Chitinophaga taiwanensis sp. nov., isolated from the rhizosphere of Arabidopsis thaliana

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An aerobic, Gram-stain-negative, rod-shaped bacterium (strain CC-ALB-1T) was isolated from the rhizosphere of Arabidopsis thaliana. Strain CC-ALB-1T was able to grow at 20–30 °C, pH 5.0–8.0 and with up to 1.0 % (w/v) NaCl. 16S rRNA gene sequence analysis showed that strain CC-ALB-1T had the highest sequence similarity to Chitinophaga ginsengisegetis Gsoil 040T (96.9 %) and Chitinophaga niastensis JS16-4T (96.7 %); lower levels of similarity (<97.0 %) were observed to strains of all other species of the genus Chitinophaga. The fatty acid profile consisted of iso-C₁₅:₀, iso-C₁₇:₀ 3-OH, C₁₅:₁ω5c, C₁₆:₁ω5c and summed feature 3 (C₁₆:₁ω7c or C₁₆:₁ω6c). The polar lipid profile contained phosphatidylethanolamine, two unidentified aminolipids and five unidentified lipids. The predominant quinone system was menaquinone 7 (MK-7). The DNA G+C content was 53.4 ± 0.4 mol%. Based on its phylogenetic, phenotypic and chemotaxonomic features, strain CC-ALB-1T is proposed to represent a novel species within the genus Chitinophaga, for which the name Chitinophaga taiwanensis sp. nov. is proposed. The type strain is CC-ALB-1T (=BCRC 80570T=JCM 18895T).

The genus Chitinophaga, the type genus of the family Chitinophagaceae (Kämpfer et al., 2011), was described by Sangkhobol & Skerman (1981). Members of the genus Chitinophaga have Gram-staining-negative, non-motile, non-spore-forming, rod-shaped cells and are oxidase-variable. Kämpfer et al. (2006) described Chitinophaga skermanii and reclassified [Flexibacter] sancti, [Flexibacter] filiformis, [Flexibacter] japonensis and [Cytophaga] arvensicola as members of the genus Chitinophaga. At the time of writing, the genus Chitinophaga includes 15 species with validly published names (http://www.bacterio.net/c/chitinophaga.html).

While investigating the bacterial diversity that inhabits roots of Arabidopsis thaliana, strain CC-ALB-1T was isolated from the rhizosphere. Plants were grown in pots in the laboratory. Briefly, rhizosphere soil (1 mm on the roots) was collected and soil samples (10 g) were added to physiological saline (0.85 % NaCl) and shaken at 25 °C for 2 h. This sample was subsequently serially diluted (10-fold dilutions), spread (100 μl per plate) on nutrient agar (10-fold diluted NA; HiMedia), coated with different concentrations of dichlorodiphenyltrichloroethane (DDT) (50, 100, 150 and 200 mg l⁻¹ in acetone) and incubated in darkness for 1 week. Yellow colonies that appeared were picked, purified and subcultured on NA. Strain CC-ALB-1T was preserved as a glycerol suspension (30 %, v/v) at −80 °C for further characterization. For taxonomic purposes, reference strains Chitinophaga ginsengisegetis KCTC 12654T and Chitinophaga niastensis JCM 15441T were purchased from the respective culture collection centres. For direct comparative analysis, all strains were grown on NA at 30 °C for 2 days, unless specified otherwise.

Colony morphology and the presence of flagella were investigated after growth on NA for 72 h. Cell morphology was studied by transmission electron microscopy (JEM-1400; JEOL) after staining with 0.2 % uranyl acetate as well as by light microscopy (model A3000; Zeiss). Gram-staining was performed as described by Murray et al. (1994). Growth was tested using nutrient broth (NB; HiMedia) at 20–50 °C (in 5 °C increments) and pH 5–10 (in 1 pH unit increments). Salt tolerance was determined by cultivating the organism in NB supplemented with NaCl at final concentrations of 0–5 % (in 1 % increments). The presence of flexirubin-type pigments was investigated as described by Bernardet et al. (2002). Catalase activity was determined by assessing bubble production by cells in 3 % (v/v) H₂O₂ and oxidase activity was determined by using...

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CC-ALB-1T is KC479802.

Two supplementary figures and a supplementary table are available with the online version of this paper.
1\% (w/v) N,N,N',N'-tetramethyl 1,4-phenylenediamine (bioMérieux). DNase was tested for by using DNase test agar (HiMedia). Hydrolysis of chitin (1\%, w/v) was also tested by adding the substrate to NA and incubating the strain for 2 weeks. The carbon source utilization pattern was determined by using the GN2 MicroPlate (Biolog). Nitrate reduction, indole production, activities of \(\beta\)-galactosidase and urease, hydrolysis of asesculin and gelatin and assimilation of 12 substrates were tested with API 20 NE strips (bioMérieux) and activities of various enzymes were determined by using the API ZYM system (bioMérieux).

Strain CC-ALB-1\(^T\) stained Gram-negative and cells were short rods, 1.2–1.4 \(\mu\)m long and 0.6–0.8 \(\mu\)m in diameter (Fig. 1). Colonies were circular, smooth and deep yellow after 2 days of incubation on NA and R2A agar. In NB, strain CC-ALB-1\(^T\) was able to grow at 20–30 \(^\circ\)C, pH 5.0–8.0 and 0–1.0 \% (w/v) NaCl. Strain CC-ALB-1\(^T\) showed a positive reaction for flexirubin-type pigments. Strain CC-ALB-1\(^T\) was able to utilize adonitol, D-gluconic acid, D-glucosaminic acid, \(\alpha\)-ketovaleric acid, DL-lactic acid, L-alaninamide, L-alanine, L-alanyl glycine, glycy1 L-glutamic acid, L-ornithine and uridine as carbon sources, to reduce nitrate and to assimilate potassium gluconate and to show activities of \(\beta\)-galactosidase, \(\beta\)-glucuronidase and \(\alpha\)-mannosidase. These features were not observed in the reference strains \(C.\) ginsengiseggetis KCTC 12654\(^T\) and \(C.\) niastensis JCM 15441\(^T\). A comparison of phenotypic properties between strain CC-ALB-1\(^T\) and the reference type strains is given in Table 1. The detail phenotypic characteristics of strain CC-ALB-1\(^T\) are given in the species description.

Bacterial genomic DNA was isolated by using the UltraClean microbial genomic DNA isolation kit (MO BIO) following the manufacturer’s instructions. The extracted DNA was used as a template to amplify the 16S rRNA gene. The PCR was performed with bacterial universal primers 1F (5\' - GAGTTTGATCATGCTGAGA-3\') and 9R (5\' - AAGGAAGTGGATCCACCCGCA-3\'). Primers 3F (5\' - CCTACGGGAGGCAGCGAG-3\'), 5F (5\' - AAATCCAAATGAAATTTGACG-3\') and 4R (5\' - TTACCGCGGGCTGTGCGAC-3\') were used for sequencing (Edwards et al., 1989). Gene sequencing was performed by using the BigDye terminator kit (Heiner et al., 1998), and the nucleotide sequence of the PCR products was determined by using an automatic DNA sequencer (ABI PRISM 310; Applied Biosystems) (Watts & MacBeath, 2001). DNA sequences were then assembled using the Fragment Assembly System program from the Wisconsin package (GCG, 1995). For identification, the almost-complete 16S rRNA gene sequence (1446 bp) of strain CC-ALB-1\(^T\) was uploaded to the EzBioCloud server (EzTaxon-e database; Kim et al., 2012) and the NCBI server for BLAST searches. Subsequently, closely related 16S rRNA gene sequences were retrieved from the EzTaxon-e or GenBank databases and aligned by using the CLUSTAL_X program version 1.83 (Thompson et al., 1997). Phylogenetic analysis was performed with MEGA 5 software (Tamura et al., 2011) and the topology of the resultant neighbour-joining, maximum-likelihood and maximum-parsimony trees was evaluated by bootstrap analyses (Felsenstein, 1985) after 1000 replications.

Comparison of the 16S rRNA gene sequence of strain CC-ALB-1\(^T\) revealed the highest similarity to the sequences of \(C.\) ginsengiseggetis Gsoil 040\(^T\) (96.9 \%) and \(C.\) niastensis JS16-4\(^T\) (96.7 \%); other strains showed lower levels of similarity (<97.0 \%) to strain CC-ALB-1\(^T\). These similarity values suggested that strain CC-ALB-1\(^T\) could be considered to represent a novel species, since sequence divergence values \(\geq 3\%\) are thought to provide strong evidence that the organisms are not related at the species level (Stackebrandt & Goebel, 1994). Phylogenetic trees were reconstructed by using 16S rRNA gene sequences with the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods; only the neighbour-joining tree is shown, with confirmed branching points indicated in Fig. 2. Based on different evolutionary comparisons, strain CC-ALB-1\(^T\) falls within the cluster of the genus Chitinophaga.

For analysis of DNA G+C content, DNA samples were prepared and degraded enzymically into nucleosides as

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**Fig. 1.** Cell morphology of strain CC-ALB-1\(^T\) after growth on NA at 30 \(^\circ\)C for 2 days, observed by transmission electron microscopy. Bars, 2 \(\mu\)m (top) and 0.5 \(\mu\)m (bottom).
Table 1. Characteristics that differentiate strain CC-ALB-1T from type strains of closely related species

Strains: 1, CC-ALB-1T; 2, C. ginsengisgetis KCTC 12654T; 3, C. niastensis JCM 15441T. Data were obtained in this study unless indicated. All three strains grow at pH 5.0–8.0. Strain CC-ALB-1T and C. niastensis JCM 15441T were able to utilize N-acetyl-D-glucosamine, D-fructose, L-fucose, D-galactose, gentiobiose, x-D-glucose, lactose, lactulose, maltose, D-mannose, melibiose, methyl β-D-glucoside, raffinose, L-rhamnose, sucrose, trehalose, turanose, pyruvic acid methyl ester, succinic acid monomethyl ester, D-gluconic acid, L-asparagine, L-aspartic acid, L-glutamic acid, L-proline, L-serine, DL-2-glycerol phosphate and 2-D-glucose 1-phosphate as carbon sources and they assimilated β-galactosidase, D-glucose, L-arabinose, D-mannose, N-acetylglucosamine and maltose; these features were not observed in C. niastensis JCM 15441T. All three strains were positive for activities of alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, z-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and Nα-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase, and negative for urease, arginine dihydrolase, lipase (C14) and indole production. +, Positive; −, negative; w, weakly positive.

Table 1. cont.

<table>
<thead>
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<th>Characteristic</th>
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<tr>
<td>Maltose</td>
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<td>−</td>
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<tr>
<td>N-Acetylglucosamine</td>
<td>+</td>
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<tr>
<td>Potassium gluconate</td>
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<td>+</td>
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<td>DNA G+C content (mol%)</td>
<td>53.4 ± 0.4</td>
<td>47.1†</td>
<td>47.0b</td>
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*Weak growth observed at 1% (w/v) NaCl.
†Data from: a, Lee et al. (2007); b, Weon et al. (2009).

described by Mesbah et al. (1989). The nucleoside mixture obtained was then separated and analysed by HPLC [Hitachi L-2130 chromatograph equipped with a Hitachi L-2200 autosampler, Hitachi L-2455 diode array detector and reversed-phase C18 column (Phenomenex Synergi 4u Fusion-RP80, 250 × 4.60 mm)]. Polyamines were extracted as described by Scherer & Kneifel (1983) and analysed by HPLC. The dansyl derivatives were separated by using a Hitachi L-2130 chromatograph equipped with a Hitachi L-2200 autosampler, Hitachi L-2485 fluorescence detector (excitation at 360 nm and emission at 520 nm) and a reversed-phase C18 column (as above). Polar lipids were extracted and analysed by two-dimensional TLC; isoprenoid quinones were purified by the methods of Minnikin et al. (1984) and analysed by HPLC as described by Collins (1985). For the extraction of fatty acid methyl esters (FAMEs), strain CC-ALB-1T and the reference type strains were cultured simultaneously on NA for 48 h at 30 °C (the strains exhibited similar growth rates). Harvested biomass was subjected to saponification, methylation and extraction (Miller, 1982). FAMEs were prepared, separated and identified according to the standard protocol (Paisley, 1996) of the Microbial Identification System (MIDI) (Sasser, 1990) by gas chromatography (Agilent 7890A) fitted with a flame-ionization detector. Identification was achieved and comparisons were made by using the Aerobe (RTSBA6) database of the MIDI System (Sherlock version 6.0).

The DNA G+C content of strain CC-ALB-1T was 53.4 ± 0.4 mol%. The predominant quinone system was menaquinone MK-7. The polar lipid profile of strain CC-ALB-1T was similar to those of C. ginsengisgetis KCTC 12654T and C. niastensis JCM 15441T, with phosphatidylethanolamine, two unidentified aminolipids and five unidentified lipids as major components (Fig. S1, available in IJSEM Online). The polyamine pattern of strain CC-ALB-1T showed sym-homospermidine as the major polyamine, which is similar to recognized species of the genus Chitinophaga (Fig. S2). The major fatty acids in strain CC-ALB-1T were iso-C₁₅:₀, iso-C₁₇:₀ 3-OH, C₁₅:₀ 3-OH, C₁₆:₀ 10:0 and summed feature 3 (C₁₆:₁ 9c and/or C₁₁:₀ 10:0c) (Table S1). The fatty acid profile of strain CC-ALB-1T was similar to those of recognized species of the genus Chitinophaga. Based on the distinct phylogenetic, phenotypic, biochemical and chemotaxonomic properties observed, strain CC-ALB-1T represents a novel species of
the genus Chitinophaga, for which the name Chitinophaga taiwanensis sp. nov. is proposed.

Description of Chitinophaga taiwanensis sp. nov.

Chitinophaga taiwanensis (Tai.wa.nen’sis. N.L. fem. adj. taiwanensis of or pertaining to Taiwan, where the type strain was isolated).

Cells are Gram-stain-negative rods, 1.2–1.4 μm long and 0.6–0.8 μm in diameter. Colonies are circular, smooth and deep yellow after 2 days of incubation on NA. Grows at 20–30 °C, pH 5.0–8.0 and 0–1% (w/v) NaCl. Oxidase- and catalase-positive. Positive for flexirubin-type pigments. Positive for reduction of nitrate to nitrite but negative for further reduction to dinitrogen gas. Assimilates D-glucose, L-arabinose, D-mannose, N-acetylglycerol, maltose, and nitrilotriacetic acid, L-alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycol L-glutamic acid, L-ornithine, L-proline, L-serine, uridine, glycerol, DL-α-glycerol phosphate and α-D-glucose 1-phosphate. Alkaline phosphatase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are positive in the API ZYM system. Positive for reduction of nitrate to nitrite but negative for further reduction to dinitrogen gas. Assimilates D-glucose, L-arabinose, D-mannose, N-acetylglycerol, maltose, and potassium gluconate in the API 20NE system. The fatty acid profile consists of iso-C15:0, iso-C17:0 3-OH, C15:1 ω6c, C16:1 ω7c and summed feature 3 (C16:1 ω7c and/or C16:1 ω6c). The polar lipid profile contains phosphatidylethanolamine, two unidentified aminolipids and five unidentified lipids as major lipids. The predominant quinone is MK-7.

The type strain, CC-ALB-1T (isolated from the rhizosphere of Arabidopsis thaliana), was isolated from the rhizosphere of Arabidopsis thaliana. The DNA G+C content of the type strain is 53.4 ± 0.4 mol%.

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Reference


