Occallatibacter riparius gen. nov., sp. nov. and Occallatibacter savannae sp. nov., acidobacteria isolated from Namibian soils, and emended description of the family Acidobacteriaceae

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Three Gram-negative, non-spore-forming, encapsulated bacteria were isolated from a Namibian river-bank soil (strains 277T and 307) and a semiarid savannah soil (strain A2-1cT). 16S rRNA gene sequence analyses placed them within subdivision 1 of the Acidobacteria and revealed 100% similarity between strains 277T and 307 and 98.2% similarity between A2-1cT and the former two strains. The closest relatives with validly published names were Telmatobacter bradus, Acidicapsa borealis and Acidicapsa ligni (94.7–95.9% similarity to the type strains). Cells of all three strains were rod-shaped and motile and divided by binary fission. Ultrastructural analyses revealed a thick cell envelope, resulting mainly from a thick periplasmic space. Colonies of strains 277T and 307 were white to cream and light pink, respectively, while strain A2-1cT displayed a bright pink colour. All three strains were aerobic, chemoheterotrophic mesophiles with a broad temperature range for growth and a moderately acidic pH optimum. Sugars and complex proteinaceous substrates were the preferred carbon and energy sources. A few polysaccharides were degraded. The major quinone in all three strains was MK-8; MK-7 occurred in strain A2-1cT as a minor compound. Major fatty acids were iso-C15:0 and iso-C17:16c. In addition, iso-C17:0 accounted for significant amounts. The DNA G+C contents of strains 277T, 307 and A2-1cT were 59.6, 59.9 and 58.5 mol%, respectively. Based on these characteristics, the three isolates are assigned to two novel species of the novel genus Occallatibacter gen. nov., Occallatibacter riparius sp. nov. [type strain 277T (=[DSM 25168T=LMG 26948]) and reference strain 307 (=[DSM 25169=LMG 26947]) and Occallatibacter savannae sp. nov. [type strain A2-1cT (=[DSM 25170T=LMG 26946])]. Together with several other recently described taxa, the novel isolates provide the basis for an emended description of the established family Acidobacteriaceae.

Abbreviation: DDH, DNA–DNA hybridization.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 277T, 307 and A2-1cT are HQ995659, HQ995660 and HQ995661, respectively.

Two supplementary figures, four supplementary tables and supplementary methods are available with the online Supplementary Material.

Although the description of the type species of the genus Acidobacterium, Acidobacterium capsulatum, dates back to 1991 (Kishimoto et al., 1991a), the family Acidobacteriaceae was described based on phylogenetic analysis of 16S rRNA gene sequences and the name was then validly published only 20 years later (Thrash & Coates, 2011b, 2012). To date, it constitutes the sole family of the order Acidobacteriales within the class Acidobacteria (Cavalier-Smith,
The order is commonly known as subdivision 1 of the currently 26 phylotaxonomic coherent groups within the phylum Acidobacteria (Barns et al., 2007; Thrash & Coates, 2011a, 2012). At the time of its original description, the family Acidobacteriaceae included the three genera Acidobacterium (one species; Kishimoto et al., 1991a, b), Edaphobacter (two species; Koch et al., 2008) and Terriglobus (one species; Eichorst et al., 2007). However, over the past 5 years, the list of described genera of subdivision 1 of the Acidobacteria has been extended to eight, with the addition of Acidicapssa (two species; Kulichevskaya et al., 2012), Acidipila (one species; Okamura et al., 2011), Bryocella (one species; Dedysj et al., 2012), Granulicella (nine species; Männistö et al., 2012; Pankratov & Dedysj, 2010; Yamada et al., 2014) and Telmatobacter (one species; Pankratov et al., 2012). Four additional species have been described for the genus Terriglobus (Baik et al., 2013; Männistö et al., 2011; Pascual et al., 2015; Whang et al., 2014). Moreover, the candidate species ‘Candidissimus Koribacter versatilis’ (Ward et al., 2009) is affiliated with this group. All isolates described so far are chemo-organoheterotrophic mesophiles with an acidic to moderately acidic pH optimum that were mostly isolated from terrestrial environments (Table 1). Here, we describe the isolation and characterization of three novel strains that extend the number of slightly acidophilic soil isolates of subdivision 1 of the Acidobacteria and represent a novel genus with two novel species.

Strains 277T and 307 were isolated from a sandy river-bank soil with moderately acidic pH (pH 5.1 in distilled water) from the Okavango River, northern Namibia (17° 51’ 59” S 19° 54’ 24” E, 1065 m elevation above sea level), sampled in spring 2010. At the time of sampling, the site was covered by water (approx. 30 cm in depth) and grown with the wild rice species Oryza longistaminata. Strain A2-1cT originated from a loamy sand of similar pH (pH 5.0 and 6.1 measured in 2 mM CaCl2 and in distilled water, respectively) sampled in spring 2009 at Erichsfelde in central Namibia (21° 36’ 41.4” S 16° 52’ 13.4” E, 1481 m elevation above sea level). The vegetation of this site was a typical open thornbush savannah dominated by Acacia mellefa and the grass Stipagrostis uniplumis that receives a mean annual precipitation of approximately 360 mm, which falls during the summer rainy season.

All three isolates were obtained from high-throughput cultivation experiments set up in sterile 96-well microtitre plates containing 180 μl soil solution equivalent (SSE)/Cmix medium per well buffered at pH 5.5 (Erichsfelde samples) or pH 5.8 (Okavango samples) with MES (see Supplementary Methods, available in the online Supplementary Material). After 6–8 weeks of incubation at 15 °C (strains 277T and 307) or 25 °C (strain A2-1cT) in the dark as static cultures, growth wells were screened for the presence of acidobacteria by group-specific PCR using the primers Acid31f (Barns et al., 1999) and 907r (Lane, 1991). Acidobacteria-positive cultures from the Okavango cultivation experiment were transferred and subcultured in 1:10-diluted liquid HD medium (DSMZ medium 1124; https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium1124.pdf). Strains 277T and 307 were purified by plating, picking and restreaking on a low-nutrient isolation medium described previously (Foesel et al., 2013), which was solidified with purified agar (Oxoid) instead of gellan gum. Of the cultures obtained from the Erichsfelde samples, 10 μl aliquots of acidobacteria-positive wells were streaked directly on SSE/HD1: 10 medium solidified with 1.5 % (w/v) purified agar (Foesel et al., 2013) and a pure culture of A2-1cT was obtained by restreaking. Unless otherwise noted, SSE/HD 1 : 10 (pH 5.5) was also used for further physiological tests and biomass production.

On solid media within 1–2 weeks, strains 277T and 307 formed soft and slimy colonies up to 2 mm in diameter that were translucent, smooth, shiny and convex with entire margins. While colonies of strain 277T were white to cream-coloured, those of strain 307 developed a light pink colour and older colonies occasionally turned brown. With a diameter of 0.5 mm at most, colonies of strain A2-1cT were smaller than those of the other two strains. They were of a very rigid consistency, displayed a bright pink colour and were translucent, smooth, shiny and convex to hemispherical with entire margins.

Cells of all three strains were rod-shaped, with sizes of 1.0–2.0 × 0.6–0.7 μm (strains 277T and 307; Fig. S1a, d) and 1.0–2.5 × 0.5–0.7 μm (strain A2-1cT; Fig. S1g). Depending on the growth phase of cultures, longer rods and bent cells were also observed. All three strains performed non-directional tumbling movements. They divided by binary fission and were Gram-negative in standard staining procedures (Smibert & Krieg, 1994) as well as the KOH test (Buck, 1982). Similar to many members of subdivision 1 of the Acidobacteria, the novel strains formed capsules (India ink staining), while spore formation (malachite green staining; Beveridge et al., 2007) was not observed (Table 1). For scanning and transmission electron micrographs, cells were fixed chemically with glutaraldehyde/formaldehyde and processed further as described previously (Huber et al., 2014). Cells seemed to shrink strongly during the chemical fixation procedure for scanning electron microscopy and negatively stained samples. Therefore, images of ultrathin sections were taken from high-pressure-frozen and cryosubstituted cells (Wanner et al., 2008).

Cells of strains 277T and 307 possessed single fimbria-like structures, whereas cells of strain A2-1cT carried multiple fimbria-like structures (arrowheads in Fig. S1c, g, k); for strains 277T and 307, the presence of capsules was evident in negative-stained samples and field-emission scanning electron microscopy images (arrows in Fig. S1b, c, f, g). Scanning electron micrographs of strain 307 revealed possible self-aggregation through the fimbria-like structures (Fig. S1f). Flagella could not be detected. Therefore, we speculate that the unusual tumbling movements observed under the light microscope might be caused by windind
Table 1. Characteristics of *Occallatibacter* gen. nov. (strains 277\(^T\), 307 and A2-1c\(^T\)) compared with all described members of subdivision 1 of the *Acidobacteria*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1a</th>
<th>1b</th>
<th>1c</th>
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<th>3</th>
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<td>Isolation source(s)</td>
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<td>River bank soil</td>
<td>Sphagnum peat</td>
<td>Sphagnum peat</td>
<td>Sphagnum peat, decaying wood</td>
<td>Acidic mine drainage, soil</td>
<td>Acidic mine drainage</td>
<td>Alpine and forest soil</td>
<td>Soil, termite hindgut, freshwater reservoir</td>
<td>Peat soil, tundra soil, lichen, cherry bark</td>
<td>Sphagnum peat</td>
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<tr>
<td>Cell shape</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods</td>
<td>Short rods</td>
<td>Coccii</td>
<td>Rods</td>
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<td>+</td>
<td>-</td>
<td>ND</td>
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<td>Motility</td>
<td>(+)*</td>
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<td>+</td>
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<td>ND</td>
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<td>Pigmentation</td>
<td>White–cream</td>
<td>White–cream</td>
<td>Bright pink</td>
<td>White, beige</td>
<td>Colourless, pink</td>
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<td>Orange</td>
<td>Beige</td>
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<td>White, pink, red</td>
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<td>NaCl tolerance (%)</td>
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<td>≤ 1.0</td>
<td>≤ 0.5</td>
<td>≤ 0.1</td>
<td>≤ 2.0</td>
<td>≤ 1.0</td>
<td>&lt; 3.5</td>
<td>ND</td>
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<td>Range</td>
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<td>3.5–6.5</td>
<td>3.0–7.5</td>
<td>3.5–7.3</td>
<td>3.0–6.0</td>
<td>4.0–7.0</td>
<td>3.5–10.0</td>
<td>3.0–8.5</td>
<td>3.2–6.6</td>
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<td>Optimum</td>
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<td>5.0–6.5</td>
<td>4.0–5.5</td>
<td>4.5–5.0</td>
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<td>ND</td>
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<td>5.0–7.0</td>
<td>3.8–5.5</td>
<td>4.7–5.2</td>
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<td>DNA G+C content (mol%)</td>
<td>59.6</td>
<td>59.9</td>
<td>58.5</td>
<td>57.6</td>
<td>51.7–54.1</td>
<td>59.5</td>
<td>59.7–60.8</td>
<td>55.8–56.9</td>
<td>57.3–63.2</td>
<td>56.0–61.2</td>
<td>60.7</td>
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All strains are Gram-negative and non-spore-forming, divide by binary fission, prefer sugars as growth substrates and, to variable extents, degrade polysaccharides. The major quinone in all strains is MK-8. All grow aerobically, although *Telmatobacter bradus* TP6017\(^T\) grows only very weakly/slowly at full oxygen tension. +, Positive; −, negative; (+), weak activity/reaction detected; ND, no data.
Table 1. cont.

<table>
<thead>
<tr>
<th>Characteristic</th>
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*Cells showed a non-directional tumbling movement, although flagella could not be detected.

†Determined as iso-C<sub>17</sub>:1<sub>v</sub> (according to MIDI annotation) and iso-C<sub>17</sub>:1<sub>c</sub> (checked by DMDS) for several strains by using GC/MS (Kulichevskaya et al., 2012) and/or iso-C<sub>15</sub>:0 2-OH (according to MIDI System, but identified as C<sub>16</sub>:1<sub>c</sub>) given for other taxa in the table, summed feature 3 was defined as C<sub>16</sub>:1<sub>c</sub> and/or C<sub>16</sub>:1<sub>c</sub> 2-OH according to the ARB neighbour-joining tool without the use of evolutionary substitution model (Pankratov et al., 2012). The pairwise 16S rRNA gene sequence similarity (ARB neighbour-joining tool) between strains 277<sup>T</sup> and 307 was more than 99.0 % (A2-1<sup>c</sup>T), 98.2 % (16S rRNA gene sequence similarity to the novel isolates, but belonged to a different phylogenetic clade (Fig. 2). Together with all other described members of subdivision 1 of the Acidobacteria, the novel isolates form a coherent cluster with a minimal similarity of >91 % between the most distantly related 16S rRNA gene sequences, which is close to the proposed threshold of 92 % for family definition (Rossello-Móra & Amann, 2015). ‘Candidatus K. versatilis’ was the exception, showing
16S rRNA gene sequence similarity of only around 89–92 % to the above-mentioned sequence cluster. As the calculated 16S rRNA gene sequence similarities between strains A2-1c T, 277 T and 307 were within or above the threshold values of 98.2–99.0 % for which DNA–DNA hybridization (DDH) is considered to be mandatory according to the latest findings (Meier-Kolthoff et al., 2013), DDH experiments were carried out to verify the status of A2-1cT as a member of a distinct species and of 277T and 307 as two strains of the same species. Therefore, cells were disrupted in a Constant Systems TS 0.75 kW (IUL Instruments) and DNA was purified from the crude lysate by chromatography on hydroxyapatite (Cashion et al., 1977). Hybridization was carried out according to established protocols (De Ley et al., 1970; Huss et al., 1983) using a model Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multiecell changer and a temperature controller with in situ temperature probe (Varian). Duplicate measurements in 2 × SSC at 70 °C yielded hybridization values of 67.3 and 73.8 % between strains 277T and 307, of 9.1 and 13.0 % between strains 277T and A2-1cT and of 16.1 and 12.2 % between strains 307 and A2-1cT. Thus, the DDH levels for strain A2-1cT with the two other strains were far below the threshold value of 70 % relatedness generally accepted for the definition of a novel species (Wayne et al., 1987). Hybridization values between strains 277T and 307 were around 70 %. Taking a conservative approach, these two strains were therefore included in one species.

For DNA G+C content determination, cells were disrupted and the DNA was purified as described for DDH. After treatment with P1 nuclease and bovine alkaline phosphatase (Mesbah et al., 1989), the resulting deoxyribonucleosides were analysed by HPLC (Shimadzu) under conditions adapted from Tamaoka & Komagata (1984). The molar DNA G+C content was calculated from the ratio of deoxyguanosine (dG) and thymidine (dT) (Mesbah et al., 1989) to be 59.6, 59.9 and 58.5 mol%, respectively, for strains 277T, 307 and A2-1cT. These values range within the DNA G+C contents reported for all other subdivision 1 acidobacteria (Table 1) and are only slightly higher than those of the nearest relatives Telmatobacter bradus TPB6017T (57.6 mol%), Acidicapsa borealis KA1T (54.1 mol%) and Acidicapsa ligni WH120T (51.7 mol%).

Isoprenoid quinones were extracted from dried biomass with chloroform/methanol (2 : 1, v/v) (Collins & Jones, 1981) and analysed via HPLC (Tindall, 1990). The quinone

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**Fig. 1.** Electron micrographs of ultrathin sections from high-pressure-frozen and cryosubstituted cells of strains 277T (a, b), 307 (c, d) and A2-1cT (e, f). All strains show typical Gram-negative cell envelopes with an outer membrane (om), a thin, electron-dense peptidoglycan layer (pg) and an inner membrane (im). Cells of all strains contain electron-dense granules, presumably representing polyphosphate (PP) (f). Strain A2-1cT contains polygonal particles in dense packages obviously representing phage capsids (circled in e). Bars, 100 nm.
systems of the three novel strains comprised menaquinone MK-8 as sole assignable component (277T and 307; 99.9 and 96.8 %, respectively) or as the major component (A2-1cT; 93.3 %); strain A2-1cT also contained MK-7 (6.7 %). With respect to cellular quinones, the three novel strains thus resemble the other described subdivision 1 acidobacteria, which all contain MK-8 as the predominant quinone (Table 1).

Fatty acid profiles were acquired for the three novel strains together with their closest phylogenetic relatives Telmatobacter bradus DSM 23630T, Acidicapsa borealis DSM 23886T and Acidicapsa ligni DSM 25248T. As far as growth requirements permitted, cultures were grown under identical conditions (SSE/HD 1 : 10 agar plates, pH 5.5, 18 days at 20°C). Only Telmatobacter bradus DSM 23630T was grown in static liquid culture rather than on agar plates to obtain enough biomass. About 40 mg wet weight of fresh cells was harvested and extracted according to the standard protocol (Sasser, 1990) of the Microbial Identification System (MIDI Inc.; version 6.1). Compounds were identified by comparison to the TSBA40 and 60 peak-naming table databases. All three strains possessed straight-chain or methyl-branched saturated and monounsaturated fatty acids, while acids carrying hydroxyl groups played only a marginal role. Their full profiles varied only slightly in the amounts of particular compounds and the presence/absence of a few minor compounds <2 % (Table S1). The major fatty acids of strains 277T, 307 and A2-1cT were iso-C15:0 (61.9, 61.6 and 54.8 %, respectively) and iso-C17:1v7c (29.0, 28.0 and 30.7 %). In addition, iso-C17:0 (5.3, 7.6 and 9.5 %, respectively) occurred in significant amounts. This is in agreement with the fatty acid profiles of the type strains of the nearest relatives Telmatobacter bradus and the two species of the genus Acidicapsa. While all characterized members of subdivision 1 of the Acidobacteria possess iso-C15:0 as the most abundant component, the second most-abundant fatty acid in the genera ‘Acidipila’, Bryocella, Edaphobacter, Granulicella and Terriglobus is C16:1v7c, followed by C16:0 as the third most-abundant component (Table 1). Furthermore, large amounts of the unusual membrane-spanning lipid 13,16-dimethyl octadecanoic acid (isodiabolic acid) were reported previously in the three novel isolates and all other subdivision 1 acidobacteria studied after direct acid hydrolysis of the cell material (Kulichevskaya et al., 2012; Sinninghe Damste et al., 2011).

Growth ranges and optima of temperature and pH were determined under oxic conditions in liquid SSE/HD 1 : 10 medium. Temperatures of 11–56°C and pH 2.5–10.0 were tested. Depending on the desired pH, MES, HEPES, HEPPS or CHES (Sigma-Aldrich or Applichem) were used as buffers at a final concentration of 10 mM. Since SSE/HD1 : 10 already contained large amounts of...
different salts, salt tolerance was tested in liquid medium HD1 : 10 (DSMZ medium 1124) amended with NaCl to final concentrations of 0–10 % (w/v). Growth was determined by following the OD_{660}. All three strains grew at approximately 11–40 °C (Table 1) and also displayed very similar growth rates (defined as ≥75 % of the highest growth rate) of 26–37 °C (strain 277'), 28–36 °C (strain 307) and 29–40 °C (strain A2-1c'). Their pH ranges for growth ranged from acidic to around neutral (strains 277' and A2-1c') or slightly basic (strain 307). Strains 277' and 307 grew optimally at pH 5.0–6.5, strain A2-1c' showed a slightly lower optimum, of pH 4.0–5.5. Minimal doubling times were 8.9, 14.0 and 7.1 h, respectively, for strains 277', 307 and A2-1c'. While strains 277' and 307 tolerated NaCl concentrations of ≤1.0 % and showed the best growth at 0.5 % NaCl, strain A2-1c' grew best in the absence of NaCl and tolerated only 0.5 % NaCl. In comparison to the other species of subdivision 1 of the Acidobacteria, the three novel isolates show elevated temperature ranges and optima for growth. The only exception is the recently described species Terriglobus albidus, which reaches similar values (Pascual et al., 2015). This might be explained by the origin of these isolates, sub-Saharan Africa, while the majority of the other isolates originated from soils of temperate and boreal zones. In contrast, preferential growth at acidic to moderately acidic pH is a feature that the novel isolates share with all other characterized members of subdivision 1 of the Acidobacteria. No tolerance of salt or only low salt tolerance is a further common characteristic of this group.

Anaerobic growth with alternative electron acceptors was evaluated in liquid medium HD1 : 10 with N₂ as the gas phase and 10 mM NaNO₃, 10 mM Na₂SO₄, 10 mM iron(III) pyrophosphate [Fe₃(PO₄)₂]₃. H₂O] or 2 mM NaN₂O₃ as electron acceptor. Fermentative growth was assessed using the API 20E test system (bioMérieux). Concentrations of electron acceptors were determined colorimetrically (Cataldo et al., 1975; Gadkari, 1984; Harrigan & McCance, 1966; Tabatabai, 1992; Tamura et al., 1974). None of the three strains reduced nitrate, nitrite, sulfate or iron(III) or showed fermentative growth. The novel isolates thus resemble the majority of the strictly aerobic subdivision 1 acidobacteria, whereas their closest phylogenetic relative Telmatobacter bradus, Acidipasa borealis, B. elongata, Terriglobus saanensis and species of the genus Granulicella that inhabit peat and tundra soils (Dedysh et al., 2012; Kulichevskaya et al., 2012; Männistö et al., 2011, 2012; Pankratov & Dedysh, 2010; Pankratov et al., 2012; Rawat et al., 2012). Of the polysaccharides tested in the present work, strains 277' and 307 degraded starch only. Strain A2-1c' was positive for pectin degradation and weakly positive for chitin degradation. Furthermore, strains 277' and 307 showed significant laccase activity, whereas strain A2-1c' was weakly positive. Like most members of subdivision 1 of the Acidobacteria, the three novel strains expressed almost the whole variety of exoenzymes included in the API ZYM test system (Table S4). While strain 277' expressed all enzymes tested at least weakly, strain 307 lacked N-acetyl-ß-glucosaminidase and strain A2-1c' lacked N-acetyl-ß-glucosaminidase, esterase lipase (C8) and ß-glucosidase. Additional physiological characteristics are detailed in the species descriptions.

In summary, strains 277', 307 and A2-1c' are aerobic, chemo-organoheterotrophic, white- to pink-pigmented mesophiles. Although they fit well into the series of moderately acidophilic soil isolates of subdivision 1 of the Acidobacteria, they can be clearly differentiated from their closest relatives Telmatobacter and Acidipasa based on their phylogeny, morphology and physiology. We therefore propose that they form the novel genus Occallatibacter gen. nov., including the two novel species Occallatibacter riparius sp. nov. and Occallatibacter savannah sp. nov.
Description of Occallatibacter gen. nov.

Occallatibacter (Occ.al.la’ti.bac’ter. L. masc. adj. occallatus thick-skinned, referring to the thick cell envelope; N.L. masc. n. bacte r a rod; N.L. masc. n. Occallatibacter rod-shaped bacterium with thick cell envelope).

Gram-negative, non-spore-forming, rod-shaped cells that divide by binary fission. Perform non-directional tumbling movements, although flagella are not detected. Capsules are formed. Strictly aerobic, chemo-organotrophic mesophiles. Catalase-positive, cytochrome-c-oxidase-negative. Sugars are the preferred carbon and energy source. Several single amino acids and complex, protein-containing substrates are also utilized. Individual polysaccharides are degraded. Major fatty acids after Bligh–Dyer extraction are iso-C₁₅ : ₀, iso-C₁₇ : ₀ 7c and iso-C₁₇ : ₀ 6c. After direct acid hydrolysis of cell material, large amounts of 13,16-dimethyl octacosanedioic acid (isodiabolic acid) are found. The major quinone is MK-8. The DNA G+C content is 59–60 mol%. The type species is Occallatibacter riparius.

Description of Occallatibacter riparius sp. nov.

Occallatibacter riparius (ri.pa’ri.u.s. N.L. masc. adj. riparius inhabiting the banks of rivers, referring to the isolation source of the type strain).

Displays the following characteristics in addition to those given in the genus description. Cells are 1.0–2.0 μm long and 0.6–0.7 μm in diameter. On solid media, forms soft and slimy colonies, up to 2 mm in diameter, that are white to cream or light pink, translucent, smooth, shiny and convex with entire margins. Grows at 11–40 °C and pH 3.5–8.5; optimal growth at 26–37 °C and pH 5.0–6.5. Minimal doubling time is 8.9 h. Tolerates NaCl concentrations of up to 1.0 % (w/v); optimal growth occurs at 0.5 % NaCl. Grows on cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, melezitose, raffinose, rhamnose, sucrose, trehalose, xylose, aspartate, glutamate, ornithine, lysine, tyrosine, gluconate, succinate, glycerol, Casamino acids, casein hydrolysate, peptone, yeast extract and starch. Strain-specific growth occurs on fucos, sorbitol, arginine, acetate and laminarin. No growth is observed on arabinose, lyxose, sorbose, adonitol, arabitol, mannitol, myo-inositol, xylitol, alanine, cysteine, glycine, histidine, leucine, isoleucine, methionine, hydroxyproline, serine, threonine, tryptophan, valine, butyrate, citrate, crotonate, formate, fumarate, glycolate, 3-ketoisocaproate, 3-ketovalerate, lactate, malate, malonate, nitrilotriacetic acid, oxaloacetate, propionate, pyruvate, tartarate, butanol, ethanol, methanol, propanol, chitin, cellulose, pectin, Tween 80 or xylan. Aesculin and gelatin are hydrolysed. Formation of indole and acetoin is not observed. Urease- and arginine dihydrolase-negative. The following additional enzyme activities (API ZYM) are present: acid and alkaline phosphatases, naphthol-AS-Bl-phosphohydrolase (weak), esterase (C4), esterase lipase (C8), lipase (C14) (weak), leucine arylamidase, valine arylamidase, cysteine arylamidase (weak), trypsin (partially weak), α-chymotrypsin (partially weak), α- and β-galactosidases, β-glucuronidase, α-glucosidase (partially weak), β-glucosidase, N-acetyl-β-glucosaminidase and α-fucosidase. α-Mannosidase activity is weak or absent. Oxygen is the sole electron acceptor. The type strain is MK-8.

The type strain is 277T (=DSM 25168T=LMG 26948T). An additional strain is 307 (=DSM 25169T=LMG 26947). Both were isolated from a sandy river-bank soil from the Okavango River, northern Namibia (17° 51’ 59” S 19° 54’ 24” E, 1065 m elevation above sea level). The DNA G+C content of the type strain is 59.6 mol%.

Description of Occallatibacter savannae sp. nov.

Occallatibacter savannae [sa.van’nae. N.L. gen. n. savannae derived from savannah (soil), referring to the isolation source of the type strain].

Shows the following features in addition to those given in the genus description. Cells are 1.0–2.5 μm long and 0.5–0.7 μm wide. Colonies reach a maximum size of 0.5 mm, are of a very firm consistency, display a bright pink colour and are translucent, smooth, shiny and convex to hemispherical with entire margins. Grows at 11–40 °C and pH 3.5–6.5; optimal growth occurs at 29–40 °C and pH 4.0–5.5. Minimum doubling time is 7.1 h. Tolerates NaCl concentrations of up to 0.5 % (%(v/v)); optimal growth occurs in the absence of NaCl. Grows on cellobiose, fucose, fructose, galactose, glucose, lactose, maltose, mannose, melezitose, raffinose, rhamnose, sucrose, trehalose, xylose, aspartate, glutamate, ornithine, lysine, tyrosine, gluconate, succinate, glycerol, Casamino acids, casein hydrolysate, peptone, yeast extract and starch. No growth is observed on arabinose, lyxose, sorbose, adonitol, arabitol, mannitol, myo-inositol, xylitol, alanine, cysteine, glutamate, glycine, histidine, leucine, isoleucine, lysine, methionine, hydroxyproline, serine, threonine, tryptophan, valine, acetate, butyrate, citrate, crotonate, formate, fumarate, glycolate, isovalerate, lactate, malate, malonate, nicotinic acid, oxaloacetate, propionate, tartrate, butanol, ethanol, methanol, propanol, cellulose, starch, Tween 80 or xylan. Aesculin and gelatin are hydrolysed, though gelatin is hydrolysed only very slowly. Indole and acetoin are not formed. Urease- and arginine dihydrolase-negative. The following additional enzyme activities are present (API ZYM): acid and alkaline phosphatases, naphthol-AS-Bl-phosphohydrolase (weak), esterase (C4) (weak), esterase lipase (C8) (weak), leucine arylamidase, valine arylamidase (weak), cysteine arylamidase (weak), trypsin (weak), α-chymotrypsin (weak), α- and β-galactosidases, β-glucuronidase (weak), α- and β-glucosidases and α-fucosidase. Lipase (C14), N-acetyl-β-glucosaminidase and α-mannosidase activities are absent. Oxygen is the sole electron acceptor. The major quinone is MK-8; MK-7 occurs as a minor constituent.

The type strain is A2-1cT (=DSM 25170T=LMG 26946T), which was isolated from a sandy tropical savannah soil in Erichsfelde, central Namibia (21° 38’ 15.8” S 16° 52’
03.9°E, 1497 m elevation above sea level). The DNA G+C content of the type strain is 58.5 mol%.

**Emended description of the family Acidobacteriaceae Thrash and Coates 2012**

The description is as given by Thrash & Coates (2011b) with the following amendments. All members are Gram-negative cocci to rods. Mostly catalase-positive and oxidase-negative. Capsule formation is prevalent; motility is variable. Aerobic or facultatively anaerobic, in some cases cold-adapted, mesophiles with a preference for sugars as carbon and energy source. Polysaccharide degradation is a common feature. The sole or predominant quinone is MK-8. After Bligh–Dyer extraction, characteristic combinations of major fatty acids are (i) iso-C₁₅∶₀, C₁₆∶₁ and C₁₈∶₀, (ii) iso-C₁₅∶₀ and iso-C₁₇∶₁, mostly accompanied by iso-C₁₇∶₀, or (iii) iso-C₁₅∶₀ and C₁₈∶₁ and C₁₆∶₀. After direct acid hydrolysis of cell material, large amounts of 13,16-dimethyl octacosanedioic acid (isodiabolic acid) are found. The DNA G+C content ranges from 51.7 to 62.1 mol%.

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