus *Xanthobacter*

Taxa: 1, *Azorhizobium oxalatiphilum* sp. nov. NS12^T; 2, *Xanthobacter* (Wiegel, 2005; Doronina & Trotsenko, 2003). +, Positive; (+) variable; −, negative.

Characteristic	1	2
Autotrophic growth with hydrogen	−	+
Yellow pigment	−	+
Accumulation of polyhydroxylated acids	−	(+)*
Utilization of methanol and ethanol	−	+
Fatty acids: †		
Summed feature 2	+	−
$C_{16:0}$ 3-OH	−	+

*No data available for *X. viscosus* or *X. aminoxidans*.
†Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 2 consisted of $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$.

For the analysis of fatty acids, cells were grown on TSA according to the standards set by the MIDI system even though the medium is suboptimal for most strains. Large amounts of biomass had to be harvested and prepared because of the large amount of slime produced by the cells. After incubation for 48 h at 28 °C, cells were harvested and whole-cell fatty acid methyl esters were obtained by methods described by Kämpfer & Kroppenstedt (1996) and separated by GC (model 6890N; Agilent Technologies). Peaks were automatically integrated and fatty acid names and percentages were determined using

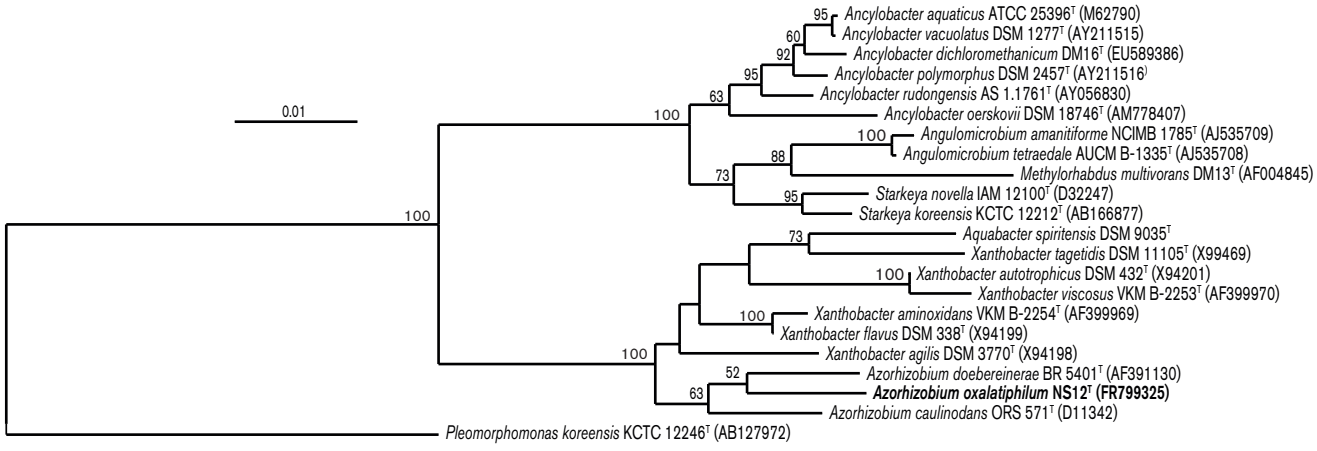

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships of strain NS12^T with members of the genera *Azorhizobium* and *Xanthobacter* and other phylogenetically related genera. Bootstrap values (>50 %) based on 1000 datasets are shown at branch nodes. Bar, 1 % difference in nucleotide sequence.

the Sherlock Microbial Identification System version 4.5 (MIDI; Sasser, 1990). Summed features were identified by GC/MS (Singlequad 320 instrument; Varian). The fatty acids of strain NS12^T were dominated by $C_{18:1}\omega7c$ (58.4 %), cyclo-$C_{19:0}\omega8c$ (27.3 %), 11-methyl-$C_{18:1}\omega7c$ (3.9 %) and $C_{16:0}$ (2.2 %) (Table 2). This composition agreed well with those obtained for *A. caulinodans* DSM 5975^T and *A. doebereinerae* DSM 18977^T. The major difference between strain NS12^T and these two reference strains was the ratio of octadecenoic acid and cyclo-$C_{19:0}$. However, the conversion of the unsaturated acid $C_{18:1}$ into the cyclic derivative cyclo-$C_{19:0}$ at the onset of cell aging is a known phenomenon (Huisman *et al.*, 1996). Obviously, cyclo-$C_{19:0}\omega8c$ was built from $C_{18:1}\omega7c$ at a much higher rate in strain NS12^T than in the reference strains. The high proportion of cyclic nonadecanoic acid in strain NS12^T reflects the fact that the standard medium for fatty acid determination was suboptimal for this organism and can, for that reason, not be taken as a criterion to discriminate the isolate from its closest relatives. The fatty acid compositions corroborated the assignment of strain NS12^T to the genus *Azorhizobium* rather than the genus *Xanthobacter* because of the following features. Strain NS12^T as well as *A. caulinodans* DSM 5975^T contained an unknown component with an equivalent chain-length (ECL) 13.957 and all members of the genus *Azorhizobium* contained summed feature 2 (identified by GC/MS as $C_{14:0}$ 3-OH), neither of which were detected in the genus *Xanthobacter*. On the other hand, $C_{16:0}$ 3-OH was found consistently in the genus *Xanthobacter* whereas it was absent in strain NS12^T and the genus *Azorhizobium*. These results are in accordance with compositions given elsewhere. All members of *X. autotrophicus*, *X. agilis* and *X. flavus* measured by Reding (1991) contained $C_{16:0}$ 3-OH but not summed feature 2 or $C_{14:0}$ 3-OH (Wiegel, 2005). The fatty acid compositions of *X. tagetidis*, *X. viscosus* and *X. aminoxidans* are not documented in literature and those of *A. caulinodans* or *A. doebereinerae* were not given in their species descriptions. However, a study of symbionts of *Aeschynomene* species included the fatty acid compositions of 14 *Azorhizobium* strains (So *et al.*, 1994). Even though the conditions for growth prior to fatty acid determination were different from the conditions applied in our study, the major components were the same as in this study, but maybe differing in quantity. The results given for the *Aeschynomene* isolates confirm the presence of $C_{14:0}$ 3-OH and absence of $C_{16:0}$ 3-OH in the genus *Azorhizobium*.

Polar lipids were extracted from cells cultivated in nutrient broth according to the method described by Minnikin *et al.* (1979) and separated by two-dimensional TLC. Chloroform/methanol/water (65 : 25 : 4, v/v) was used in the first direction and chloroform/acetic acid/methanol/water (80 : 15 : 12 : 4, v/v) was used in the second direction. To identify spots, the chromatographic behaviour of lipid components was compared with those of authentic standard substances (phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine; Sigma). The specific spray reagents ninhydrin and molybdenum blue were used while total lipids were visualized by spraying with molybdophosphoric acid and heating at 150 °C for 10 min (Embley & Wait, 1994). Strain NS12^T contained phosphatidylcholine, phosphatidylglycerol, phosphatidylmonomethylethanolamine and phosphatidylethanolamine as well as minor amounts of two unidentified aminolipids and two unidentified phospholipids (Fig. S1, available in IJSEM Online).

Isoprenoid quinones were extracted according to the method of Collins *et al.* (1977) and analysed by HPLC (Shimadzu LC 20A; Groth *et al.*, 1997). Strain NS12^T contained ubiquinone Q-10 as the major component and ubiquinones Q-9 and Q-11 as minor components, with a ratio of peak area of 94 : 3 : 1. The occurrence of ubiquinone Q-10 as the predominant quinone is in accordance with earlier reports on members of the family *Xanthobacteraceae* (summarized by Hwang & Cho, 2008). Ubiquinone Q-9 has been detected in species of the genus *Xanthobacter* (Wiegel, 2005) and the phylogenetically more distant *Pseudoxanthobacter soli* (Arun *et al.*, 2008), and Q-11 has been found in the distantly related genus *Devosia* (Yoon *et al.*, 2007).

Physiological tests were performed according to standard methods (Smibert & Krieg, 1994) after incubation for up to 10 days at 28 °C. The Gram-reaction was tested by the non-staining KOH method as described by Buck (1982) and by testing for aminopeptidase using ready-made sticks (Merck). Cell morphology and sporulation were determined after growth on nutrient agar for 2, 5 and 10 days by phase-contrast microscopy. Motility of cells was tested by observing swarming in soft agar (per litre: 1.0 g yeast extract, 0.1 g K_2HPO_4, and 2.0 g agar) incubated at 22 °C for up to 5 days. Anaerobic growth was checked in oxygen-poor OF medium with D-glucose covered by paraffin (Hugh & Leifson, 1953). Substrate utilization tests were carried out in mineral medium (Stanier *et al.*, 1966) with vitamins (DSM medium 461) because members of the genera *Azorhizobium* and *Xanthobacter* have been described as being vitamin dependent (Dreyfus *et al.*, 1988; Wiegel, 2005), which was confirmed in our laboratory for strain NS12^T and *A. caulinodans* DSM 5975^T. Chemolithoautotrophy with hydrogen was tested in mineral medium under a microaerobic atmosphere containing 60 % hydrogen (Malik, 1988). API 20 NE strips (bioMérieux) and GN2 MicroPlates (Biolog) were used according to the manufacturers' instructions. Results were recorded after incubation at 28 °C for up to 6 days (API 20 NE) or up to 2 days (GN2 MicroPlates).

Physiologically, strain NS12^T showed properties similar to those of the genera *Azorhizobium* and *Xanthobacter*. For all strains tested, the substrate spectrum – as tested by utilization as the sole source of carbon and energy – generally comprised carbonic acids but not carbohydrates (Tables 3 and 4). The Biolog system nicely demonstrated this substrate pattern in that strain NS12^T reacted

Table 2. Whole-cell fatty acid compositions of strain NS12^T and members of the genera *Azorhizobium* and *Xanthobacter*

Strains: 1, *Azorhizobium oxalatiphilum* sp. nov. NS12^T; 2, *A. caulinodans* DSM 5975^T; 3, *A. doebereinerae* DSM 18977^T; 4, *X. flavus* DSM 338^T; 5, *X. autotrophicus* DSM 432^T; 6, *X. aminoxidans* DSM 15009^T; 7, *X. agilis* DSM 3770^T; 8, *X. tagetidis* DSM 11105^T; 9, *X. viscosus* DSM 21355^T. All data were taken from this study. Cells were grown on TSA at 28 °C for 2 days. ECL, Equivalent chain-length; −, <0.3 % of total.

Fatty acid (%)	1	2	3	4	5	6	7	8	9
Unknown ECL 13.957	0.6	0.6	−	−	−	−	−	−	−
Summed features*									
2	1.5	1.7	0.5	−	−	−	−	−	−
3	0.4	1.1	0.8	0.05	5.7	0.5	1.5	1.3	6.3
$C_{16:0}$	2.2	2.3	1.2	2.6	2.7	2.2	8.2	11.5	3.0
cyclo-$C_{17:0}$	0.6	−	−	−	−	−	−	−	0.4
$C_{17:0}$	0.8	0.2	0.4	0.3	−	−	−	1.2	−
$C_{16:0}$ 3-OH	−	−	−	1.6	1.1	1.5	1.4	1.1	1.0
$C_{18:1}\omega7c$	58.4	80.0	86.6	91.1	86.3	82.8	70.8	70.0	84.3
$C_{18:0}$	1.9	2.6	2.0	2.9	1.6	1.4	1.7	0.8	1.5
11-methyl-$C_{18:1}\omega7c$	3.9	5.6	2.5	−	−	0.5	−	−	−
cyclo-$C_{19:0}\omega8c$	27.3	4.1	5.4	0.4	−	9.2	9.9	13.4	1.3
$C_{18:0}$ 3-OH	−	0.6	−	0.4	−	0.5	0.9	−	0.3
$C_{20:2}\omega6,9c$	0.9	−	−	−	−	0.5	−	−	−
$C_{20:1}\omega7c$	1.1	0.4	0.9	0.5	2.3	0.4	4.9	0.3	1.8

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 2 consisted of $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I) and was identified by GC/MS as $C_{14:0}$ 3-OH. Summed feature 3 consisted of $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and was identified by GC/MS as $C_{16:1}$.

positively with several carbonic acid and amino acid cavities but not with sugar or sugar alcohol cavities. The lack of yellow pigments and polyhydroxycarboxylate inclusions and the inability of strain NS12^T to utilize methanol and ethanol or to grow chemolithoautotrophically with dihydrogen are arguments against the assignment of the isolate to the genus *Xanthobacter*. Strain NS12^T could be differentiated from the members of the genus *Azorhizobium* by its ability to utilize *meso*-tartrate and its inability to utilize malonate, malate, L-aspartate and glutarate (Table 4). The isolate grew on nutrient agar at 10–36 °C, but not at 5 or 37 °C. In contrast, *A. caulinodans* DSM 5975^T grew at 44 °C, which is in accordance with the species description (Dreyfus *et al.*, 1988). Strain NS12^T responded to formic acid, L-aspartic acid and glycerol in the Biolog plates but did not utilize these as the sole source of carbon for growth in mineral medium. Citrate was utilized by the isolate in mineral medium but not under the conditions prevailing in API 20 NE strips. The specific growth rate (μ) of strain NS12^T at 30 °C in mineral medium (DSMZ medium 81) with 4 g potassium oxalate l^{-1} as the sole source of carbon and energy was 0.068 h^{-1} (t_d=10 h). *A. caulinodans* is described as being unable to utilize oxalate as a sole substrate (Dreyfus *et al.*, 1988). Resistance of strain NS12^T to antibiotics, as well as resistance to heavy metal ions, have been described by Sahin *et al.* (2002). The results are indicated in the species description.

For *X. aminoxidans* DSM 15009^T, we found growth at 40 °C on nutrient agar slants while the species description gives 34 °C as the maximum growth temperature (Doronina & Trotsenko, 2003). Motility of *X. aminoxidans* DSM 15009^T seems to depend on cultivation and testing conditions. as for *X. flavus*, which first was described as a non-motile species and for which active locomotion and peritrichous flagella were detected later (Reding *et al.*, 1992). While *X. aminoxidans* is described as being non-motile, we observed swarming of *X. aminoxidans* DSM 15009^T in soft agar, and also motile cells when harvested from nutrient agar. Also deviating from the species description (Dreyfus *et al.*, 1988), no utilization of L-lysine by *A. caulinodans* DSM 5975^T was detected in this study.

Strain NS12^T was tested for nitrogenase activity by the acetylene reduction assay (Stewart *et al.*, 1967). Cultures in 5 ml Wiegel's mineral medium (Wiegel, 2006) with 2 g lactate l^{-1} and vitamins were grown under microaerobic conditions (0.6 %, v/v, oxygen in dinitrogen) at 28 °C. After 24 h, 10 % of the gas phase was substituted with acetylene and the cultures were reincubated at 28 °C. Ethylene production was measured after 24, 48 and 72 h by GC (model 3700 with a N_2 Chromopak column and flame-ionization detector; Varian). To determine cell growth, the optical density (OD_{600}) of cultures was measured using a CO8000 cell density meter over 72 h. *X. flavus* DSM 338^T and *A. doebereinerae* DSM 18977^T were used as positive controls. For negative controls, non-inoculated medium and cultures of *X. flavus* DSM 338^T without acetylene substitution were used (data not shown). The results of the acetylene reduction assay are summarized in Table S1.

Table 3. Phenotypic characteristics of strain NS12^T and members of the genera *Azorhizobium* and *Xanthobacter* using API 20 NE

Strains: 1, *Azorhizobium oxalatiphilum* sp. nov. NS12^T; 2, *A. caulinodans* DSM 5975^T; 3, *A. doebereinerae* DSM 18977^T; 4, *X. flavus* DSM 338^T; 5, *X. autotrophicus* DSM 432^T; 6, *X. aminoxidans* DSM 15009^T; 7, *X. agilis* DSM 3770^T; 8, *X. tagetidis* DSM 11105^T; 9, *X. viscosus* DSM 21355^T. All strains were positive for utilization of malate and oxidase. All strains were negative for indole production, acidification of glucose, arginine dihydrolase, hydrolysis of aesculin and gelatin, *β*-galactosidase and utilization of glucose, arabinose, mannose, mannitol, maltose, *N*-acetylglucosamine and caprate. +, Positive; W, weakly positive; DEL, positive delayed for ⩾5 days; −, negative.

Characteristic	1	2	3	4	5	6	7	8	9
Nitrate reduction	+	+	+	+	+	+	+	−	+
Urease	+	+	+	−	DEL	DEL	DEL	+	+
Utilization of:									
Gluconate	−	+	DEL	+	+	+	DEL	+	+
Adipate	−	DEL	−	+	−	−	−	−	−
Citrate	−	+	−	+	DEL	+	−	−	W
Phenylacetate	−	−	−	+	+	+	−	−	−

Table 4. Morphological and physiological characteristics of strain NS12^T and closely related members of the genera *Azorhizobium* and *Xanthobacter*

Strains: 1, *Azorhizobium oxalatiphilum* sp. nov. NS12^T; 2, *A. caulinodans* DSM 5975^T; 3, *A. doebereinerae* DSM 18977^T; 4, *X. flavus* DSM 338^T; 5, *X. autotrophicus* DSM 432^T; 6, *X. aminoxidans* DSM 15009^T. All data were taken from this study. All strains were positive for growth with 2 % NaCl and utilization of fumarate, succinate, gluconate, lactate, propionate, pyruvate and L-glutamine. All strains were negative for utilization of lactose, sucrose and L-lysine. BY, Bright yellow; C, cream; Y, yellow; +, positive; W, weakly positive; DEL, positive delayed for ⩾5 days; V, variable; −, negative; ND, no data available.

Characteristic	1	2	2	4	5	6
Motility in semisolid agar	+	+	+	−	−	+
Autotrophic growth with H_2	−	−	+	+	+	+
Colony colour	C	C	C	Y	BY	Y
Slime production on:						
Nutrient agar	+	W	−	−	+	−
Tryptone soya agar	−	W	+	W	W	−
Maximum growth temperature (°C)	36	44	36	37	37	40
Utilization in mineral medium						
D-Fructose	−	−	−	−	+	−
Malonate	−	+	+	DEL	−	DEL
Malate	−	+	DEL	DEL	−	DEL
Citrate	+	+	−	+	+	+
L-Aspartate	−	+	+	V	+	+
L-Alanine	+	+	+	+	−	+
Formate	−	−	+	+	+	+
Methanol	−	−	−	+	+	+
Ethanol	−	−	+	+	+	+
Glycerol	+	+	+	+	−	+
DL-Proline	−	+	V	−	−	−
Glutarate	−	+	+	+	+	+
D-Saccharate	−	+	ND	ND	ND	ND
Quinate	−	+	ND	ND	ND	ND
meso-Tartrate	+	−	−	−	+	ND

Acetylene was converted to ethylene by strain NS12^T as well as the two control strains. For all three strains, the amount of ethylene increased over time and turbidity of the cultures remained constant after the first 24 h. After 24 h, strain NS12^T revealed much lower ethylene production than *X. flavus* DSM 338^T, but this difference decreased to 18 % after 48 h and 13 % after 72 h. After this period, ethylene production by strain NS12^T reached the same level of production as *X. flavus* DSM 338^T.

The genomic, chemotaxonomic and phenotypic properties suggested the assignment of strain NS12^T to the genus *Azorhizobium*. On the basis of low DNA–DNA relatedness with its closest phylogenetic neighbours and of distinguishing biochemical traits, it can be concluded that strain NS12^T represents a novel species within this genus. Thus, a novel species, *Azorhizobium oxalatiphilum* sp. nov., is proposed.

Description of *Azorhizobium oxalatiphilum* sp. nov.

Azorhizobium oxalatiphilum [o.xa.la.ti′phi.lum. N.L. n. *oxalas -atis* oxalate; N.L. neut. adj. *philum* (from Gr. neut. adj. *philon*), friend, loving; N.L. neut. adj. *oxalatiphilum* oxalate-loving].

Gram-reaction-negative rods, motile by peritrichous flagella. Cells of fresh cultures are 1 × 2.5–3 μm; some are 4 μm long. Cells are slightly irregular with rounded ends and occur singly or in pairs. After 2 days in nutrient broth with 1 g succinate l^{-1}, cells become elongated or pleomorphic. No spores or gas vesicles. Non-pigmented. On nutrient agar, colonies are round, translucent, convex and cream, 0.3–0.5 mm in diameter after 2 days of incubation and become slimy with age. Fixes dinitrogen (acetylene reduction assay). Does not grow autotrophically with hydrogen. Grows on nutrient agar slopes at 10 °C but not at 5 °C; maximum growth temperature is 36 °C. Grows in nutrient broth with 2 % NaCl. Oxidase-, catalase- and urease-positive. Negative for acid production from glucose,

β-galactosidase activity, indole production, arginine dihydrolase activity and gelatin and aesculin hydrolysis (API 20 NE). Utilizes malate (API 20 NE), lactate, propionate, pyruvate, succinate, citrate, fumarate, 2-ketoglutarate, D-galacturonate, gluconate (weakly), glycerol, glutamine and L-alanine (in mineral medium with vitamins), but does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, maltose, *N*-acetylglucosamine, gluconate, caprate, adipate, citrate, phenylacetate (API 20 NE), D-fructose, sucrose, lactose, malonate, malate, glutarate, formate, L-saccharate, quinate, DL-proline, L-aspartate, methanol or ethanol (in mineral medium with vitamins). With GN2 MicroPlates, metabolizes methylpyruvate, monomethylsuccinate, acetic acid, formic acid, α-, β- and γ-hydroxybutyric acids, α-ketobutyric acid, DL-lactic acid, propionic acid, succinic acid, bromosuccinic acid, succinamic acid, L-asparagine, L-aspartic acid, L-glutamic acid, L-pyroglutamic acid and glycerol. Contains phosphatidylcholine, phosphatidylglycerol, phosphatidylmonomethylethanolamine and phosphatidylethanolamine as the major polar lipids and ubiquinone Q-10 as the major quinone, with traces of Q-9 and Q-11. The major fatty acids (>2 %) are $C_{18:1}\omega7c$, cyclo-$C_{19:0}\omega8c$, 11-methyl-$C_{18:1}\omega7c$ and $C_{16:0}$; also contains $C_{14:0}$ 3-OH. The fatty acid $C_{16:0}$ 3-OH is not detected. Resistant to (μg per disc) erythromycin (15), ampicillin (10) and bacitracin (10), but sensitive to gentamicin (10), streptomycin (10) and chloramphenicol (30). Sensitive to (all 2.5 μg per disc) $HgCl_2$, but resistant to $ZnSO_4 . 7H_2O$; $NiCl_2 . 6H_2O$, $CoCl_2 . 6H_2O$, $CuSO_4 . 5H_2O$, Pb acetate and K_2CrO_7.

The type strain, NS12^T (DSM 18749^T=CCM 7897^T), was isolated from macerated *Rumex* sp. petioles after enrichment in mineral medium with oxalate.

Emended description of the genus *Azorhizobium* Dreyfus *et al.* 1988

Azorhizobium [A.zo.rhi.zo′bi.um. N.L. n. *azotum* (from Fr. n. *azote* [from Gr. prep. *a* not; Gr. n. *zôê* life; N.Gr. n. *azôê* not sustaining life]) nitrogen; N.L. pref. *azo-* pertaining to nitrogen; N.L. neut. n. *Rhizobium* a bacterial generic name; N.L. neut. n. *Azorhizobium* a nitrogen (using) *Rhizobium*].

The description of the genus is as given by Dreyfus *et al.* (1988) with following amendments. Urease activity may be present. Utilization of DL-proline and malonate is variable. The main fatty acids are $C_{18:1}\omega7c$, cyclo-$C_{19:0}\omega8c$, 11-methyl-$C_{18:1}\omega7c$ and $C_{16:0}$. Also contains $C_{14:0}$ 3-OH, but not $C_{16:0}$ 3-OH.

Acknowledgements

We are grateful to G. Pötter (DSMZ) for performing the fatty acid analysis. We thank P. Aumann, I. Brandes, M. Kopitz, I. Kramer, B. Sträubler, J. Swiderski, H. Thierfelder, A. Wasner and S. Welnitz for excellent technical assistance.

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