

***Occallatibacter riparius* gen. nov., sp. nov. and *O. savannae* sp. nov. two novel acidobacterial species isolated from Namibian soils and emended description of the family *Acidobacteriaceae***

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**Running title:** *Occallatibacter riparius* gen. nov., sp. nov. and *O. savannae* sp. nov.

**Subject category:** New Taxa, subsection *Acidobacteria*

**Footnote**

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Occallatibacter riparius* 277<sup>T</sup> and 307 and *Occallatibacter savannae* A2-1c<sup>T</sup> are HQ995659, HQ995660, and HQ995661, respectively.

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**Isolation medium SSE/Cmix (1 L):**

MES	10 mM (final concentration)
SSE (double concentrated) <sup>a</sup>	500 ml
Distilled water	500 ml (less the volume of the solutions added after autoclaving)

Strain dependent adjust to pH 5.5 or pH 5.8 and autoclave.

After cooling add the following sterile filtered solutions:

Trace element solution SL-10 <sup>b</sup>	1.0 ml
Vitamin solution <sup>c</sup>	1.0 ml
Mix of carbohydrates <sup>d</sup>	20 µM each compound (final)
Mix of organic acids <sup>e</sup>	20 µM each compound (final)
Mix of amino acids <sup>f</sup>	20 µM each compound (final)
Aromatic compounds <sup>g</sup>	20 µM each compound (final)
Inducers <sup>h</sup>	2 µM each compound (final concentration)

**<sup>a</sup> SSE (Soil Solution Equivalent) after Angle *et al.* (1991) - double concentrated:**

CaCl <sub>2</sub> x 2H <sub>2</sub> O	0.2938 g
NH <sub>4</sub> Cl	0.1069 g
MgCl <sub>2</sub> x 6H <sub>2</sub> O	0.2036 g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.1983 g
MgSO <sub>4</sub> x 7H <sub>2</sub> O	0.7390 g
CaSO <sub>4</sub> x 2H <sub>2</sub> O	0.8606 g
Ca(NO <sub>3</sub> ) <sub>2</sub> x 4H <sub>2</sub> O	0.2360 g
NaNO <sub>3</sub>	0.4240 g
KH <sub>2</sub> PO <sub>4</sub> (100 mM solution)	0.5000 ml
FeSO <sub>4</sub> x 7H <sub>2</sub> O	0.0111 g
K <sub>2</sub> SO <sub>4</sub>	0.0870 g
Distilled water	1000 ml

Stir over night and use directly or autoclave and store for future use.

**<sup>b</sup> Trace element solution SL-10 (Tschech & Pfennig, 1984):**

HCl (25%; 7.7 M)	10 ml
FeCl <sub>2</sub> x 4H <sub>2</sub> O	1.50 g
CoCl <sub>2</sub> x 6H <sub>2</sub> O	190.0 mg
MnCl <sub>2</sub> x 4H <sub>2</sub> O	100.0 mg
ZnCl <sub>2</sub>	70.0 mg
Na <sub>2</sub> MoO <sub>4</sub> x 2H <sub>2</sub> O	36.0 mg
NiCl <sub>2</sub> x 6H <sub>2</sub> O	24.0 mg
H <sub>3</sub> BO <sub>3</sub>	6.0 mg
CuCl <sub>2</sub> x 2H <sub>2</sub> O	2.0 mg
Distilled water	990 ml

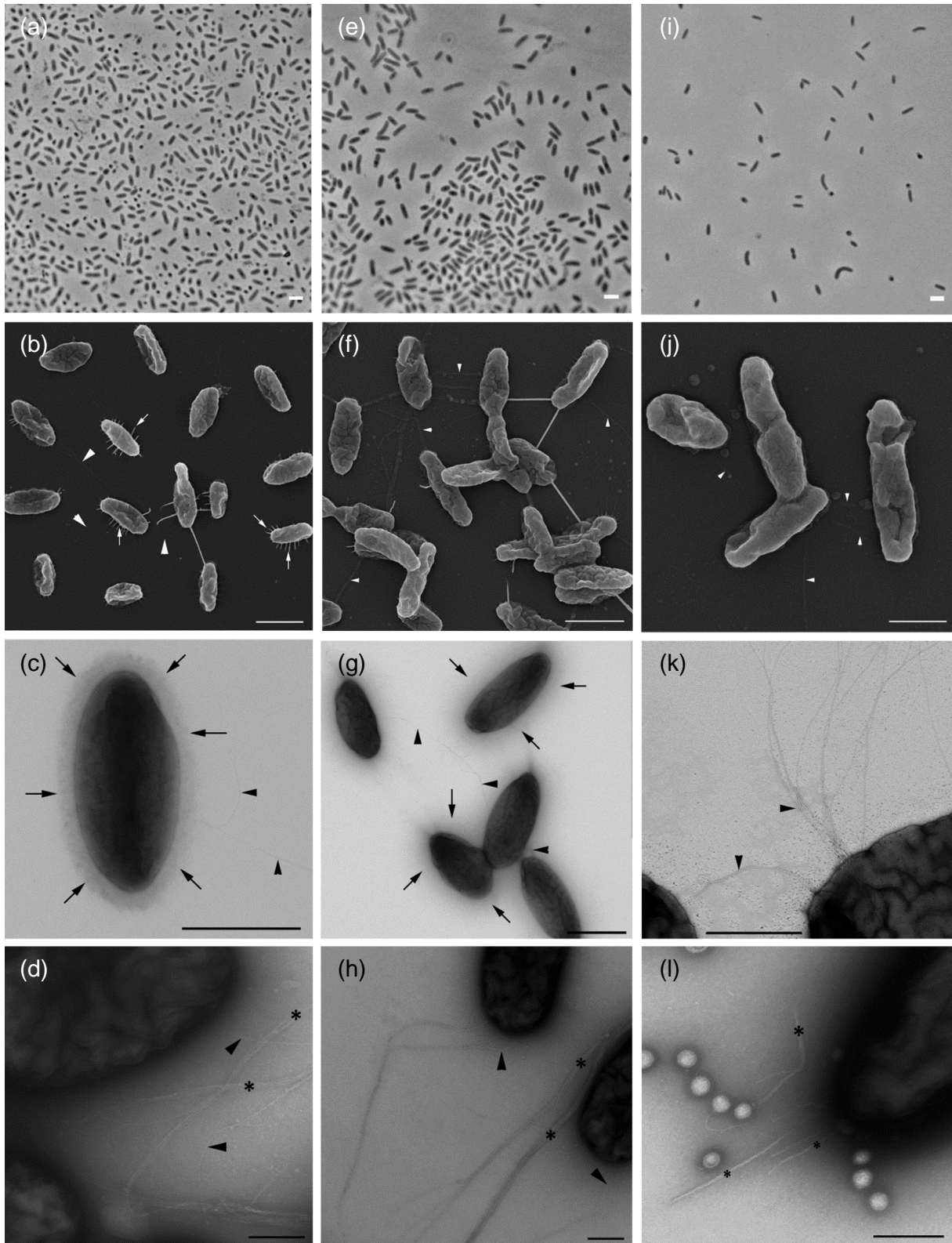
First dissolve FeCl<sub>2</sub> in HCl, then dilute with water, add and dissolve the other salts. Finally make up to 1000 ml.

**<sup>c</sup> Balch's vitamin mixture (Balch *et al.*, 1979):**

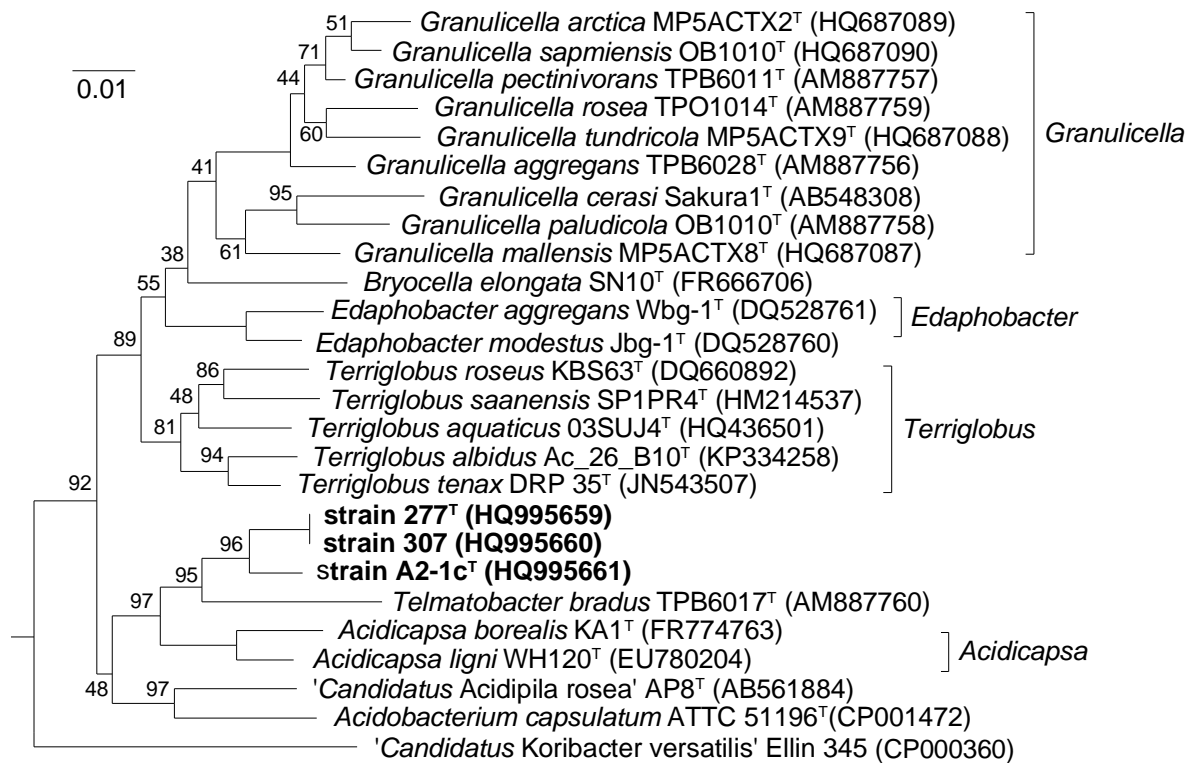
Biotin	2 mg
Folic acid	2 mg
Pyridoxine hydrochloride	10 mg
Thiamine hydrochloride	5 mg
Riboflavin	5 mg
Nicotinic acid	5 mg
DL-Ca-pantothenate	5 mg
Vitamin B <sub>12</sub>	0.1 mg
p-Aminobenzoic acid	5 mg
Lipoic acid	5 mg
Distilled water	1000 ml

- <sup>d</sup> **Mix of carbohydrates:** arabinose, fucose,  $\beta$ -gentibiose, glucose, rhamnose, trehalose, xylose, N-acetyl-D-galactosamine, glucosamine, and mannit.
- <sup>e</sup> **Mix of organic acids:** acetate, butyrate, citrate, formate, lactate, succinate, malate, oxaloacetate, 2-oxoglutarate, propionate, pyruvate, and valerate.
- <sup>f</sup> **Mix of amino acids:** alanine, arginine, asparagine, aspartate, cysteine, glutamine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.
- <sup>g</sup> **Aromatic compounds:** sodium benzoate and sodium salicylate.
- <sup>h</sup> **Inducers:** cyclic adenosine monophosphate (cAMP), N-(oxohexanoyl)-DL-homoserine lactone (OHHL), and N-(butyryl)-DL-homoserine lactone (BHL).

**Supplementary Figure 1.** Phase-contrast photomicrographs (a, e, i), scanning electron micrographs (b, f, j), and transmission electron micrographs (c, d, g, h, k, l) of strains 277<sup>T</sup> (a-d), 307 (e-h), and A2-1c<sup>T</sup> (i-l). Arrows show capsule, arrow heads point to single fimbria-like structures, and stars mark aggregated fimbria-like structures. Scale bars, 2  $\mu\text{m}$  (a, b, e, f, i), 1  $\mu\text{m}$  (c, g), 0.5  $\mu\text{m}$  (j), 0.25  $\mu\text{m}$  (k), and 0.2  $\mu\text{m}$  (d, h, l).



**Supplementary Figure 2.** Rooted neighbor-joining phylogenetic tree (Felsenstein correction) based on almost full-length 16S rRNA gene sequences showing the relationship of strains 277<sup>T</sup>, 307, and A2-1c<sup>T</sup> to each other and to further *Acidobacteria* subdivision 1 taxa. Bootstrap values (expressed as a percentages of 1000 replicates) below 100% are indicated at the respective branching points. The following sequences were used as outgroup: *Rubinisphaera brasiliensis* DSM 5305<sup>T</sup> (AJ231190), *Gimesia maris* DSM 8797<sup>T</sup> (AJ231184), and *Planctopirus limnophilus* DSM 3776<sup>T</sup> (X62911). Bar indicates 1% nucleotide divergence.



### Supplementary Table 1.

Full cellular fatty acid profiles of the three novel strains and its closest phylogenetic relatives. **1**, 277<sup>T</sup>; **2**, 307; **3**, A2-1c<sup>T</sup>, **4**, *Telmatobacter bradus* DSM 23630<sup>T</sup>; **5**, *Acidicapsa borealis* DSM 23886<sup>T</sup>; **6**, *Acidicapsa ligni* DSM 25248<sup>T</sup>; **7**, *Bryocella elongata* DSM 22489<sup>T</sup>.

All data were gained under comparable conditions (SSE/HD1:10, pH 5.5, 18d, 20°C) within this study.

Values given are percentages of total fatty acids identified with standard methods of the Microbial Identification System (MIDI Inc.; version 6.1) (Sasser, 1990). Summed features represent two fatty acids that could not be separated by GLC with the MIDI system.

Summed feature 1 contained 15:1 ISO H and/or 13:0 3OH, summed feature 3 contained 16:1 ω7c and/or 15:0 iso 2-OH, summed feature 4 contained 17:1 iso I and/or 17:1 anteiso B, summed feature 5 contained 18:2w6,9c and/or 18:0 ANTE; bold, major components ≥ 10%; -, not detected.

Fatty acid	1	2	3	4	5	6	7
<b>Saturated</b>							
12 : 0	-	-	-	-	-	-	0.2
14 : 0	-	-	-	0.5	0.5	0.7	1.1
15 : 0	-	-	-	-	-	-	0.2
16 : 0	0.5	0.6	0.6	3.8	1.2	1.4	<b>12.8</b>
17 : 0	-	-	-	-	-	0.4	-
18 : 0	-	-	-	3.8	-	-	0.6
20 : 0	-	-	-	-	-	-	0.5
<b>Unsaturated</b>							
14 : 1 ω5c	-	-	-	-	-	-	0.5
15 : 1 ω6c	-	-	-	-	-	-	0.2
16 : 1 ω5c	-	-	-	-	-	-	0.6
18 : 1 ω7c	-	-	-	0.3	-	-	0.8
18 : 1 ω9c	-	-	-	1.2	-	-	0.3
<b>Methyl-branched</b>							
13 : 0 iso	-	-	-	0.6	-	-	0.2
14 : 0 iso	-	-	-	-	0.4	-	-
15 : 0 iso	<b>61.9</b>	<b>61.6</b>	<b>54.8</b>	<b>61.3</b>	<b>64.8</b>	<b>60.2</b>	<b>45.8</b>
15 : 1 iso F	-	-	0.5	3.0	-	-	-
16 : 0 iso	0.4	-	2.0	-	-	-	-
17 : 1 iso ω7c <sup>a</sup>	<b>29.0</b>	<b>28.0</b>	<b>30.7</b>	<b>16.7</b>	<b>17.0</b>	<b>25.3</b>	1.3
17 : 0 iso	5.3	7.6	9.5	4.6	<b>12.4</b>	7.2	3.5
17 : 0 anteiso	1.1	1.4	0.6	0.6	0.4	0.4	-
17 : 0 10 methyl	-	-	-	-	0.9	0.9	-
<b>Hydroxy</b>							
11 : 0 2-OH	-	-	-	0.2	-	-	-
13 : 0 iso 3-OH	-	-	-	0.5	-	-	-
17 : 0 iso 3-OH	-	-	-	1.3	-	-	-

Summed Feature							
1	0.7	0.4	0.5	0.3	1.1	1.3	0.1
3 <sup>b</sup>	0.4	0.6	0.9	0.4	1.4	2.2	<b>31.2</b>
4	0.6	-	-	0.2	-	-	-
5	-	-	-	0.3	-	-	-
Unknown							
11.543	-	-	-	-	-	-	0.2
13.565	-	-	-	0.6	-	-	-

<sup>a</sup> Determined as *iso*-C<sub>17:1</sub> ω<sub>9</sub>c by the DSMZ service unit according to the MIDI System, but identified as *iso*-C<sub>17:1</sub> ω<sub>7</sub>c via GC/MS (Kulichevskaya *et al.*, 2012; Sinninghe Damsté *et al.*, 2011), although erroneously designated as *iso*-C<sub>17:1</sub> ω<sub>8</sub>c in the latter work (see main text).

<sup>b</sup> Determined as summed feature 3 (16:1 ω<sub>7</sub>c and/or 15:0 iso 2-OH) by the DSMZ service unit according to the MIDI System, but identified as C<sub>16:1</sub> ω<sub>7</sub>c by (Sinninghe Damsté *et al.*, 2011) using GC/MS.

### Supplementary Table 2.

Substrate range of strains 277<sup>T</sup>, 307, and A2-1c<sup>T</sup>.

All three strains grew on cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, melizitose, raffinose, rhamnose, sucrose, trehalose, xylose, ornithine, tyrosine, gluconate, succinate, glycerol, casamino acids, casein hydrolysate, peptone, yeast extract. Substrates tested but not utilized are given in the respective species descriptions.

Carbon source	277 <sup>T</sup>	307	A2-1c <sup>T</sup>
Fucose	-	+	+
Sorbitol	+	-	-
Xylitol	-	-	+
Alanine	-	-	+
Arginine	-	+	-
Aspartate	+	+	-
Glutamate	+	+	-
Lysine	+	+	-
Phenylalanine	-	-	+
Proline	-	-	+
Acetate	+	-	-
Pyruvate	-	-	+
Chitin	-	-	+
Laminarin	-	+	+
Pectin	-	-	+
Starch	+	+	-



### Supplementary Table 3.

Polysaccharide degradation by *Acidobacteria* subdivision 1 genera.

Genera: **1, *Occallatibacter***, including summarized data of strains 277<sup>T</sup>, 307, and A2-1c<sup>T</sup> (all this study); **2, *Telmatobacter***, including *T. bradus* TPB6017<sup>T</sup> (Pankratov *et al.*, 2012); **3, *Acidicapsa***, including *A. borealis* KA1<sup>T</sup> and *A. ligni* WH120<sup>T</sup> (Kulichevskaya *et al.*, 2012); **4, ‘*Acidipila*’,** including ‘*A. rosea*’ AP8<sup>T</sup> (Okamura *et al.*, 2011); **5, *Acidobacterium***, including *A. capsulatum* 161<sup>T</sup> (Kishimoto *et al.*, 1991); **6, *Edaphobacter***, including *E. aggregans* DSM 19364<sup>T</sup> (Pankratov & Dedysh, 2010); **7, *Terriglobus***, including *T. roseus* DSM 18391<sup>T</sup> (Pankratov & Dedysh, 2010), *T. saanensis* SP1PR4<sup>T</sup> (Männistö *et al.*, 2011), *T. tenax* DRP 35<sup>T</sup> (Whang *et al.*, 2014), *T. aquaticus* 03SUJ4<sup>T</sup> (Baik *et al.*, 2013), and *T. albidus* Ac\_26\_B10<sup>T</sup> (Pascual *et al.*, 2015); **8, *Granulicella***, including *G. paludicola* OB1010<sup>T</sup>, *G. pectinivorans* TPB6011<sup>T</sup>, *G. aggregans* TPB6028<sup>T</sup>, *G. rosea* TPO1014<sup>T</sup> (Pankratov & Dedysh, 2010), *G. arctica* MP5ACTX2<sup>T</sup>, *G. mallensis* MP5ACTX8<sup>T</sup>, *G. tundricola* MP5ACTX9<sup>T</sup>, *G. sapmiensis* S6CTX5A<sup>T</sup> (Männistö *et al.*, 2012), and *G. cerasi* Sakura1<sup>T</sup> (Yamada *et al.*, 2014); **9, *Bryocella***, including *B. elongata* SN10<sup>T</sup> (Dedysh *et al.*, 2012).

+, positive; (+), weakly positive; -, negative; ND, no data.

When characteristics differ among strains, numbers in brackets give the number of strains showing the respective feature (first number) compared to the number of all strains considered (second number).

Polysaccharide	1	2	3	4	5	6	7	8	9
Alginate	ND	-	ND	ND	ND	-	-(2/5), ND(3/5)	-(8/9), ND(1/9)	ND
Chitin	+(1/3), -(2/3)	-	-	ND	ND	-	-(4/5), ND(1/5)	-(8/9), ND(1/9)	-
Chitosan	ND	-	-	ND	ND	-	-(2/5), ND(3/5)	-(8/9), ND(1/9)	ND
Cellulose	-	+	-	ND	ND	-	-(4/5), ND(1/5)	-(8/9), ND(1/9)*	-
Chondroitine	ND	-	ND	ND	ND	-	-(1/5), ND(4/5)	-(4/9), ND(5/9)	+
Fuoidan	ND	-	+(1/2), -(1/2)	ND	ND	-	-(1/5), ND(4/5)	-(4/9), ND(5/9)	+
Laminarin	+(2/3), -(1/3)	+	+(1/2), -(1/2)	ND	ND	+	+(1/5), +(1/5), ND(3/5)	+(8/9), ND(1/9)	ND
Lichenan	ND	-	ND	ND	ND	+	-(2/5), ND(3/5)	+(6/9), -(2/9), ND(1/9)	+
Pectin	+(1/3), -(2/3)	+	+(1/2), -(1/2)	ND	ND	-	+(2/5), -(1/5), ND(2/5)	+(8/9), ND(1/9)	+
Pullulan	ND	-	-	ND	ND	-	-(2/5), ND(3/5)	+(1/9), -(7/9), ND(1/9)	ND
Starch	+(2/3), -(1/3)	+	+(1/2), -(1/2)	ND	+	(+)	+(4/5), -(1/5)	+(7/9), -(1/9), ND(1/9)	-
Xylan	-	+	+(1/2), -(1/2)	ND	ND	(+)	+(1/5), -(2/5), ND(2/5)	+(7/9), -(1/9), ND(1/9)	-

\* *G. cerasi* not tested for cellulose, but able to hydrolyze CM-cellulose.

#### Supplementary Table 4.

Enzyme activities of strains 277<sup>T</sup>, 307, and A2-1c<sup>T</sup> and other described *Acidobacteria* subdivision 1 members as determined by the API ZYM test system (Biomérieux).

Genera: **1, *Occallatibacter***, including strains (a) 277<sup>T</sup>, (b) 307, and (c)A2-1c<sup>T</sup> (all this study); **2, *Telmatobacter***, including *T. bradus* TPB6017<sup>T</sup> (Pankratov *et al.*, 2012); **3, *Acidicapsa***, including *A. borealis* KA1<sup>T</sup> and *A. ligni* WH120<sup>T</sup> (Kulichevskaya *et al.*, 2012); **4, ‘*Acidipila*’**, including ‘*A. rosea*’ AP8<sup>T</sup> (Okamura *et al.*, 2011); **5, *Acidobacterium***, including *A. capsulatum* DSM 11244<sup>T</sup> (Koch *et al.*, 2008); **6, *Edaphobacter***, including *E. modestus* Jbg-1<sup>T</sup> and *E. aggregans* Wbg-1<sup>T</sup> (Koch *et al.*, 2008); **7, *Terriglobus***, including *T. roseus* DSM 18391<sup>T</sup>, *T. saanensis* SP1PR4<sup>T</sup> (Männistö *et al.*, 2011), *T. tenax* DRP 35<sup>T</sup> (Whang *et al.*, 2014), *T. aquaticus* 03SUJ4<sup>T</sup> (Baik *et al.*, 2013), and *T. albidus* Ac\_26\_B10<sup>T</sup> (Pascual *et al.*, 2015); **8, *Granulicella***, including *G. arctica* MP5ACTX2<sup>T</sup>, *G. mallensis* MP5ACTX8<sup>T</sup>, *G. tundricola* MP5ACTX9<sup>T</sup>, *G. sapmiensis* S6CTX5A<sup>T</sup>, *G. paludicola* DSM 22464<sup>T</sup>, *G. pectinivorans* DSM 21001<sup>T</sup>, *G. rosea* DSM 18704<sup>T</sup> (Männistö *et al.*, 2012), and *G. cerasi* Sakura1<sup>T</sup> (Yamada *et al.*, 2014); **9, *Bryocella***, including *B. elongata* SN10<sup>T</sup> (Dedysh *et al.*, 2012).

+, positive; -, negative; (+), weak enzyme activity detected; ND, no data.

When characteristics differ among strains, numbers in brackets give the number of strains showing the respective feature (first number) compared to the number of all strains considered (second number).

Enzyme	1a	1b	1c	2	3	4	5	6	7	8	9
Alkaline phosphatase	+	+	+	-	+	ND	-	+	+	+(7/8), -(1/8)	-
Acid phosphatase	+	+	(+)	+	+	ND	+	+	+	+	+
Naphthol-AS-BI-phosphohydrolase	+	+	(+)	+	+	ND	+	+	+	+	+
Esterase (C 4)	+	+	(+)	+	+	ND	+	+	+(1/5), -(4/5)	+(2/8), +(5/8), -(1/8)	+
Esterase Lipase (C 8)	(+)	(+)	-	-	+	ND	-	+	+(1/5), +(1/5), -(3/5)	+(3/8), +(3/8), -(2/8)	+
Lipase (C 14)	+	+	+	-	-	ND	-	-	+(1/5), -(4/5)	-	-
Leucine arylamidase	+	+	(+)	-	+	ND	-	+(1/1), +(1/1)	+(3/5), +(2/5)	+	+
Valine arylamidase	(+)	(+)	(+)	+	+	ND	-	+(1/1), +(1/1)	+(1/5), +(1/5), -(3/5)	+(5/8), -(3/8)	+
Cysteine arylamidase	+	(+)	(+)	-	+	ND	-	-	+(1/5), -(3/5)	+(1/8), +(4/8), -(3/8)	-
Trypsin	+	(+)	(+)	-	ND	ND	-	-	+(2/5), -(3/5)	+(4/8), +(2/8), -(2/8)	-
$\alpha$ -Chymotrypsin	+	+	+	-	+(1/1), -(1/1)	ND	-	+(1/1), +(1/1)	+(2/5), -(3/5)	+(5/8), +(2/8), -(1/8)	-
$\alpha$ -Galactosidase	+	+	+	+	+	ND	(+)	+	+	+	-
$\beta$ -Galactosidase	+	+	(+)	+	+	ND	+	+	+	+	+
$\beta$ -Glucuronidase	+	(+)	+	+	+	ND	+	+	+	+(7/8), +(1/8)	+
$\alpha$ -Glucosidase	+	+	+	+	+	ND	(+)	+(1/1), +(1/1)	+(4/5), +(1/5)	+	+
$\beta$ -Glucosidase	+	+	-	+	+	ND	+	+(1/1), +(1/1)	+	+	+
N-Acetyl- $\beta$ -glucosaminidase	(+)	-	-	+	+	ND	+	+(1/1), -(1/1)	+(4/5), +(1/5)	+(7/8), -(1/8)	+
$\alpha$ -Mannosidase	+	+	+	-	+	ND	-	-	+(4/5), -(1/5)	+(5/8), +(1/8), -(2/8)	-
$\alpha$ -Fucosidase	+	+	+	+	+	ND	-	+(1/1), +(1/1)	+	+(5/8), -(3/8)	-

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