

# *Acidicapsa acidisoli* sp. nov., from the acidic soil of a deciduous forest

Heizo Matsuo,† Chisaki Kudo,† Juan Li and Akio Tonouchi\*

## Abstract

A bacterial strain designated strain  $SK-11^{T}$  was isolated from the acidic soil of a deciduous forest in the Shirakami Mountains in Japan. Cells of strain  $SK-11^{T}$  were aerobic, non-motile, Gram-stain-negative rods,  $0.7-1.0 \,\mu$ m in width and  $1.0-1.4 \,\mu$ m in length. The pH range for growth was between pH 4.0 and 5.5, with an optimum at pH 5.0. The temperature range for growth was between 10 and  $35^{\circ}$ C, with an optimum at around  $25-30^{\circ}$ C. Strain  $SK-11^{T}$  utilized various carbohydrates as growth substrates as well as yeast extract and protein hydrolysates. The major cellular fatty acids (>10 % of total fatty acid contents) were iso- $C_{15:0}$  (55.4 %), iso- $C_{17:0}$  (16.7 %) and iso- $C_{17:1}\omega9c/10$  methyl-hexadecanoic acid (17.7 %). The major respiratory quinone was MK-8. The polar lipids consisted of phosphatidylethanolamine, phosphatidylglycerol, two unidentified phospholipids and an unidentified polar lipid. The DNA G+C content of strain SK-11<sup>T</sup> was 56.9 %. Phylogenetic analysis based on the 16S rRNA gene sequences revealed that strain SK-11<sup>T</sup> belonged to the family *Acidobacteriaceae* within subdivision 1 of the phylum *Acidobacteria*, and the closest relatives of strain SK-11<sup>T</sup> were *Acidicapsa ligni* WH120<sup>T</sup> and *Acidicapsa borealis* KA1<sup>T</sup>, with 16S rRNA gene sequence similarities of 96.6 and 96.5 %, respectively. On the basis of the evidence from our polyphasic study, we concluded that strain SK-11<sup>T</sup> represents a novel species of the genus *Acidicapsa*, and propose the name *Acidicapsa acidisoli* sp. nov. The type strain of *Acidicapsa acidisoli* pnov. is SK-11<sup>T</sup> (=DSM 100508<sup>T</sup>=NBRC 111227<sup>T</sup>).

Recent culture-independent approaches have revealed that the phylum Acidobacteria is a highly diverse bacterial group (currently divided into at least 26 subdivisions) comparable to the phylum Proteobacteria, and its members are considered ubiquitous and dominant in various soil environments, making up an average of 20 % (ranging between 5 and 46 %) of the all soil bacteria [1-3]. The first description of a species of the phylum Acidobacteria, Acidobacterium capsulatum, dates back to 1991. However, currently, 25 years after its description, there are few species with validly published names (www.bacterio.net/) in the phylum Acidobacteria although the members of the phylum Acidobacteria are extremely diverse, as estimated by 16S rRNA gene-based molecular surveys. This is primarily attributable to the difficulty in the cultivation of these bacteria. The Shirakami Mountains, located at the northern area of Honshu Island in Japan, is composed of primeval Japanese Beech forests with acidic brown forest soils, and was registered as a UNESCO World Natural Heritage site in 1993. Our preliminary culture-independent approach revealed that the bacterial communities of the soils in the Shirakami Mountains are dominated by the members of the phylum Acidobacteria as well as those of the phylum *Proteobacteria* (unpublished), suggesting that these species play an important role in the soil ecosystems of the Shirakami Mountains. Here, we describe a novel acidophilic bacterial strain from the soil of a deciduous forest in the Shirakami Mountains.

The medium used for isolation of bacteria, designated IS medium (pH 5.0), contained the following ingredients: Nutrient broth (Oxoid),  $0.065 \text{ g} \text{ l}^{-1}$ ; MgSO<sub>4</sub>•7H<sub>2</sub>O,  $1.0 \text{ g} \text{ l}^{-1}$ ; 2-(N-morpholino) ethanesulfonic acid (MES),  $2.0 \text{ g } \text{l}^{-1}$ ; cycloheximide, 0.1 g  $l^{-1}$ ; soil extract, 500 ml  $l^{-1}$ ; and gellan gum,  $8.0 \text{ g l}^{-1}$ . The soil extract was prepared from the soil collected at the same sampling site described below according to the procedure described by George et al. [4]. For normal cultivation, modified IS medium (MIS medium), in which cycloheximide was omitted from IS medium and supplemented with  $8.0 \text{ g} \text{ l}^{-1}$  of glucose was used. For biochemical and biophysical characterization, a medium designated YNG (pH 5.0) containing 6.7 g l<sup>-1</sup> of Yeast Nitrogen Base (Difco), 8.0 g l<sup>-1</sup> of glucose, and  $2.0 \text{ g} \text{ l}^{-1}$  of MES was used. If needed, it was solidified with  $8.0 \text{ g} \text{ l}^{-1}$  of gellan gum). Incubations on solid media were performed at 30 °C and incubations in liquid

Author affiliation: Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan. \*Correspondence: Akio Tonouchi, symbio@cc.hirosaki-u.ac.jp

Keywords: Acidobacteria; subdivision I.

Abbreviations: ML, maximum-likelihood; NJ, neighbour-joining.

<sup>†</sup>These authors contributed equally to this work.

The DDBJ/EMBL/GenBank accession number for the 16S rRNA gene sequence of *Acidicapsa acidisoli* SK-11<sup>T</sup> is LC027464.

One supplementary table and one supplementary figure are available with the online Supplementary Material.

media were performed at 30 °C with shaking at 100 r.p.m. on a reciprocal shaker, unless stated otherwise.

The soil used for isolation of bacteria was sampled on Jun 14, 2013 in a ridge of the forest (N40 30.916, E140 12.894; 346 m elevation above the sea level) at the Shirakami Natural Science Park of the Shirakami Institute for Environmental Sciences, Hirosaki University, Kawaratai, Nishimeya village, Nakatsugaru District, Aomori. The vegetation of the site included several deciduous trees such as Querqus cripsula, Fagus crenata, Magnolia obovata and Acer pictum. After removing visible litter from the forest floor, soil was collected with a sterilized trowel from a depth of 10 cm below the surface, transferred to a sterilized plastic bag, transported to the laboratory in an icepack-cooled box, and immediately subjected to bacterial isolation upon arrival. As shown in Table S1 (available in the online Supplementary Material), the soil sampled was acidic with the pH values of 4.5 [pH (H2O)] and 3.4 [pH (KCl)]. The soil was serially diluted to 10<sup>-5</sup> in sterilized distilled water and 0.1 ml of each dilution was spread onto the surface of IS medium; the inoculated plates were incubated at 30 °C. After 60 days of incubation, some small colonies that formed on the plates were picked, streaked on the same fresh medium, and recultivated. The discrete colonies formed on the plates were picked and subjected to the identification procedure based on the analysis of 16S rRNA gene sequence. A strain designated SK-11<sup>T</sup> showing relatively good growth on IS medium was selected for further taxonomic analysis. The reference strains, Acidicapsa borealis DSM  $23886^{T}$  and Acidicapsa ligni DSM 25248<sup>T</sup>, were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and were compared with strain SK-11<sup>T</sup>; all strains were cultured in the same conditions. All experiments were performed at least in duplicates.

Gram staining was performed with a Favor G Nissui kit (Nissui Pharmaceutical). Catalase and oxidase were assayed using ID Color Catalase (bioMérieux) and oxidase identification sticks (Oxoid), respectively. Some other biochemical features were examined using API ZYM and API 20NE strips (bioMérieux). Susceptibility to antibiotics (ampicillin, chloramphenicol, gentamycin, kanamycin, novobiocin, and streptomycin) was evaluated on MIS medium using BBL Sensi-Disc Susceptibility Test Discs (Becton, Dickinson and Company). Anaerobic growth was examined on MIS medium using an AnaeroPack System (Mitsubishi Gas Chemical). For these tests, cells cultureed for 2 weeks on MIS medium were used.

The temperature range for growth was determined in YNG medium at 4, 10, 15, 20, 25, 30, 33, 35, 37, 40 and 45  $^{\circ}$ C. The pH range for growth was determined in YNG medium in the range of pH 3.5–7.5, in increments of 0.5 pH units. Tolerance to NaCl was examined in MIS medium containing 0– 5 % NaCl, in increments of 0.5 %. Growth was examined in modified YNG medium in which glucose was replaced by 0.05 % of each of the following substrates: D-arabinose, L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose,

D-mannose, L-sorbose, L-rhamnose, D-tagatose, D-fucose, Lfucose, D-cellobiose, D-lactose, D-maltose, D-melibiose, Dsucrose, D-trehalose, D-turanose, D-melezitose, D-raffinose, D-ribitol, D-xylitol, *myo*-inositol, D-mannitol, D-sorbitol, D-galacturonate, aesculin, arbutin, D-salicin, inulin, soluble starch, carboxymethyl cellulose (CMC), xylan, pectin, polygalacturonate, chitin (colloidal), lignin, yeast extract, peptone (casein derived), casamino acid. For these tests, the incubations were carried out for up to 30 days and the growth was assessed by monitoring OD<sub>420 nm</sub> using a photoelectric colorimeter (mini photo 5; Sanshin).

For the determination of genomic G+C content and for the DNA-DNA hybridization experiments, genomic DNA was extracted and purified from cells harvested from the culture at the exponential growth stage using a QIAGEN Blood and Cell Culture DNA kit (QIAGEN) according to the manufacturer's instructions. The purified genomic DNA was hydrolyzed to nucleotides by nuclease P1 provided in a DNA GC kit (Yamasa) and the nucleotides were separated by HPLC to determine the genomic G+C content according to the manufacturer's instructions. DNA-DNA hybridization was performed using the microtiter plate technique developed by Ezaki *et al.* [5] and modified by Dianou *et al.* [6].

For the extraction of cellular fatty acids, respiratory quinones, and polar lipids, cells grown on MIS medium for 20 days were used; the cells were recovered by suspending in water, harvested by centrifugation, washed twice with water, and then lyophilized. Extraction of fatty acids and their esterification were performed using a Fatty Acid Methylation Kit and a Fatty Acid Methyl Ester Purification Kit (Nacalai) according to the manufacture's instructions. The purified fatty acid methyl esters were analyzed by GC at TechnoSuruga Laboratory (Shizuoka, Japan) with the MIDI Sherlock Microbial Identification System (MIDI Corporation). Respiratory quinones were extracted according to the method described by Nishijima et al. [7] and identified by HPLC at TechnoSuruga Laboratory. Polar lipids were extracted and identified by two-dimensional thin layer chromatography using silica gel HPTLC plates (HPTLC Silica gel 60, Merck Millipore) as described by Minnikin et al. [8].

For the phylogenetic analysis, the nearly full-length 16S rRNA gene of strain SK-11<sup>T</sup> was PCR-amplified using primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1541r (5'-AAG GAGGTGATCCAGCCGCA-3') and sequenced as described previously [9]. The 16S rRNA gene sequence was deposited in GenBank/EMBL/DDBJ under the accession number LC027464. The 16S rRNA gene sequences of the close phylogenetic relatives included in subdivision 1 of the phylum Acidobacteria were obtained by running a BLAST search against the database in EzBioCloud (http://www.ezbiocloud.net/) and NCBI. The phylogenetic analysis was performed using the MEGA7 software [10]. The 16S rRNA gene sequences were aligned using the MUSCLE program [11]. Phylogenetic trees were inferred by the neighbour-joining (NJ) [12] and maximum-likelihood (ML) methods [13]. Evolutionary distances were calculated in accordance with Kimura's two-parameter

model [14] for the NJ tree, whereas, for the ML tree, Kimura's two-parameter model combined with the gamma distribution with invariant sites (G+I) model [13] was adopted. Gaps and missing data were eliminated. The reliabilities of the reconstructed phylogenetic trees were estimated by the bootstrap method performed with 1000 resamplings.

A bacterial strain designated SK-11<sup>T</sup> was isolated from the acidic soil of a deciduous forest in the Shirakami Mountains in the course of enumeration of cultivable bacterial cells. The colonies of strain SK-11<sup>T</sup>, which formed on MIS medium after 10 days of incubation, were white, circular, convex, smooth, and 0.4-2.8 mm in diameter with entire margins. Cells of strain SK-11<sup>T</sup> were Gram-stain-negative rods, non-motile, non-spore forming, capsule forming, 0.7-1.0 µm in width and 1.0-1.4 µm in length, and occurred as single cells or in pairs. Chain forms were found often, especially in young cultures. Strain SK-11<sup>T</sup> could not grow in the absence of molecular oxygen. Catalase and oxidase activities were positive. Gelatin was not liquefied. H<sub>2</sub>S was not produced. Nitrate was not reduced. Indole was not produced. The temperature range for growth was 10-35 °C, with an optimum at around 25-30 °C. The pH range for growth was 4.0-5.5, with an optimum at 5.0. The salinity range for growth of the isolate was 0-0.4 % NaCl, with an optimum at around 0-0.1 %. The enzyme activities obtained using the API systems are described in detail in the species description section and are summarized in Table 1. Strain SK-11<sup>T</sup> was resistant to ampicillin, chloramphenicol, gentamycin, kanamycin, and streptomycin, and was sensitive to novobiocin.

Strain SK-11<sup>T</sup> could utilize the following substrates as a carbon source for its growth: D-arabinose (weak), L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-fucose (weak), L-fucose, D-cellobiose, D-lactose, D-maltose, D-melibiose, D-sucrose, D-trehalose, D-turanose, D-melezitose (weak), D-raffinose, D-galacturonate, arbutin, D-salicin, inulin (weak), soluble starch, xylan, pectin, yeast extract, peptone (casein-derived), and casamino acid. Among these substrates, the best growth occurred on D-xylose rather than D-glucose. The following substrates could not be utilized: D-ribose, D-tagatose, *myo*-inositol, D-mannitol, D-ribitol, D-xylitol, L-sorbose, D-sorbitol, aesculin, CMC, polygalacturonate, lignin and chitin (colloidal).

Cellular fatty acids contents in the cells of strain SK-11<sup>T</sup> and its close relatives (*Acidicapsa borealis* and *Acidicapsa ligni*) are listed in Table 2. The following were the major cellular fatty acids of strain SK-11<sup>T</sup> at quantity >10%: iso- $C_{15:0}$  (55.4%), iso- $C_{17:1}$   $\omega$ 9*c*/10-methyl-hexadecanoic acid (17.7%), and iso- $C_{17:0}$  (16.7%). The major respiratory quinone was MK-8. The polar lipids consisted of phosphoethanolamine (PE), phosphatidylglycerol (PG), two unidentified phospholipids, and an unidentified polar lipid (Fig. S1). The genomic DNA G+C content was 56.9 mol%.

DNA-DNA hybridization experiments of strain SK-11<sup>T</sup> with *Acidicapsa ligni* DSM 25248<sup>T</sup> and *Acidicapsa borealis* 

**Table 1.** Differential characteristics of strain  $\mathsf{SK-11}^\mathsf{T}$  and its closest neighbors

Taxa: 1, *Acidicapsa acidisoli* SK-11<sup>T</sup>; 2, *A. ligni* DSMZ 25248<sup>T</sup>; 3, *A. borealis* DSM 23886<sup>T</sup>. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3
Cell size (µm)	0.7- 1.0×1.0-1.4	0.5- 0.8×1.0-2.0	0.6- 0.9×1.0-3.0
Color of colony	White	White	Pale Pink
DNA G+C content (mol%)	56.9	51.7*	54.1*
Growth at 35 °C	+	_	+
Growth at pH6.0	-	+	+
Oxidase activity	+	_	-
Growth in the presence of 0.5 % NaCl Substrate utilization:	_	+	+
D-ribose	-	+	+
D-fucose	W	_	-
D-xylitol	-	+	-
Arbtin	+	_	+
Xylan	+	+	-
Pectin	+	-	+
Enzyme activities:			
Cystine arylamidase	+	W	-
Chymotrypsin	-	_	+
$\alpha$ -glucosidase	-	_	+
Susceptibility to antibiotics:			
Kanamycin	-	_	+

\*Data from Kulichevskaya *et al.* [19].

DSM  $23886^{T}$  revealed relatedness values of 13% (reciprocal 17%) and 10% (reciprocal 11%), respectively.

Phylogenetic analysis based on the 16S rRNA gene sequences revealed that strain SK-11<sup>T</sup> shared a sequence similarity of less than 97 % with the type strains of the family *Acidobacteriaceae* within subdivision 1 of the phylum *Acidobacteria. A. ligni* WH120<sup>T</sup> and *A. borealis* KA1<sup>T</sup> were the closest relatives of strain SK-11<sup>T</sup> with 96.6 and 96.5 % 16S rRNA gene sequence similarities, respectively. In the NJ phylogenetic tree reconstructed based on the 16S rRNA gene sequences, strain SK-11<sup>T</sup> was clustered with the two type strains of the genus *Acidicapsa* with a high bootstrap value of 90 % and shared a branching node with *A. borealis* KA1<sup>T</sup> with a bootstrap value of 65 %; these results were also reproduced in the ML tree with relatively high bootstrap values (>60 %; Fig. 1).

The 16S rRNA gene sequence-based phylogenetic analysis revealed a close relationship between strain SK-11<sup>T</sup> and the two type strains of the genus *Acidicapsa*, i.e. *A. ligni* WH120<sup>T</sup> and *A. borealis* KA1<sup>T</sup>. To distinguish strain SK-11<sup>T</sup> from *A. ligni* WH120<sup>T</sup> and *A. borealis* KA1<sup>T</sup>, we conducted comparative analyses using strain SK-11<sup>T</sup>, *A. ligni* DSM 25248<sup>T</sup> (=WH120<sup>T</sup>), and *A. borealis* DSM 23886<sup>T</sup> (=KA1<sup>T</sup>). Strain SK-11<sup>T</sup> shared many characteristics with

**Table 2.** Cellular fatty acids profiles (%) of strain SK-11<sup>T</sup> and its closest neighbors

Taxa: 1, Acidicapsa acidisoli SK-11<sup>T</sup>; 2, A. ligni DSMZ 25248<sup>T</sup>; 3, A. borealis DSM 23886<sup>T</sup>. Fatty acids of any organisms more than 0.5% are shown. TR, Trace (<0.5%); -, not detected;  $\omega$ , Position of the double bond from the methyl end; c, cis isomer. Major fatty acids more than 10% are given in bold type.

Fatty acid	1	2	3
Saturated:			
C <sub>12:0</sub> iso	TR	TR	TR
C <sub>11:0</sub> 20H	TR	-	-
C <sub>13:0</sub> 2OH	TR	-	-
C <sub>14:0</sub> iso	TR	-	-
C <sub>15:0</sub> anteiso	-	-	TR
C <sub>15:0</sub> iso	55.4	51.4	52.2
C <sub>16:0</sub> iso	1.0	TR	0.8
C <sub>16:0</sub>	2.9	4.0	2.1
C <sub>17:0</sub> iso	16.7	8.3	9.4
C <sub>17:0</sub> anteiso	TR	TR	-
C <sub>17:0</sub>	0.5	1.1	1.7
C <sub>18:0</sub> iso	-	-	TR
C <sub>18:0</sub>	0.5	2.5	3.0
C <sub>19:0</sub> iso	-	TR	TR
C <sub>19:0</sub> anteiso	TR	-	-
Unsaturated:			
$C_{15:1}\omega 8c$	TR	TR	TR
$C_{17:1}\omega 8c$	-	TR	TR
$C_{18:1}\omega 9c$	0.5	0.7	0.9
Summed features*:			
C <sub>15:1</sub> iso H/C <sub>13:0</sub> 3OH	0.6	0.9	1.0
$C_{16:1}\omega 7c/C_{16:1}\omega 6c$	2.2	3.5	3.2
$C_{17:1}$ iso I/ $C_{17:1}$ anteiso B	0.6	0.6	0.6
$C_{18:2}\omega 6,9c/C_{18:0}$ anteiso	-	TR	TR
$C_{18:1}\omega7c/C_{18:1}\omega6c$	-	TR	-
iso-C <sub>17 : 1</sub> ω9c/ 10-methyl-hexadecanoic acid†	17.7	25.5	22.8

\*Summed features represent fatty acids that could not be separated by GC with the MIDI system.

†iso-C<sub>17:1</sub>ω9c/10 methyl-hexadecanoic acid was not detected in the type strains of *A. ligni* and *A. borealis* in a previous study by Kulichevskaya *et al.* [19]; instead, iso-C<sub>17:1</sub>ω8c was detected as a major fatty acid in these strains. This is likely because different procedures were used for fatty acid analysis between our study and that by Kulichevskaya *et al.*; we used the MIDI system for fatty acid analysis, whereas Kulichevskaya *et al.* analyzed fatty acids by GC and GC-MS without using the MIDI system.

A. ligni DSM 25248<sup>T</sup> and A. borealis DSM 23886<sup>T</sup>. All these strains were Gram-strain-negative, capsule forming, nonspore-forming, and non-motile rods. Additionally, they were strictly aerobic, acidophilic, and mesophilic; positive for catalase activity; and containing  $C_{15:0}$  iso and iso- $C_{17:1}$  $\omega$ 9c/10-methyl-hexadecanoic acid as the major fatty acids. However, as shown in Tables 1 and 2, there are some biochemical, biophysical, and chemotaxonomic differences distinguishing SK-11<sup>T</sup> from *A. ligni* DSM 25248<sup>T</sup> and *A. borealis* DSM 23886<sup>T</sup>. Moreover, as mentioned above, the 16S rRNA gene sequence similarity of strain SK-11<sup>T</sup> with the other members of the phylum *Acidobacteria* was lower than 97 %, which is the generally accepted threshold value for species delineation proposed by Stackebrandt and Goebel [15]; this suggests that strain SK-11<sup>T</sup> is a representative of a novel species within the phylum *Acidobacteria*. Furthermore, as described above, the DNA–DNA hybridization experiments indicated low relatedness between SK-11<sup>T</sup> and the two type strains of the genus *Acidicapsa*; the relatedness values were much lower than the threshold value of 70 % for species delineation proposed by Wayne *et al.* [16] and acknowledged as the standard by Stackebrandt *et al.* [17].

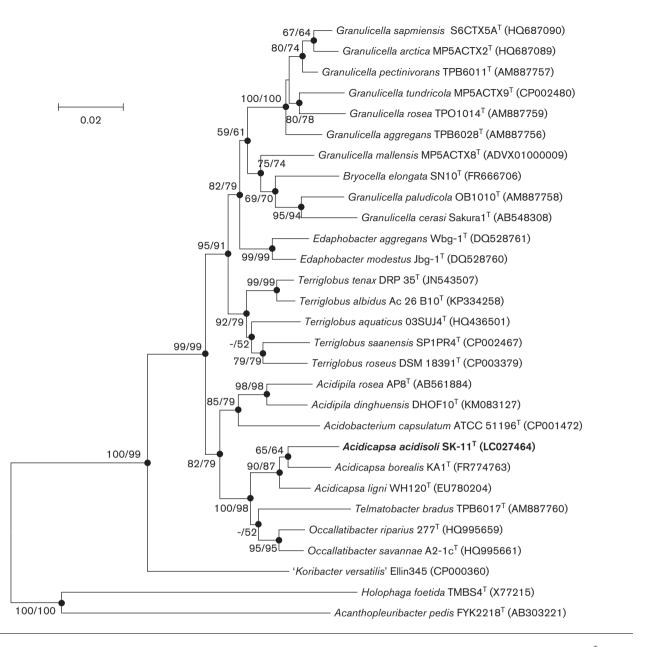
Strain SK-11<sup>T</sup> has many characteristics typical of previously described species from *Acidobacteria* subdivision 1. For example, strain SK-11<sup>T</sup> is aerobic, acidophilic, heterotrophic, and unable to use chitin and CMC as a carbon source [18]. Interestingly, strain SK-11<sup>T</sup> can utilize D-fucose, which is minor in plants and in soils, as a carbon source, whereas the majority of subdivision 1 [16] species, including *A. ligni* WH120<sup>T</sup> and *A. borealis* KA1<sup>T</sup>, cannot use this sugar [18, 19]. Strain SK-11<sup>T</sup> prefers acidic culture conditions (pH 4.0–5.5) and is capable of using plant-derived polymers (inulin, pectin, starch, and xylan) as a carbon source, which suggests that SK-11<sup>T</sup> plays a role in decomposing plant materials in its acidic-soil habitat in the deciduous forest of the Shirakami Mountains.

In conclusion, on the basis of the evidence presented here, strain SK-11<sup>T</sup> is distinct from *A. ligni* WH120<sup>T</sup> and *A. borealis* KA1<sup>T</sup> and is a novel species of the genus *Acidicapsa*, for which the name *Acidicapsa acidisoli* sp. nov. represented by strain SK-11<sup>T</sup> is proposed.

# DESCRIPTION OF ACIDICAPSA ACIDISOLI SP. NOV.

Acidicapsa acidisoli (a.ci.di.so'li. L. adj. acidus, acidic; L. n. solum, soil; N.L. gen. n. acidisoli, of acidic soil).

Cells are Gram-staining-negative rods, aerobic, 0.7-1.0 µm in width and 1.0-1.4 µm in length, non-motile, occurring singly, in pairs, or occasionally in chains. Colonies on MIS medium after 10 days of incubation at 30 °C are white, 0.4-2.8 mm in diameter, convex, smooth and circular with entire margins. Growth occurs at 10-35 °C (optimum 25-30°C), pH 4.0-5.5 (optimum pH 5.0) and 0-0.4% NaCl (optimum 0-0.1%). Growth occurs on D-arabinose (weak), L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-fucose (weak), L-fucose, D-cellobiose, D-lactose, D-maltose, D-melibiose, D-sucrose, D-trehalose, D-turanose, D-melezitose (weak), D-raffinose, Dgalacturonate, arbutin, D-salicin, inulin (weak), soluble starch, xylan, pectin, yeast extract, peptone (casein derived), casamino acid. Growth does not occur on D-ribose, D-tagatose, myo-inositol, D-mannitol, D-ribitol, D-xylitol, L-sorbose, D-sorbitol, aesculin, CMC, polygalacturonate, lignin



**Fig. 1.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationship of strain SK-11<sup>T</sup> with related representatives of the family *Acidobacteriaceae* within subdivision 1 of the phylum *Acidobacteria*. The sequences of *Holophaga foetida* TMBS4<sup>T</sup> and *Acanthopleuribacter pedis* FYK2218<sup>T</sup> from subdivision 8 of the phylum *Acidobacteria* were used as the outgroups. The two values at each node represent the percent (>50 %) of NJ/ML bootstrap from 1000 resamplings. The filled circles at the nodes indicate the generic branches obtained by both the neighbour-joining and maximum likelihood methods. Bar, 0.02 substitutions per site.

and chitin (colloidal). Catalase- and oxidase-positive. Negative for nitrate reduction, H<sub>2</sub>S production, gelatin liquefaction, and indole production. In the API ZYM system, positive for the activities of alkaline phosphatase, C4 esterase, C8 esterase lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase,  $\alpha$ -galactosidase (weak),  $\beta$ -galactosidase,  $\beta$ -glucuronidase,  $\beta$ -glucosidase. N-acetyl- $\beta$ -glucosaminidase, and  $\alpha$ -fucosidase; negative for the activities of C14 lipase,  $\alpha$ -chymotrypsin,  $\alpha$ -gulcosidase and  $\alpha$ -mannosidase. Phosphoethanolamine (PE), phosphatidylglycerol (PG), two unidentified phospholipids, and an unidentified polar lipid are present as polar lipids. Resistant to ampicillin, chloramphenicol, gentamycin, kanamycin, and streptomycin, whereas sensitive to novobiocin. The major cellular fatty acids are iso-C<sub>15:0</sub>, iso-C<sub>17:1</sub> $\omega$ 9c/10methyl-hexadecanoic acid, and iso-C<sub>17:0</sub>. The major menaquinone is MK-8. The type strain, SK-11<sup>T</sup> (=DSM 100508<sup>T</sup>=NBRC 111227<sup>T</sup>), was isolated from acidic soil of a deciduous forest in the Shirakami Mountains in Japan. The genomic DNA G+C content of type strain is 56.9 mol%.

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# Conflicts of interest

The authors declare that there are no conflicts of interest.

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