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 and/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-stain-negative, non-motile, non-spor-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...
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 β-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-1T stained Gram-negative and cells were short rods, 1.2–1.4 μm long and 0.6–0.8 μm 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-1T was able to grow at 20–30 °C, pH 5.0–8.0 and 0–1.0 % (w/v) NaCl. Strain CC-ALB-1T showed a positive reaction for flexirubin-type pigments. Strain CC-ALB-1T was able to utilize adonitol, D-gluconic acid, D-glucosaminic acid, α-ketovleric acid, DL-lactic acid, L-alaninamide, L-alanine, L-alanyl glycine, glycol L-glutamic acid, L-ornithine and uridine as carbon sources, to reduce nitrate and to assimilate potassium gluconate and to show activities of β-galactosidase, β-glucuronidase and α-mannosidase. These features were not observed in the reference strains C. ginsengisegetis KCTC 12654T and C. niastensis JCM 15441T. A comparison of phenotypic properties between strain CC-ALB-1T and the reference type strains is given in Table 1. The detail phenotypic characteristics of strain CC-ALB-1T 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′-GAGTTTGATCATGCTGAGC-3′) and 9R (5′-AAGGAGTGTGCTCAACCG-3′). Primers 3F (5′-CCTACGGGAGCGACGAG-3′), 5F (5′-AAACTCAAATGAATTGAC-3′), 9F (5′-AAGGAAGTTTGATCATGGCTCA-3′) and 4R (5′-TTACCGCGGCTGCTGGCAC-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-1T 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-1T revealed the highest similarity to the sequences of C. ginsengisegetis Gsoil 040T (96.9 %) and C. niastensis JS16-4T (96.7 %); other strains showed lower levels of similarity (<97.0 %) to strain CC-ALB-1T. These similarity values suggested that strain CC-ALB-1T could be considered to represent a novel species, since sequence divergence values ≥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-1T 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
Table 1. Characteristics that differentiate strain CC-ALB-1<sup>T</sup> from type strains of closely related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
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<tbody>
<tr>
<td><strong>Ranges for growth</strong></td>
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<tr>
<td>NaCl concentration (%, w/v)</td>
<td>0–1</td>
<td>0–1</td>
<td>0–1&lt;sup&gt;*&lt;/sup&gt;</td>
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<tr>
<td>Temperature (°C)</td>
<td>20–30</td>
<td>20–40</td>
<td>20–30</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>±</td>
<td>–</td>
</tr>
<tr>
<td>Protease (gelatin hydrolysis)</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Reduction of nitrate to nitrite</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Reduction of nitrate to nitrogen</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Carbon source utilization</strong></td>
<td></td>
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<tr>
<td>Dextrin</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Adonitol</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Cellobiose</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>D-Gluconic acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Glucosaminic acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2-Ketovaleric acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>DL-Lactic acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>L-Alaninamide</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>L-Alanine</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>L-Alanyl glycine</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>L-Ornithine</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Uridine</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Glycerol</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td><strong>Enzyme activities</strong></td>
<td></td>
<td></td>
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<tr>
<td>Esterase lipase (C8)</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>2-Galactosidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>–</td>
<td>±</td>
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<tr>
<td>β-Glucuronidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>2-Glucosidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>2-Mannosidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2-Fucosidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td><strong>Assimilation of (API 20NE):</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>d-Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>l-Arabinose</td>
<td>+</td>
<td>+</td>
<td>–</td>
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Table 1. cont.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Potassium glutonate</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>53.4 ± 0.4</td>
<td>47.1&lt;sup&gt;†&lt;/sup&gt;</td>
<td>47.0&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>†</sup>Weak growth observed at 1 % (w/v) NaCl.

The DNA G+C content of strain CC-ALB-1<sup>T</sup> was 53.4 ± 0.4 mol%. The predominant quinone system was menaquinone MK-7. The polar lipid profile of strain CC-ALB-1<sup>T</sup> was similar to those of C. ginsengisgetis KCTC 12654<sup>T</sup> and C. niastensis JCM 15441<sup>T</sup>, with phosphatidyglycocolamine, two unidentified aminolipids and five unidentified lipids as major components (Fig. S1, available in IJSEM Online). The polyamine pattern of strain CC-ALB-1<sup>T</sup> 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-1<sup>T</sup> were isoc<sub>14:0</sub>-iso, iso<sub>17:0</sub>-3-OH, C<sub>15:1</sub>~<sub>trans</sub>, C<sub>16:0</sub>, C<sub>16:1</sub>~<sub>7c</sub> and summed feature 3 (C<sub>16:1</sub>~<sub>7c</sub>) (Table S1). The fatty acid profile of strain CC-ALB-1<sup>T</sup> 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-1<sup>T</sup> represents a novel species of C. ginsengisgetis, which is similar to recognized species of the genus Chitinophaga.

**References:**
- S. Y. Lin and others.
- Mesbah et al. (1989).
- Collins et al. (1985).
- Miller, 1982.
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. Hydrolysis of chitin is not observed when 1 % chitin is added to NA. The following carbon sources are utilized in the Biolog GN2 system: dextrin, N-acetyl-D-glucosamine, adonitol, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, D-glucose, L-arabinose, D-mannose, N-acetyl-D-glucosamine, maltose and potassium gluconate in the API 20NE system. Assimilates maltose and potassium gluconate in the API 20NE system. Assimilates L-aspartic acid, L-glutamic acid, glycyl L-glutamic acid, L-alanine, L-alanyl glycine, L-asparagine, L-melibiose, methyl D-glucoside, raffinose, L-rhamnose, D-glucuronic acid, D-glucosamine, D-gluconic acid, D-glucosidase, D-galactosidase, D-glucuronidase, β-glucosidase, N-acetyl-β-D-glucosaminidase, α-mannosidase and D-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-acetylglucosamine, maltose and potassium gluconate in the API 20NE system. The fatty acid profile consists of iso-C15:0, C16:1ω7c, C16:1ω8c and summed feature 3 (C16:1ω6t and/or C16:1ω5t). 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 (KC479802), was isolated from the rhizosphere of Arabidopsis thaliana. The DNA G+C content of the type strain is 53.4 ± 0.4 mol%.

**Acknowledgements**

The authors would like to thank Mrs Mariyam Shahina for technical assistance. This research work was kindly supported by grants from the National Science Council, the Council of Agriculture, Executive Yuan, and in part by the Ministry of Education, Taiwan, ROC, under the ATU plan.

**Reference**


**Fig. 2.** Phylogenetic analysis of strain CC-ALB-1T based on 16S rRNA gene sequences. Distances were determined and clustering was performed by using the neighbour-joining method with MEGA software version 5. Filled circles indicate that the corresponding nodes were also recovered in trees reconstructed based on the maximum-likelihood and maximum-parsimony algorithms. Bootstrap values (>50 %) based on 1000 replications are listed as percentages at branching points. Bar, 0.05 substitutions per nucleotide position.


