**Aridibacter famidurans** gen. nov., sp. nov. and **Aridibacter kavangonensis** sp. nov., two novel members of subdivision 4 of the Acidobacteria isolated from semiarid savannah soil

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Acidobacteria constitute an abundant fraction of the soil microbial community and are currently divided into 26 subdivisions. Most cultivated members of the Acidobacteria are affiliated with subdivision 1, while only a few representatives of subdivisions 3, 4, 8, 10 and 23 have been isolated and described so far. Two novel isolates of subdivision 4 of the Acidobacteria were isolated from subtropical savannah soils and are characterized in the present work. Cells of strains A22_HD_4HT and Ac_23_E3T were immotile rods that divided by binary fission. Colonies were pink and white, respectively. The novel strains A22_HD_4HT and Ac_23_E3T were aerobic mesophiles with a broad range of tolerance towards pH (4.0–9.5 and 3.5–10.0, respectively) and temperature (15–44 and 12–47 °C, respectively). Both showed chemo-organoheterotrophic growth on some sugars, the amino sugar N-acetylgalactosamine, a few amino acids, organic acids and various complex protein substrates. Major fatty acids of A22_HD_4HT and Ac_23_E3T were iso-C15 : 0, summed feature 1 (C13 : 0:OH/iso-C15 : 1 : H), summed feature 3 (C16 : 1ω7c/C16 : 1ω6c) and anteiso-C17 : 0. The major quinone was MK-8; in addition, MK-7 occurred in small amounts. The DNA G+C contents of A22_HD_4HT and Ac_23_E3T were 53.2 and 52.6 mol%, respectively. The closest described relative was Blastocatella fastidiosa A2-16T, with 16S rRNA gene sequence identity of 93.2 and 93.3 %, respectively. Strains A22_HD_4HT and Ac_23_E3T displayed 16S rRNA gene sequence similarity of 97.4 % to each other. On the basis of the low DNA–DNA hybridization value, the two isolates represent different species. Based on morphological, physiological and molecular characteristics, the new genus Aridibacter gen. nov. is proposed, with two novel species, the type species **Aridibacter famidurans** sp. nov. (type strain A22_HD_4HT=DSM 26555T=LMG 27985T) and a second species, **Aridibacter kavangonensis** sp. nov. (type strain Ac_23_E3T=DSM 26558T=LMG 27597T).

Over recent decades, the majority of newly described bacterial species have been representatives of the phyla Actinobacteria, Firmicutes and Proteobacteria (Hugenholtz et al., 1998; Janssen et al., 2002). In contrast, only a few members of the Acidobacteria have validly published names, although this phylum accounts for up to 70% of the soil microbial community (Janssen, 2006; Jones et al., 2009; Lauber et al., 2009) and acidobacteria are also abundant in other types of habitats such as hot springs (Hobel et al., 2005), polar deserts of Antarctica (Pointing et al., 2009), wastewater (LaPara et al., 2000) and cave paintings (Schabereiter-Gurtner et al., 2004). The phylum Acidobacteria is divided into 26 subdivisions (Barns et al., 1999), and only subdivisions 1, 3, 4, 8, 10 and 23 contain at least one described species. Eight genera [Acidobacterium (Kishimoto et al., 1991), Terriglobus (Bäck et al., 2013; Eichorst et al., 2007; Männistö et al., 2011; Whang et al., 2014), Edaphobacter (Koch et al., 2008), Acidicapsa (Kulichevskaya et al., 2013),...
et al. (2012), *Granulicella* (Männistö et al., 2012; Pankratov & Dedysk, 2010), *Telmato bacter* (Pankratov et al., 2012), *Acidithiobacillus* (Okamura et al., 2011) and *Bryocella* (Dedysk et al., 2012) belong to subdivision 1, one genus (*Bryobacter*; Kulichevskaya et al., 2010) belongs to subdivision 3, three genera (*Holophaga* (Liesack et al., 1994), *Geothrix* (Coates et al., 1999) and *Acanthopleuribacter* (Fukunaga et al., 2008)) belong to subdivision 8, one genus (*Thermotomaculum*; Izumi et al., 2012) belongs to subdivision 10 and one genus (*Thermoanaerobaculum*; Losey et al., 2013) belongs to subdivision 23. Members of subdivision 4 of the *Acidobacteria* are abundant in different soils (Barns et al., 1999; Foesel et al., 2014; Jones et al., 2009) and have been shown to be cultivable (Bryant et al., 2007; George et al., 2011; Joseph et al., 2003; Stott et al., 2008). Recently, the first two members of this subdivision with validly published names, *Blastocatella fastidiosa* (Foesel et al., 2013) and *Pyrimononas methylaliphagogenes* (Crowe et al., 2014), have been described. The existence of the co-cultured strain ‘*Candidatus Chlororacidobacterium thermophilum*’ (Bryant et al., 2007) was reported earlier. While *B. fastidiosa* and *P. methylaliphagogenes* are slightly acidophilic or neutrophilic chemoheterotrophic soil bacteria (Crowe et al., 2014; Foesel et al., 2013), ‘*Candidatus C. thermophilum*’ is a basophilic phototroph that thrives in microbial mats of hot springs (Bryant et al., 2007). *B. fastidiosa* is a mesophile; the other two strains show thermophilic traits. In the present study, two novel isolates are described that extend the group of cultivated members of subdivision 4 of the *Acidobacteria*.

Strain A22_HD_4H<sup>T</sup> was isolated from a clayey sand soil that was slightly basic (pH 7.4 and 8.2, measured in 2 mM CaCl<sub>2</sub> and in distilled water, respectively). The soil sample was collected in spring 2009 from a pasture at the farm Erichsfelde in central Namibia (21° 38' 15.8" S 16° 52' 03.9" E, 1497 m above sea level). Strain Ac_23_E3<sup>T</sup> originated from a sandy fallow with a similar pH (pH 7.3 and 8.2, measured in 2 mM CaCl<sub>2</sub> and in distilled water, respectively) sampled in spring 2011 at Mashare, Kavango region, in northern Namibia (17° 53' 37.93" S 20° 14' 50.71" E, 1069 m above sea level). For the isolation of novel acidobacteria, soil suspensions (10 mM HEPPS, pH 8.0) were inoculated in 200 µl liquid soil solution equivalent (SSE)/HD 1:10 (Foesel et al., 2013). Instead of MES, 10 mM HEPPS was used to buffer the SSE/HD medium at pH 8.0. After 6 weeks of incubation at 20 °C, cultures were screened for acidobacterial growth by group-specific PCR using the primer pair Acido31f (Barns et al., 1999) and 1492r (Lane, 1991). Cultures that yielded PCR products were plated on SSE/HD 1:10 plates solidified with purified agar (15 g L<sup>-1</sup>; Oxoid). Strain Ac_23_E3<sup>T</sup> was isolated by subsequent streaking on plates. In contrast, strain A22_HD_4H<sup>T</sup> was only gained in pure culture by plating after a prolonged starvation period of an enrichment culture in pure SSE (Angle et al., 1991), pH 8.0. Unless otherwise noted, SSE/HD 1:10 was also used in the following physiological tests and for biomass production.

Liquid cultures of strain A22_HD_4H<sup>T</sup> displayed a yellow colour in initial stages. With increasing density, cultures developed a bright pink colour and formed aggregates even during shaking. On agar plates, colonies of A22_HD_4H<sup>T</sup> had a diameter of 0.1–0.2 mm and were pink, circular, translucent and convex with entire margins. Strain Ac_23_E3<sup>T</sup> formed white colonies with a light pinkish hue. The colonies had a diameter of 0.2–0.3 mm and were translucent and convex with entire margins. Liquid cultures of strain Ac_23_E3<sup>T</sup> were white and did not form aggregates. Cell morphology of both strains was examined by light microscopy (Zeiss Axio Lab.A1; Carl Zeiss). Pictures were taken with a Zeiss Axios Imager.M2 microscope (Carl Zeiss) equipped with an AxioCam MRm camera. Cells of both strains were rod-shaped and divided by binary fission. Single cells of strains A22_HD_4H<sup>T</sup> and Ac_23_E3<sup>T</sup> were 2.5–3.0 µm long and about 0.9 and 0.6–0.7 µm in diameter, respectively. In contrast to strain A22_HD_4H<sup>T</sup>, which formed single cells, strain Ac_23_E3<sup>T</sup> also formed short chains of two to four cells (Fig. 1a–d). However, formation of longer chains, as found in the closest relative *B. fastidiosa* A2-16<sup>T</sup> (Foesel et al., 2013), was not observed. Consistent with all other characterized acidobacteria, both strains stained Gram-negative (Gerhardt et al., 1994). Capsule and spore formation was examined by India ink and malachite green staining, respectively (Bast, 2011), and was not observed for strains A22_HD_4H<sup>T</sup> and Ac_23_E3<sup>T</sup>, which is similar to the properties of *B. fastidiosa* A2-16<sup>T</sup> (Foesel et al., 2013). For transmission electron microscopy, cells were prepared as described previously (Foesel et al., 2013) except that 1% (v/v) glutaraldehyde and 2% (v/v) formaldehyde was used in the initial fixation step. Both strains revealed the structure of a Gram-negative cell wall (Fig. 1e–g). Several cells of strains A22_HD_4H<sup>T</sup> and Ac_23_E3<sup>T</sup> contained intracytoplasmic inclusion bodies (Fig. 1e). Outer membrane vesicles also occurred (Fig. 1f).

Almost full-length 16S rRNA gene fragments of strains A22_HD_4H<sup>T</sup> and Ac_23_E3<sup>T</sup> were amplified and sequenced as described previously (Foesel et al., 2013). Sequences were added to the small-subunit rRNA non-redundant reference database SILVA version 108 (http://www.arb-silva.de; Pruesse et al., 2007) using the program package ARB (Ludwig et al., 2004). After automated alignment with the Fast aligner tool implemented in ARB, the alignment was refined manually based on secondary structure information. Phylogenetic trees were calculated using neighbour-joining, maximum- parsimony and maximum-likelihood algorithms (termini filter; 41 485 valid positions between positions 60 and 1438 of the *Escherichia coli* 16S rRNA reference gene; 1000 bootstrap resamplings). All three methods placed the novel strains A22_HD_4H<sup>T</sup> and Ac_23_E3<sup>T</sup> in the vicinity of *B. fastidiosa* A2-16<sup>T</sup> (Foesel et al., 2013) within subdivision 4 of the phylum *Acidobacteria* (93.2 and 93.3% sequence similarity, respectively; Fig. 2). The novel strains were only distantly related (~83% sequence similarity) to the members of subdivision 4 *P. methylaliphagogenes* K22<sup>T</sup>
Crowe et al. (2014) and ‘Candidatus C. thermophilum’ (Bryant et al., 2007). The closest phylogenetic relatives (96–98 % sequence similarity) were clone sequences derived from environments such as soils (GenBank accession numbers AB637077 and GU444118; unpublished), soil crusts (JX255145; unpublished), subsurface sediments (HM186080; Lin et al., 2012), skin (JF139787, JF175323; Kong et al., 2012) and clean rooms (DQ532357; Moissl et al., 2007). Strains A22_HD_4HT and Ac_23_E3T had 16S rRNA gene sequence identity of 97.4 %. This value is below the threshold of 98.7–99.0 % 16S rRNA gene sequence identity for which DNA–DNA hybridization is advised to be mandatory (Stackebrandt & Ebers, 2006). However, DNA–DNA hybridization was performed to confirm the existence of two species following a more conservative threshold of 97 % 16S rRNA gene sequence identity for species definition and recommendation of additional DNA–DNA hybridization experiments (Stackebrandt & Goebel, 1994; Rosselló-Mora & Amann, 2001).

The established protocol for DNA–DNA hybridization (De Ley et al., 1970) was used as modified 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). Cells of strains A22_HD_4HT and Ac_23_E3T 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). Duplicate measurements yielded hybridization values of 29.8 and 23.7 % for strains A22_HD_4HT and Ac_23_E3T that fall far below the limit for species definition of 70 % recommended by Wayne et al. (1987) and confirm the assignment of the two strains to different species.

For DNA G+C content determination, cells were disrupted in a French press. The DNA was purified using hydroxyapatite (Cashion et al., 1977). After sample treatment with P1 nuclease and analysis by HPLC (Shimadzu), the DNA

Fig. 1. (a, b) Phase-contrast photomicrographs of strains A22_HD_4HT (a) and Ac_23_E3T (b). Bars, 10 μm. (c, d) Scanning electron micrographs of strains A22_HD_4HT (c) and Ac_23_E3T (d). Bars, 1 μm. (e–g) Transmission electron micrographs of strains A22_HD_4HT (e, f) and Ac_23_E3T (g). Bars, 200 nm. CM, Cytoplasmic membrane; IC, intracytoplasmic inclusion body; OM, outer membrane; P, periplasma; V, outer membrane vesicle.
G+C content was calculated (Mesbah et al., 1989; Tamaoka & Komagata, 1984) to be 53.2 and 52.6 mol% for strains A22_HD_4HT and Ac_23_E3T, respectively. These numbers fall within the range of DNA G+C content of 51.6–62.7 mol% for other established species of subdivisions 1, 3, 4, 8, 10 and 23 of the Acidobacteria, but clearly exceed the DNA G+C content of 46.5 mol% of B. fastidiosa A2-16T (Table 1).

Isooprenoid quinones were extracted from dried biomass with chloroform/methanol (2:1, v/v) (Collins & Jones, 1981) and analysed via HPLC (Tindall, 1990). The detected quinones were MK-8 and, in very small amounts, MK-7, which is in congruence with the quinone composition of B. fastidiosa A2-16T (Table 1; Foesel et al., 2013) and the other described members of subdivision 4 of the Acidobacteria (Bryant et al., 2007; Crowe et al., 2014).

For fatty acid analysis, cells were grown in liquid SSE/HD 1:10 for 8 days at 28°C. Data taken from the literature were obtained under growth conditions comparable to those used for strains A22_HD_4HT and Ac_23_E3T. All media used contained glucose or glucuronic acid and yeast or Casamino acids (0.1–0.5 g l⁻¹) as growth factors. Protein substrates such as peptone (0.5 g l⁻¹) were also present. After harvesting about 40 mg wet weight, cells were extracted according to the standard protocol (Sasser, 1990) of the Microbial Identification System (MIDI Inc.; version 6.1) for fatty acid analysis. The fatty acids were identified by comparison to the TSBA40 peak-naming table database. Strains A22_HD_4HT and Ac_23_E3T possessed straight-chain, methyl- and/or hydroxyl-branched saturated and monounsaturated fatty acids. The major fatty acids of A22_HD_4HT and Ac_23_E3T were iso-C₁₅:0 (35.1 and 38.0%), summed feature 3 (C₁₆:1ω7c and/or C₁₆:1ω6c; 20.1 and 15.3%), summed feature 1 (C₁₃:0 3-OH and/or iso-C₁₅:1 H; 17.2 and 11.7%) and anteiso-C₁₇:0 (6.2 and 10.9%). Both strains also contained C₁₆:0 (3.6 and 5.0%), iso-C₁₃:0 (4.1 and 3.5%) and anteiso-C₁₇:0 A (3.0 and 4.1%). In summary, the fatty acid profiles of the two strains differed in the proportions of single compounds rather than in their composition (Table S1, available in the online Supplementary Material). Large amounts of iso-C₁₅:0 and summed feature 3 (C₁₆:1ω7c/C₁₆:1ω6c) are a common feature of strains A22_HD_4HT and Ac_23_E3T and members of subdivision 1 [Granulicella species (Pankratov & Dedysh, 2010), Bryocella elongata SN10 T (Dedysh et al., 2012), Terriglobus roseus KBS 63 T (Eichorst et al., 2007) and Edaphobacter aggregans Wbg-1 T (Koch et al., 2008)]. Yet, the high content of summed feature 1 (C₁₃:0 3-OH/iso-C₁₅:1 H) and higher amounts of anteiso-C₁₇:0 A distinguishes the novel strains from these representatives of the Acidobacteria. Large amounts of summed feature 1 (C₁₃:0 3-OH/iso-C₁₅:1 H) and summed feature 3 (C₁₆:1ω7c/C₁₆:1ω6c) and the presence of anteiso-C₁₇:1 A in strains A22_HD_4HT and Ac_23_E3T are in congruence with the fatty acid profile of B. fastidiosa A2-16T (Table 1). Compared with B. fastidiosa.
A2-16T, strains A22 HD_4H^T and Ac_23_E3^T contained larger amounts of iso-C_{13:0}, iso-C_{15:0} C_{16:0} and anteiso-C_{17:0}. In contrast, B. fastidiosa A2-16T contains larger amounts of iso-C_{16:0} and iso-C_{17:1}v_{9c} (Table 1). A further distinctive feature is the occurrence of iso-C_{16:1} Hi in B. fastidiosa A2-16T. The large amount of iso-C_{15:0} is a trait that strains A22 HD_4H^T and Ac_23_E3^T have in common with another member of subdivision 4, P. methylaliphatogenes K22^T, while the latter is distinguished by the presence of larger amounts of other saturated iso-branched fatty acids (iso-C_{17:0}, iso-C_{19:0}, iso-C_{21:0}; Crowe et al., 2014).

The polar lipid compositions of strains A22 HD_4H^T and Ac_23_E3^T were analysed by two-dimensional TLC (modified after Bligh & Dyer, 1959; Tindall et al., 2007). Both strains contained phosphatidycholine, phosphatidylglycerol, diphosphaetylcholine and phosphatidylethanolamine (Fig. S1). The presence of phosphatidylethanolamine and

Table 1. Characteristics of strains A22_HD_4H^T and Ac_23_E3^T compared with B. fastidiosa A2-16T

<table>
<thead>
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<th>Characteristic</th>
<th>1</th>
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<tbody>
<tr>
<td>Cell shape</td>
<td>Rod</td>
<td>Rod</td>
<td>Sphere to rod</td>
</tr>
<tr>
<td>Cell size (µm)</td>
<td>2.5–3.0 x 0.9</td>
<td>2.5–3.0 x 0.6–0.7</td>
<td>0.8–12.0 x 0.8–0.9</td>
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<tr>
<td>Cell division</td>
<td>Binary fission</td>
<td>Binary fission</td>
<td>Binary fission/budding</td>
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<tr>
<td>Motility</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>Yellow–pink</td>
<td>White (bright pinkish hue)</td>
<td>Orange–pink</td>
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<td>NaCl tolerance (% w/v)</td>
<td>≤1.0</td>
<td>≤1.0</td>
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<td>Temperature for growth (°C)</td>
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<td></td>
<td></td>
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<tr>
<td>Range</td>
<td>15–44</td>
<td>12–47</td>
<td>14–40</td>
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<tr>
<td>pH for growth</td>
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<td></td>
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<tr>
<td>Range</td>
<td>4.0–9.5</td>
<td>3.5–10.0</td>
<td>4.0–10.0</td>
</tr>
<tr>
<td>Optimum</td>
<td>5.5–9.0</td>
<td>5.5–8.0</td>
<td>5.0–7.5</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>53.2</td>
<td>52.6</td>
<td>46.5</td>
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<td>Major fatty acids (%)</td>
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<td>iso-C_{13:0}</td>
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<td>11.7</td>
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<td>C_{16:0}</td>
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<tr>
<td>C_{16:1}o9c/C_{16:1}o6c</td>
<td>20.1</td>
<td>15.3</td>
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<td>10.9</td>
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<tr>
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<td>anteiso-C_{17:1} A</td>
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<td>Xylose</td>
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<tr>
<td>Asparagine</td>
<td>(++)</td>
<td>(++)</td>
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<td>Glutamine</td>
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<td>–</td>
</tr>
<tr>
<td>N-Acetylgalactosamine</td>
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<td>–</td>
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<tr>
<td>Heptanoic acid</td>
<td>(++)</td>
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<tr>
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<tr>
<td>Starch</td>
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Strains: 1, A22 HD_4H^T; 2, Ac_23_E3^T; 3, B. fastidiosa A2-16T (data from Foesel et al., 2013). Cultivation conditions of A22 HD_4H^T, Ac_23_E3^T and B. fastidiosa A2-16T were comparable. All strains utilized protocatechuate, Casamino acids, casein hydrolysate, peptone and yeast extract. All three strains were derived from semiarid savannah soils, did not form spores or capsules and contained MK-8 as the major quinone. +, Positive; −, negative; ( + ), weak growth detected; ND, no data available. Potentially diagnostic fatty acids are highlighted in bold.
phosphatidylglycerol has also been described for the members of subdivision 1 *Acidobacterium capsulatum* 161^T and *Telmatobacter bradus* TPB6017^T (Kulichevskaya et al., 2012; Pankratov et al., 2012) and subdivision 23 *Thermoaerobaculum aquaticum* MP-01^T (Losey et al., 2013). Dedys et al. (2012) confirmed the presence of phosphatidylcholine in *Acidobacterium capsulatum* 161^T and Kulichevskaya et al. (2012) determined phosphocholine in *Acidicapsa borealis* KAI^T and *Acidobacterium capsulatum* 161^T. The presence of diphosphatidylcholine, as determined for strains A22_HD_4HT^T and Ac_c_23_E3^T, was also reported for *Thermoaerobaculum aquaticum* MP-01^T (Losey et al., 2013).

Ranges and optima of temperature and pH for growth were determined in triplicate underoxic conditions in liquid SSE/HD 1:10 medium. Growth was examined at 10–56 °C and pH 1.0–11.0. Depending on the pH, MES, HEPES, HEPPS or CHES (from Sigma-Aldrich or Applichem; 10 mM each) were used as buffers. Growth was determined by measuring the OD660. Growth of strain A22_HD_4HT^T occurred at 15–44 °C and pH 4.0–9.5. Optimal growth (defined as ≥75% of highest growth rate achieved) was determined at 24–36 °C (highest rate at 27 °C) and pH 5.5–9.0 (highest rate at pH 7.0). Strain Ac_c_23_E3^T grew at 12–47 °C and pH 3.5–10.0. Optimal growth occurred at 36–44 °C (highest rate at 39 °C) and pH 5.5–8.0 (highest rate at pH 6.5). Salt tolerance was determined in HD 1:10 medium [l^-1]: 0.25 g yeast extract, 0.5 g peptone, 0.1 g glucose, 0.1 ml ten-vitamin solution (Balch et al., 1979) and 1 ml trace element solution SL 10 (Tschech & Pfennig, 1984)] with NaCl concentrations between 0 and 10% (w/v). Strains A22_HD_4HT^T and Ac_c_23_E3^T tolerated 0–1% (w/v) NaCl. They grew best at 0.25% (w/v) NaCl, while growth of *B. fastidiosa* A2-16^T was already inhibited at this NaCl concentration (Foesel et al., 2013). The ability to tolerate a broad range of temperatures and pH is comparable to that of *B. fastidiosa* A2-16^T (Table 1) and *P. methylaliphathogenes* K22^T (Crowe et al., 2014), and thus might be an important feature of subdivision 4 of the *Acidobacteria*. Among the three mesophilic Namibian strains A22_HD_4HT^T, Ac_c_23_E3^T and *B. fastidiosa* A2-16^T, Ac_c_23_E3^T is distinguished by its elevated temperature optimum, whereas *P. methylaliphathogenes* K22^T stands out because of its thermophilic traits.

The range of growth substrates utilized by strains A22_HD_4HT^T and Ac_c_23_E3^T was tested in duplicate in liquid oxic SSE/HD 1:10 medium. For this purpose, peptone and glucose were omitted completely and yeast extract was added only in traces. The growth substrates tested comprised sugars, organic acids, keto acids, alcohols, amino acids (0.5–10 mM each; Table S2), Casamino acids, casein hydrolysate, laminarin, peptone, yeast extract (0.05% w/v each) and Tween 80 (0.001% w/v). Growth on cellulose (micocrystalline, 20 μm particle size; Sigma-Aldrich), chitin (from crab shells; Roth) and soluble starch (Merck) was tested on solidified media with a final concentration of 0.5 g substrate l^-1. Additionally, a negative control was prepared without the addition of any polymer. After 6 weeks of incubation at 20 °C, degradation of starch, cellulose and chitin was assessed by the appearance of colonies and by probing the presence of clear zones around colonies, in the case of starch and cellulose after staining with Lugol’s solution (Barrow & Feltham, 1993) and Congo red (Wood & Bhat, 1988), respectively.

Cytochrome-c oxidase and catalase activities were determined by established protocols (Barrow & Feltham, 1993; Gerhardt, 1994). Cytochrome-c oxidase was additionally tested by Bactident oxidase (Merck). Indole formation, aesculin degradation, urease activity and further exoenzyme activities were determined by using the API ZYM and API 20 NE test systems (bioMérieux). Similar to *B. fastidiosa* A2-16^T, the preferred growth substrates of strains A22_HD_4HT^T and Ac_c_23_E3^T were protein-containing, complex substrates such as Casamino acids, peptone or yeast extract, as well as protocatechuatate (Table 1). However, the novel strains showed a slightly broader substrate range, as they also grew on some sugars, the amino sugar N-acetylglactosamine and a few amino acids and organic acids. This is a feature shared with another member of subdivision 4 of the *Acidobacteria*, *P. methylaliphathogenes* K22^T, which showed an even broader range of substrate utilization (Crowe et al., 2014). The novel strains differed with respect to the sugars, amino acids and organic acids used (Table 1). In contrast to strain Ac_c_23_E3^T, strain A22_HD_4HT^T grew on pure agar. Strain Ac_c_23_E3^T grew on the polymer laminarin, while strain A22_HD_4HT^T did not. Like *B. fastidiosa* A2-16^T (Foesel et al., 2013), strain Ac_c_23_E3^T showed the ability to grow on solid media containing the polymers starch, cellulose and chitin, while this feature was not present in strain A22_HD_4HT^T (Table 1). In addition, the appearance of clearing zones after staining with Congo red verified the utilization of cellulose by strain Ac_c_23_E3^T. Strain A22_HD_4HT^T showed no clearing zones after staining with Congo red. Furthermore, both strains revealed no decolouration of starch medium after staining with Lugol’s solution. Exoenzyme profiles of strains A22_HD_4HT^T and Ac_c_23_E3^T as determined with the API ZYM test system (bioMérieux) were similar (Table S3). The main difference from *B. fastidiosa* A2-16^T was the expression of some sugar-degrading enzymes such as β-glucosidase and N-acetyl-β-glucosaminidase in the two novel strains, which is in agreement with the range of growth substrates identified.

In summary, strains A22_HD_4HT^T and Ac_c_23_E3^T are aerobic, chemo-organoheterotrophic, white- to pink-pigmented mesophiles with broad pH and temperature tolerance. Common features of the two novel isolates and the earlier described isolates of subdivision 4 of the *Acidobacteria* from soil, *B. fastidiosa* A2-16^T and *P. methylaliphathogenes* K22^T, are the preference for complex proteinaceous substrates and the broad ranges of pH and temperatures tolerated, although each strain shows individual growth ranges according to the habitat from which it was derived. ‘Candidatus C. thermophilum’, as a phototrophotroph from microbial mats, differs in its habitat
preferences and primary metabolism. The different G+C content of genomic DNA and the fatty acid compositions distinguish the novel strains from their nearest phylogenetic relative, B. fastidiosa A2-16T. Beside the formation of cell chains, the results of DNA–DNA hybridization clearly differentiate strains Ac_23_E3T and A22_HD_4H\textsuperscript{T} from each other. Additionally, the optimal growth temperature of strain Ac_23_E3T (36–44 °C) clearly exceeds the optimal growth temperature of strain A22_HD_4H\textsuperscript{T} (24–36 °C). Based on phylogeny, morphology and physiology of strains A22_HD_4H\textsuperscript{T} and Ac_23_E3T, the novel genus Aridibacter gen. nov. is proposed, including two novel species, Aridibacter famidurans sp. nov. and Aridibacter kavangonensis sp. nov.

**Description of Aridibacter gen. nov.**

Aridibacter [A.ri’di.bac’ter. L. masc. adj. aridis dry; N.L. masc. n. bacte a short rod; N.L. masc. n. Aridibacter rod-shaped bacterium isolated from dry (soil)].

Gram-negative, non-spore-forming, non-motile rods that occur as single cells or in short chains and divide by binary fission. Negative for cytochrome-c oxidase. Catalase-positive. No capsule formation. Aerobic, chemo-organotrophic mesophiles. Small amounts of NaCl enhance growth. Major fatty acids include iso-C\textsubscript{15:0}, summed feature 1 (C\textsubscript{13:0}-3-0H/iso-C\textsubscript{15:0} H), summed feature 3 (C\textsubscript{16:1}α/β7c and anteiso-C\textsubscript{17:0}). The major quinone is MK-8. The type species is Aridibacter famidurans.

**Description of Aridibacter famidurans sp. nov.**

Aridibacter famidurans [fa.mi.du’rans. L. fem. n. fames hunger; L. part. adj. durans enduring; N.L. part. adj. famidurans surviving hunger].

The description complies with the description of the genus and in addition includes the following characteristics. Cells are 2.5–3.0 μm long and 0.6–0.7 μm in diameter. Liquid cultures have a yellow to bright-pink colour. Dependent on the age of the culture, aggregates and flocs are formed even in shaken cultures. Colonies on agar plates are 0.1–0.2 mm in diameter, circular, white with a bright pink hue, translucent and convex with entire margins. Grows at 15–44 °C and pH 4.0–9.5. Under optimal growth conditions, the doubling time is 5.5 h. Grows at up to 1 % (w/v) NaCl, with best growth at 0.25 % (w/v) NaCl. Grows on maltose, rhamnose, N-acetylgalactosamine, fumarate, isovalerate, protocatechuate, Casamino acids, casein hydrolysate, peptone, yeast extract, laminarin and cellulose. On lactose, glutamine, L-asparagine, triprenylbacte, starch and chitin, weak growth is detected. No growth is observed on arabinose, cellobiose, erythrose, erythrylose, fructose, fucose, galactose, glucose, lyxose, mannose, melezitose, raffinose, sorbose, sucrose, trehalose, xylene, glucosamine, N-acetylgalactosamine, acetoxy, adonitol, arabinotol, dulcitol, lynitol, mannotol, myo-inositol, sorbitol, xylitol, alanine, arginine, aspartate, cysteine, glutamate, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, adipate, acetate, ascorbate, benzoate, trimethoxybenzoate, butyrate, α-hydroxybutyrate, β-hydroxybutyrate, γ-hydroxybutyrate, isobutyrate, caproate, caprylate, citrate, isocitrate, crotonate, formate, fumarate, glutonate, 2-oxoglutarate, glycine, glycolate, glyoxylate, isovalerate, lactate, maleate, malonate, nicotinic acid, oxaloacetate, propionate, pyruvate, shikimate, succinate, tartrate, 2-oxovalerate, butanol, 1,2-butandiol, 2,3-butandiol, ethanol, ethylene glycol, glycerol, methanol, propanol, 1,2-propandiol, fermented rumen extract, laminarin or Tween 80. Enzyme activities include alkaline and acid phosphatases, leucine arylaminidase, valine arylaminidase, trypsin, β-glucosidase and N-acetyl-β-glucosaminidase. Weak activities of α-glucosidase, x-chymotrypsin, esterase lipase C8, lipase C14 and naphthol-AS-BI-phosphohydrolase are detected. No activities of esterase C4, cystine arylaminidase, β-galactosidase, β-glucuronidase, x-mannosidase or x-fucosidase are determined. Aesculin and 4-nitrophenyl β-D-galactopyranoside are hydrolysed. Gelatin is hydrolysed after 1 week. Negative for urease, arginine dihydrolase and indole production.

The type strain is A22_HD_4H\textsuperscript{T} (=DSM 26555\textsuperscript{T} =LMG 27985\textsuperscript{T}), isolated from a sandy subtropical savannah soil in Erichsfelde, Namibia. The DNA G+C content of the type strain is 53.2 mol%.

**Description of Aridibacter kavangonensis sp. nov.**

Aridibacter kavangonensis [ka.van.go.nen’sis N.L. masc. adj. kavangonensis deriving from the Kavango region, Namibia].

The description complies with the description of the genus with the following additional characteristics. Cells are 2.5–3.0 μm long and 0.6–0.7 μm in diameter and form chains of two to four cells. Colonies are 0.2–0.3 mm in diameter, circular, white with a bright pink hue, translucent and convex with entire margins. No aggregates are formed in liquid culture. Grows at 12–47 °C and pH 3.5–10.0. Doubling time under optimal growth conditions is 6.1 h. Grows at up to 1 % (w/v) NaCl, with best growth at 0.25 % (w/v) NaCl. Grows on maltose, rhamnose, N-acetylgalactosamine, fumarate, isovalerate, protocatechuate, Casamino acids, casein hydrolysate, peptone, yeast extract, laminarin and cellulose. On lactose, glutamine, L-asparagine, trimethoxybenzoate, starch and chitin, weak growth is detected. No growth is observed on arabinose, cellobiose, erythrose, erythrylose, fructose, fucose, galactose, glucose, lyxose, mannose, melezitose, raffinose, sorbose, sucrose, trehalose, xylene, glucosamine, N-acetylgalactosamine, acetoxy, adonitol, arabitol, dulcitol, lynitol, mannotol, myo-inositol, sorbitol, xylitol, alanine, arginine, aspartate, cysteine, glutamate, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, threonine, tryptophan, tyrosine, valine, adipate, acetate, ascorbate, benzoate, butyrate, heptanoic acid, α-hydroxybutyrate, β-hydroxybutyrate, γ-hydroxybutyrate, isobutyrate, caproate,
caprylate, citrate, isocitrate, crotonate, formate, gluconate, 2-oxoglucconate, glucuronate, 2-oxoglutarate, glycolate, glyoxylate, laevulinate, lactate, malate, maleic acid, malonate, nicotinic acid, oxaloacetate, propionate, pyruvate, shikimate, succinate, tartrate, 2-oxoalate, butanol, 1,2-butanediol, 2,3-butanediol, ethanol, ethylene glycol, glycerol, methanol, propanol, 1,2-propanediol, fermented rumen extract or Tween 80. Enzyme activities include alkaline and acid phosphatases, leucine arylaminidase, valine arylaminidase, trypsin, α-chymotrypsin, naphtol-AS-BI-phosphohydrolase, β-glucosidase and N-acetyl-β-glucosaminidase. Weak activities of esterase lipase C8 and β-glucuronidase are detected. No activity of esterase C4, lipase C14, cystine arylaminidase, α-galactosidase, β-galactosidase, α-glucosidase, α-mannosidase or α-fucosidase is present. Aesculin and 4-nitrophophenol β-D-galactopyranoside are hydrolysed. Negative for gelatinase, urease, indole production and arginine dihydrolase.

The type strain is Ac_23_E3T (=DSM 26558T=LMG 27597T), isolated from a sandy subtropical savannah soil in Mashare, Namibia. The DNA G+C content of the type strain is 52.6 mol%.

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