The first representative of the globally widespread subdivision 6 Acidobacteria, Vicinamibacter silvestris gen. nov., sp. nov., isolated from subtropical savannah soil

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Members of the phylum Acidobacteria are abundant in a wide variety of soil environments. Despite this, previous cultivation attempts have frequently failed to retrieve representative phylotypes of Acidobacteria, which have, therefore, been discovered by culture-independent methods (13175 acidobacterial sequences in the SILVA database version 123; NR99) and only 47 species have been described so far. Strain Ac_5_C6 T is the first isolate of the globally widespread and abundant subdivision 6 Acidobacteria and is described in the present study. Cells of strain Ac_5_C6 T were Gram-stain-negative, immotile rods that divided by binary fission. They formed yellow, extremely cohesive colonies and stable aggregates even in rapidly shaken liquid cultures. Ac_5_C6 T was tolerant of a wide range of temperatures (12–40 °C) and pH values (4.7–9.0). It grew chemooorganoheterotrophically on a broad range of substrates including different sugars, organic acids, nucleic acids and complex proteinaceous compounds. The major fatty acids of Ac_5_C6 T were iso-C 17:0, C 18:1 ω7c, C 18:1 ω9c and iso-C 15:0. Summed feature 3 (C 16:1 ω7c/C 16:1 ω6c), iso-C 17:0 and C 16:0 were also detected. Phosphatidylcholine, phosphatidyglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unidentified glycolipid were identified as polar lipids. The major quinone was MK-8. The DNA G+C content of Ac_5_C6 T was 65.9 mol%. With 16S rRNA gene sequence similarities of 83 %, the closest described relatives were Acidicapsa borealis KA1 T, Acidobacterium capsulatum 161 T, Granulicella pectinovorans TPB6011 T, Occallatibacter riparius 277 T and Paludibaculum fermentans P105 T. According to the morphological, physiological and molecular characteristics, the novel genus Vicinamibacter gen. nov., and the novel species, Vicinamibacter silvestris sp. nov. (type strain Ac_5_C6 T = DSM 29464 T = LMG 29035 T) are proposed.
contain described species. The eleven genera, *Acidobacterium* (Kishimoto et al., 1991), *Terriglobus* (Baik et al., 2013; Eichorst et al., 2007; Männistö et al., 2011; Pascoal et al., 2015; Whang et al., 2014), *Edaphobacter* (Koch et al., 2008; Wang et al., 2015), *Acidicapsa* (Kulichevskaya et al., 2012), *Granulicella* (Männistö et al., 2012; Pankratov & Dedys, 2010; Yamada et al., 2014), *Telmatobacter* (Pankratov et al., 2012), *Acidipila* (Jiang et al., 2015; Okamura et al., 2011), *Bryocella* (Dedys et al., 2012), *Occellatibacter* (Foesel et al., 2015), *Terracidiphilus* (Garcia-Fraile et al., 2015) and *Silvibacterium* (Lladó et al., 2016) belong to sd1. The two genera, *Bryobacter* (Kulichevskaya et al., 2010) and *Paludibaculum* (Kulichevskaya et al., 2014), fall into sd3. The eight genera *Blastocatella* (Foesel et al., 2013), *Pyrinomonas* (Crowe et al., 2013), *Aridibacter* (Huber et al., 2014), *Chloracidobacterium* (Tank & Bryant, 2015), *Tellurimicrobium* (Pascoal et al., 2015), *Stenotrophobacter* (Pascual et al., 2015), *Brevitalea* (Wüst et al., 2016) and *Arenimicrobium* (Wüst et al., 2016) into sd4 and the three genera *Holophaga* (Liesack et al., 1994), *Geothrix* (Coates et al., 1999) and *Acanthopleuribacter* (Fukunaga et al., 2008) into sd8. Finally, the genus *Thermostomaculum* (Izumii et al., 2012) is a representative of sd10 and the genus *Thermoanaebobacterium* (Losey et al., 2013) a member of sd23. In particular, no isolate of sd6 is available, although the latter represents the dominant subdivision in many soils (Barns et al., 1999; Foesel et al., 2014; Jones et al., 2009). 16S rRNA sequence analyses revealed that members of sd6 dominate in soils with a neutral or (slightly) basic pH, together with bacteria of sd4, whereas members of sd1 and 3 prevail in soils which are (slightly) acidic (Foesel et al., 2014; Jones et al., 2009). About half of the soils on earth have a neutral or (slightly) basic pH (www.fao.org/soils-portal/soil-survey/soil-properties/chemical-properties/en/). Thus, they would constitute suitable sources for the cultivation of representatives of sd6. However, no isolate of this subdivision has been described in detail so far, but the successful isolation of 11 *Acidobacteria* sd6 strains from Belgian soil samples has been reported previously (George et al., 2011).

In the present study we describe the first representative of sd6 *Acidobacteria, Vicinamibacter silvestris* gen. nov., sp. nov., which was isolated from a semiarid subtropical savannah soil.

Strain Ac. _5_C6^T_ was isolated from a riparian woodland soil collected from south of the Okavango river (17°53′35.52″S; 20°14′57.52″E; 1061 m above sea level) near the village of Mashare in the North-East of Namibia. The soil was a clayey sand with a slightly basic pH (7.5 and 8.0 measured in 10 mM CaCl₂ and in distilled water, respectively). Total bacterial cell numbers were determined in the clayey soil with fluorescent microscopy, as described in Lunau et al., 2005. For the enrichment of *Acidobacteria* the soil was diluted with 10 mM HEPES (pH 7.0) and 20 µl of the diluted soil suspension containing approximately 10 cells were inoculated in 180 µl of liquid SE (soil extract)/HD 1:10 medium (pH 7.0). This medium is based on a mixture of the DSMZ media number 12 and 1124 (instead of garden soil a mixture of Namibian sandy savannah soils was used; Wüst et al., 2016; available in the online Supplementary Material), which was supplemented with a 10-vitamin solution (Balch et al., 1979; Supplementary Material) and trace element solution SL-10 (Tschech & Pfennig, 1984; Supplementary Material). After 9 weeks of incubation at 20 °C in the dark, cultures were screened for acidobacterial growth by phyllum-specific PCR using the primer pair Acid31f (Barns et al., 1999) and 1492r (Lane, 1991). Cultures that yielded positive PCR products were plated on SE/HD 1:10 plates (pH 7.0) solidified with purified agar (15 g l⁻¹; Oxoid). A pure culture of _Ac. _5_C6^T_ was finally obtained by subsequent restreaking on plates. Unless otherwise noted, SSE/HD 1:10 (pH 7.0) was used in the following physiological tests and for biomass production (Supplementary Material). The SSE/HD 1:10 (pH 7.0) medium was modified medium DSMZ number 1426, as it was buffered with HEPES instead of MES. The medium was based on the soil solution equivalent (SSE; Angle et al., 1991), supplemented with 10-vitamin solution (Balch et al., 1979), a trace element solution SL-10 (Tschech & Pfennig, 1984) and solidified with purified agar (Oxoid). Data used from the literature had been obtained under comparable growth conditions to those implemented here for strain _Ac. _5_C6^T_. All media employed contained glucose or gluconic acid, and yeast or casamino acids (0.1–0.5 g l⁻¹) as growth factors. Proteinaceous substrates such as peptone (0.5 g l⁻¹) were also present.

Strain Ac. _5_C6^T_ formed solid colonies on agar plates with a diameter of about 1 mm; they were yellow, circular, convex-hemispherical and opaque with entire margins. These features are comparable to the soil isolates described by George et al., 2011. Even during shaking liquid cultures of strain _Ac. _5_C6^T_ formed yellow aggregates, which were very solid and could not be disrupted without damaging the cells. Light microscopic observations (Zeiss Axioscope.A1; Carl Zeiss, with AxioCam Mrm camera) confirmed that the cells of strain _Ac. _5_C6^T_ were rod-shaped, 1.3–2.0 µm long, approximately 0.6–0.7 µm in diameter and that they divided by binary fission. Strain _Ac. _5_C6^T_ formed aggregates and single cells also occurred occasionally (Fig. 1a). Consistent with all other *Acidobacteria* characterized so far, strain _Ac. _5_C6^T_ was Gram-stain-negative (Gerhardt et al., 1994). India ink and malachite green staining (Bast, 2011) confirmed, respectively, that capsules and spores were not formed in strain _Ac. _5_C6^T_. To dissolve the ultrastructure of strain _Ac. _5_C6^T_, cells were high-pressure frozen and cryosubstituted for electron microscopy to prevent artefact formation due to cell shrinkage as a reaction to chemical fixation (Wanner et al., 2008). Like other representatives of the *Acidobacteria*, strain _Ac. _5_C6^T_ revealed a Gram-negative structure of the cell wall and intracellular inclusion bodies (Fig. 1b). As shown by Foesel et al. (2015) and Wüst et al. (2016), high numbers of ribosomes could be detected in strain _Ac. _5_C6^T_.

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The almost full-length 16S rRNA gene fragment of strain Ac_5_C6\textsuperscript{T} was amplified by colony-PCR using primer pair 8I (Turner et al., 1999) and 1492r (Lane, 1991). The resulting sequence comprised 1476 unambiguous nucleotides between *Escherichia coli* positions 28 and 1491 (Brosius et al., 1978). The sequence was added to the 16S rRNA-based ‘All-Species Living Tree’ Project (LTP) database (Yarza et al., 2008) release 123 (September 2015) using the program package ARB version 6.0 (Ludwig et al., 2004). After automated alignment with the Fast aligner tool implemented in ARB, the alignment was manually refined based on secondary structure information. Phylegetic trees were constructed using neighbour-joining, maximum parsimony and maximum likelihood algorithms (40 % maximum frequency filter, resulting in 1418 valid columns between positions 64 and 1439 of the *E. coli* 16S rRNA reference gene; 1000 bootstrap resamplings). All three methods placed the three novel isolates within sd6 *Acidobacteria* where they constituted a stable cluster together with strains IG0-001–IG0-009 isolated from a Belgian soil (George et al., 2011) (Fig. 2). The pairwise 16S rRNA gene sequence similarities (ARB neighbour-joining tool without the use of an evolutionary substitution model) between strain Ac_5_C6\textsuperscript{T} and the former isolates were 97.2–99.2 %. Strains IG0-010 (91.7 %) and IG0-011 (94.5 %) were more distantly related. The closest relatives of strain Ac_5_C6\textsuperscript{T} were representatives of *Acidobacteria* sds 1 and 3, namely *Acidicyclus borealis* KAI\textsuperscript{1}, *Granulumella pectinovorans* TPB601\textsuperscript{1}, *Acidobacterium capsulatum* 161\textsuperscript{1}, *Ocellatibacter riparius* 277\textsuperscript{1} and *Paludibaculum fermentans* P105\textsuperscript{1} with 16S rRNA nucleotide similarities of 83–84 %.

To determine the G+C content of strain Ac_5_C6\textsuperscript{T}, cells were disrupted in a French press and the DNA was subsequently purified using hydroxyapatite (Cashon et al., 1977). The sample was treated with P1 nuclease and bovine alkaline phosphatase (Mesbah et al., 1989) and the resulting deoxyribonucleosides were analysed by HPLC (Shimazu), employing conditions adapted from Tamaoka & Komagata (1984). The G+C content of strain Ac_5_C6\textsuperscript{T} was 65.9 mol % (calculated by the method introduced by Mesbah et al., 1989); this exceeds the G+C content of 46.5–62.7 mol% of the other described species of *Acidobacteria* sds 1, 3, 4, 8, 10 and 23 including the thermophilic representatives *Pyrimonas methylyaliphaticovora* K22\textsuperscript{1}, *Chloracidobacterium thermophilium* B\textsuperscript{1}, *Thermotomaculum hydrothermale* AC55\textsuperscript{1} and *Thermoaeroebaculum aquaticum* MP-01\textsuperscript{1} (Table S1, available in the online Supplementary Material). Only *Geothrix fermentans* H-5\textsuperscript{1} and *Arenimicrobium luteum* Ac_12_G8\textsuperscript{1} exhibit higher G+C contents with 68.9 mol% and 66.9 mol %, respectively (Table S1).

Isoprenoid quinones were extracted from dried biomass with chloroform/methanol (2:1, v/v) as introduced by Collins & Jones (1981). The subsequent analysis by HPLC (Tindall, 1990) identified MK-8 as the major quinone of strain Ac_5_C6\textsuperscript{T}, which is in congruence with the majority of the described representatives of the *Acidobacteria* sds 1, 3 and 4 (Table S1). In addition, minor amounts of MK-9 were detected in strain Ac_5_C6\textsuperscript{T}.

The polar lipid composition was determined by two-dimensional TLC, as described previously (modified after Bligh & Dyer, 1959; Tindall et al., 2007; Fig. S1). Phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unidentified glycolipid were identified as the polar lipids of strain Ac_5_C6\textsuperscript{T}. This polar lipid composition matched that reported for the sd4 member *Tellurimicrobium multivorans* Ac_18_E7\textsuperscript{c} (Pascual et al., 2015), except for the presence of an unidentified phosphoglycolipid in Ac_18_E7\textsuperscript{c} and the presence of an unidentified glycolipid in Ac_5_C6\textsuperscript{T}. Also, apart from the additional presence of an unidentified glycolipid in Ac_5_C6\textsuperscript{T}, the polar lipid composition of strain Ac_5_C6\textsuperscript{T} also corresponded with the polar lipid composition of the savannah strains, *Aridibacter* spp. (Huber et al.,

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**Fig. 1.** a) Phase-contrast photomicrograph of strain Ac_5_C6\textsuperscript{T}. Bar, 10 µm. b) Transmission electron micrograph of an ultrathin section of strain Ac_5_C6\textsuperscript{T}. Bar, 200 nm. OM = outer membrane, P = periplasm, CM = cytoplasmic membrane, IB = inclusion body, R = ribosomes.
2014) and *Stenotrophobacter* spp. (Pascual et al., 2015). Phosphatidylethanolamine and phosphatidylglycerol were also present in the two sd1 species *Acidobacterium capsulatum* 161T and *Telmatobacter bradus* TPB6017T (Pankratov et al., 2012) and in the sd23 member *Thermoanaerobaculum aquaticum* MP-01T (Losey et al., 2013). Additionally, strain Ac_5_C6T contains phosphatidylglycine, as do other *Acidobacteria* except i.e. *Bryobacter aggregatus* MPL3T and *Thermotomaculum hydrothermale* AC55T. Apart from strain Ac_5_C6T, diphosphatidylglycerol was also determined in the sd23 member *Thermoanaerobaculum aquaticum* MP-01T (Losey et al., 2013) and in the savannah soil representatives of sd4 *Aridibacter* spp., *Tellurimicrobi um multivorans Ac_18_E7T*, *Stenotrophobacter* spp., *Brevitalea* spp. and *Arenimonibacter luteum Ac_12_G8T* (Huber et al., 2014; Pascual et al., 2015; Wüst et al., 2016).

For fatty acid analysis about 40 mg wet weight of cells were harvested and extracted according to the standard protocol (Sasser, 1990) of the Microbial Identification System (MIDI Inc.; version 6.1). The fatty acid composition was identified by the comparison to the TSBA40 peak naming table database. Strain Ac_5_C6T possessed straight chain, methyl and/or hydroxyl-branched unsaturated and monosaturated fatty acids. The major components were iso-C_{17:1} ω9c (30.1 %), C_{18:1} ω7c (28.6 %) and iso-C_{15:0} (13.4 %). Furthermore, summed feature 3 (C_{16:1}ω7c/C_{16:1} ω6c, 5.6 %), iso-C_{17:0} (5.4 %) and C_{16:0} (4.4 %) occurred in notable amounts. The full profile is given in Table S2.

Thus, more than half (58.7 %) of the fatty acids of strain Ac_5_C6T consisted of the two unsaturated (methyl-branched) fatty acids C_{18:1} ω7c and iso-C_{17:1} ω9c. This feature clearly distinguishes the sd6 strain Ac_5_C6T from all *Acidobacteria* described so far. In contrast, iso-C_{15:0} which is the major component (>35 %) in most of the described *Acidobacteria* only constituted the third most abundant compound in strain Ac_5_C6T. The presence of C_{16:0} and summed feature 3 (C_{16:1}ω7c/C_{16:1} ω6c) or C_{17:1}ω7c are a feature that strain Ac_5_C6T and some members of sd1 and sd4 such as *Granulicella* spp., *Bryocella* elongata SN10T, *Terriglobus roseus* KBS 63T, *Edaphobacter* spp., *Aridibacter* spp. have in common (Dedysh, 2010), although strain Ac_5_C6T only constituted the third most abundant feature contents compared to the other strains. No summed feature 1 (iso-C_{15:1} H/C_{13:0} 3-OH) was detected in strain Ac_5_C6T as opposed to the sd4 *Aridobacteria* such as *Blastocatella fastidiosa* A2-16T, *Aridibacter* spp., *Tellurimicrobi um multivorans Ac_18_E7T*, *Stenotrophobacter* spp., *Brevitalea* spp. and *Arenimonibacter luteum Ac_12_G8T*, which were also isolated from subtropical soils. iso-C_{17:0} was detected in strain Ac_5_C6T as in the sd1 *Acidobacteria Acidicapsa* spp., *Occalitobacter riparius* 277T and *Telmatobacter bradus* TPB6017T, in the sd4 *Aridobacteria*, *Pyroimonas methylaliphathogenes*, K22T, *Tellurimicrobi um multivorans Ac_18_E7T*, *Brevitalea* spp. and *Arenimonibacter luteum Ac_12_G8T*, in *Acanthopleuribacter pedis* FYK2218T, in *Thermotomaculum hydrothermal* AC55T and in *Thermoanaerobaculum aquaticum* MP-01T (Table S1).

The temperature and pH ranges and optima for growth were determined between 10 and 56 °C and between pH 1.0 and 11.0 under oxic conditions in liquid SSE/HD 1 : 10 medium in duplicate and triplicate, respectively. Depending on the desired pH range, MES, HEPES, HEPPS or CHES (Sigma-Aldrich; 10 mM each) were used as buffers. Growth was determined by measuring the optical density at 660 nm. Growth of strain Ac_5_C6T was detected between 12 and 40 °C and between pH 4.7 and 9.0. Optimal growth (defined as ≥75 % of the highest growth rate achieved) was reached at 26–31 °C and pH 6.5–8.1. The absolute optimum was observed at 28 °C and pH 7.0. Hence, strain Ac_5_C6T tolerates a broad range of temperatures and pH values, a feature shared with the representatives of sd4 *Acidobacteria Blastocatella fastidiosa* A2-16T, *Aridibacter* spp., *P. methylaliphathogenes* K22T, *Tellurimicrobi um multivorans Ac_18_E7T*, *Stenotrophobacter* spp., *Brevitalea* spp. and *Arenimonibacter luteum Ac_12_G8T* (Crowe et al., 2014; Foessel et al., 2013; Huber et al., 2014; Pascual et al., 2015; Wüst et al., 2016). HD 1 : 10 medium [0.25 g l^{-1} yeast extract, 0.5 g l^{-1} peptone, 0.1 g l^{-1} glucose, 0.1 ml l^{-1} 10-vitamin solution (Balch et al., 1979; Supplementary Material) and 1 ml l^{-1} trace element solution SL-10 (Tschech & Pfennig, 1984; Supplementary Material)] with varying NaCl concentrations [0–10 % (w/v)] was used to determine the salt tolerance of strain Ac_5_C6T. Strain Ac_5_C6T tolerated NaCl concentrations of 0–1 % (w/v), which is comparable to the preferences of other savannah soil isolates (Table S1). However, strain Ac_5_C6T grew best at a NaCl concentration of 0 % (w/v).

Six parallels of liquid oxic SSE/HD 1 : 10 medium without peptone, yeast extract and glucose were used to determine the growth substrates utilized by strain Ac_5_C6T. The growth medium was supplemented with different sugars, organic acids, keto acids, alcohols, amino acids (0.5–10 mM each), casamino acids, casein hydrolysate, laminarin, peptone, yeast extract (0.05 % w/v each) and Tween 80 (0.001 % w/v) (Table S3), accordingly. Unlike most of the subtropical savannah strains of sd4, strain Ac_5_C6T grew on various sugars (Table S4), a trait common to the sd1 species *Acidobacterium capsulatum* 161T, *Terriglobus roseus* KBS 63T, *Edaphobacter* spp. and *Bryocella elongata* SN10T as well as the sd4 representative *Tellurimicrobi um multivorans Ac_18_E7T* (Table S4). However, strain Ac_5_C6T also utilized proteinaceous substances like casamino acids, yeast extract and peptone as do the sd4 representatives from semiarid savannah soils *Blastocatella fastidiosa* A2-16T (Foessel et al., 2013), *Aridibacter* spp. (Huber et al., 2014), *Tellurimicrobi um multivorans Ac_18_E7T* (Pascual et al., 2015), two *Stenotrophobacter* spp. (Pascual et al., 2015), *Brevitalea* spp. and *Arenimonibacter luteum Ac_12_G8T* (Wüst et al., 2016). Additionally, the ability of strain Ac_5_C6T to degrade complex substrates like cellulose, chitin, starch, xylan, pectin and lignin was tested on solidified SSE medium supplemented with 0.005 % (w/v) yeast extract and a final concentration of 0.5 g l^{-1} of the respective polymer. Strain
Fig. 2. Rooted neighbour-joining phylogenetic tree (Felsenstein correction) based on almost full-length 16S rRNA gene sequences showing the relationship of strain Ac_5_C6T to related taxa. Bootstrap values (expressed as a percentage of 1000 replicates) are indicated at the respective branching points. The following sequences were used as outgroups: Planctopirus limnophila DSM 3776T (X62911), Gimesia maris DSM 8797T (AJ231184) and Blastopirellula marina DSM 3645T (X62912). Bar, 10% nucleotide divergence.

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Ac<sub>5</sub>C<sub>6</sub>T could not degrade any of the polymeric substrates when tested for the appearance of clearing zones with suitable aqueous staining solutions (Pascual et al., 2015).

The API ZYM and API 20NE test systems (Biomerieux) were employed to test exoenzyme activity and the fermentation of sugars. While different exoenzyme activities were determined, the fermentation of sugars could not be demonstrated (Table S5). Cytochrome c-oxidase and catalase activities were determined as described in Cowan et al., (1993) and Gerhardt (1994). In addition, the activity of cytochrome c-oxidase was verified using Bactident Oxidase (Merck).

The resistance or susceptibility of strain Ac<sub>5</sub>C<sub>6</sub>T to common antibiotics was tested on SSE/HD 1:10 agar plates supplemented with the following antibiotics (concentrations used in parenthesis): ampicillin (100 µg ml<sup>-1</sup>), carbenicillin (100 µg ml<sup>-1</sup>), kanamycin (50 µg ml<sup>-1</sup>), chloramphenicol (34 µg ml<sup>-1</sup>), erythromycin (25 µg ml<sup>-1</sup>), spectinomycin (25 µg ml<sup>-1</sup>), streptomycin (25 µg ml<sup>-1</sup>), tetracycline (12.5 µg ml<sup>-1</sup>) and gentamicin (16 µg ml<sup>-1</sup>). Strain Ac<sub>5</sub>C<sub>6</sub>T showed resistance to ampicillin, carbenicillin, kanamycin, chloramphenicol, erythromycin, tetracycline and gentamicin and susceptibility to spectinomycin and streptomycin (Table S1).

In summary, strain Ac<sub>5</sub>C<sub>6</sub>T is an aerobic, chemoorganoheterotrophic, yellow pigmented mesophile with a tolerance to a broad range of pH values and temperatures, as well as forming strong aggregates in liquid cultures. The high G+C content exceeds that of known thermophilic Acidobacteria and the high amounts of the methyl-branched and unsaturated fatty acids C<sub>18:1</sub> ω7c and iso-C<sub>17:1</sub> ω9c distinguishes this first described representative of sd6 Acidobacteria from all other Acidobacteria characterised so far. Furthermore, 16S rRNA gene sequence analysis unambiguously places strain Ac<sub>5</sub>C<sub>6</sub>T in sd6 Acidobacteria and with similarities of 83–84% clearly distinguishes strain Ac<sub>5</sub>C<sub>6</sub>T from its phylogenetically closest described relatives with validly published names: Acidicapsa borealis KA1<sup>T</sup>, Granulicella pontiniformis TPB6011<sup>T</sup>, Acidobacterium capsulatum 161<sup>T</sup>, Occallatibacter riparius 277<sup>T</sup> and Paludibaculum fermentans P105<sup>T</sup> of sds 1 and 3.

Based on the phylogeny, morphology and physiology of strain Ac<sub>5</sub>C<sub>6</sub>T, the novel genus Vicinamibacter gen. nov., including the novel species, Vicinamibacter silvestris sp. nov., is proposed.

**Description of Vicinamibacter silvestris, sp. nov.**

Vicinamibacter silvestris (sil.ves’tris. L. masc. adj. silvestris pertaining to the forest soil from which the type strain was isolated).

Cells are 1.3–2.0 µm long and 0.6–0.7 µm in diameter. Liquid cultures and colonies on agar plates are yellow. Tight aggregates are formed even in shaken cultures and cannot be disrupted. Colonies on agar plates are 1–2 mm in size and are circular, convex-hemispherical and opaque with entire margins. Grows at temperatures of 12–40 °C (best at 25.5–31.1 °C) and pH 4.7–9.0 (best at 6.5–8.1). Under optimal growth conditions the doubling time is 17.4 h. The strain grows at NaCl concentrations of up to 1% (w/v; optimum, 0% w/v).

Grows on arabinose, fructose, galactose, glucose, lactose, lypoxy, maltose, rhamnose, xylose, cellobiose, melezitose, raffinose, sucrose, trehalose, hydroxy-proline, N-acetylgalactosamine, N-acetylglucosamine, fumarate, gluconate, glutamate, casamino acids, casein hydrolysate, peptone, yeast extract and fermented rumen extract. Weak growth is observed on fucose, mannose, acetoin, ascorbate, citrate, glycerin, heptanoic acid, isovaleric acid, 2-oxoglutarate, pyruvate, alanine, asparagine, aspartate, proline, protocatechuic acid, shikimate, trimethoxybenzoate and laminarin. No growth is observed on erythrose, erythulose, sorbose, glucosamine, adonitol, arabitol, dulcitol, lyxitol, mannitol, myo-inositol, sorbitol, xylitol, arginine, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, serine, threonine, tryptophan, tyrosine, valine, adipate, acetae, benzoate, butyrate, α-hydroxybutyrate, β-hydroxybutyrate, γ-hydroxybutyrate, isobutyrate, caproate, caprylate, isocitrate, crotonate, formate, 2-oxoglutarate, succinate, γ-ketoglutarate, glycolate, glyoxylate, laevulinate, lactate, malate, malonate, nicotinic acid, oxaloacetate, propionate, succinate, tartrate, 2-oxovalerate, butanol, 1,2-butandiol, 2,3-butandiol, ethanol, ethylene glycol, methanol, propanol, 1,2-propanediol and Tween 80.

Positive for alkaline and acid phosphatase, naphtol-AS-BI-phosphohydrodrolase, α-chymotrypsin, trypsin, esterase C4, leucine arylaminidase and valine arylamidase. Cysteine fission. Cytochrome c-oxidase positive. Catalase-positive. No capsule formation. Major fatty acids are iso-C<sub>17:1</sub> ω6c, C<sub>18:1</sub> ω7c and iso-C<sub>15:0</sub> 2-C<sub>16:1</sub> ω6c and summed feature 3 (C<sub>16:1</sub> ω7c/C<sub>16:1</sub> ω6c). The major quinone is MK-8. Phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and an unidentified glycolipid are the polar lipids. Tolerant to a wide range of pH values. Aerobic, chemoorganotrophic mesophile that grows on different sugars, organic acids and complex proteinaceous substrates.

The type species is Vicinamibacter silvestris.
arylamidase and esterase lipase C8 show weak activity. Negative for the activities of the enzymes α-glucosidase, β-glucosidase (ApiZYM), α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase, α-fucosidase, N-acetyl-β-glucosaminidase, lipase C14. Aesculin and gelatine are hydrolyzed. Urease, arginine dihydrolase, indol production, para-nitrophenyl-β-D-galactopyranosidase, nitrate reduction to nitrite and/or nitrogen and fermentation of glucose are negative. Resistant to ampicillin, carbenicillin, kanamycin, chloramphenicol, erythromycin, tetracycline and gentamicin and susceptible to spectinomycin and streptomycin.

The type strain is Ac_5_C6T (= DSM 29464T = LMG 29035T) and was isolated from a sandy subtopical savannah soil from a riparian woodland in Mashare, Namibia. The genomic DNA G+C content of the type strain is 65.9 mol%.

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References


