Chryseobacterium rigui sp. nov., isolated from an estuarine wetland

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A bacterium, designated strain CJ16T, was isolated from the estuarine wetland of the Han River. Cells of the isolate were yellow-pigmented, Gram-stain-negative, non-motile and rod-shaped. Growth of strain CJ16T was observed in TSB at 5–37 °C (optimum 30 °C), at pH 5.0–9.0 (optimum pH 6.0) and with 0–3 % (w/v) NaCl (optimum 0 %). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain CJ16T was most closely related to Chryseobacterium hagamense KCTC 22545T (97.2 % 16S rRNA gene sequence similarity). Genomic relatedness based on DNA–DNA hybridization between strain CJ16T and C. hagamense KCTC 22545T was 23 % (strain CJ16T as probe) and 19 % (strain KCTC 22545T as probe). Chemotaxonomic analysis revealed that strain CJ16T possessed MK-6 as the major isoprenoid quinone and sym-homospermidine as the predominant polyamine. The predominant fatty acids were iso-C₁₅ : 0 (26.9 %), iso-C₁₇ : 0 3-OH (16.8 %) and summed feature 9 (comprising C₁₆ : 0 10-methyl and/or iso-C₁₇ : 1ω₉c; 10.5 %). The DNA G+C content of strain CJ16T was 37.9 mol%. Based on phenotypic, genotypic and phylogenetic studies, strain CJ16T represents a novel species of the genus Chryseobacterium, for which the name Chryseobacterium rigui sp. nov. is proposed. The type strain is CJ16T (=KACC 16560T =JCM 18078T).

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CJ16T is JQ071497.

A supplementary figure is available with the online version of this paper.

taenense KACC 15162T and C. taichungense KACC 11658T, which are the isolate’s closest phylogenetic relatives.

Strain CJ16T was isolated from the estuarine wetland of Han River in Korea by using a standard dilution-plating technique on tryptic soy agar (TSA; BD Difco) at 30 °C for 2 days under aerobic conditions. The isolate was sub-cultured several times to obtain a pure culture. The 16S rRNA gene was amplified from a single colony using AccuPower PCR Premix (Bioneer) and the universal bacterial primer pair pBact27F and pUniv1492R (Lane, 1991). The amplification products were purified using a gel extraction kit (Qiagen) and cloned into the T&K cloning vector (RBC Bioscience). The 16S rRNA gene sequence analysis was carried out using an automated DNA analyser (PRISM 3730XL DNA Analyzer; Applied Biosystems) according to the manufacturer’s instructions. The 16S rRNA gene sequence of strain CJ16T was compared with other sequences in the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012) using the pairwise alignment method. The 16S rRNA gene sequences of strain CJ16T and other closely related species were manually aligned using jPHYDIT software (http://plaza.snu.ac.kr/~jchun/jphydit/index.php; Jeon et al., 2005). The phylogenetic trees were inferred according to the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. Evolutionary distances were computed by the model of Jukes & Cantor (1969). Tree
Chryseobacterium rigui sp. nov.

Fig. 1. Neighbour-joining phylogenetic tree, based on almost-complete 16S rRNA gene sequences, showing the relationships between strain CJ16T and representative members of the genus Chryseobacterium. Bootstrap values (>50%) based on 1000 resampled datasets are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered with the maximum-likelihood algorithm. Elizabethkingia meningoseptica ATCC 13253T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

A nearly complete 16S rRNA gene sequence (1439 bp) of strain CJ16T was obtained. The phylogenetic analysis revealed that strain CJ16T belonged to the genus Chryseobacterium. Strain CJ16T displayed the highest 16S rRNA gene sequence similarity with C. hagamense KCTC 22545T (97.2%), followed by C. hominis KACC 14168T (96.9%), C. piscicola DSM 21068T (96.6%), C. taeanense KACC 15162T (96.6%) and other recognized species of the genus Chryseobacterium (<96.6%). Strain CJ16T formed a monophyletic clade with other type strains of members of the genus Chryseobacterium in the neighbour-joining tree (Fig. 1), which was also recovered in the maximum-likelihood tree. Furthermore, strain CJ16T and C. hagamense KCTC 22545T shared only 23% DNA–DNA relatedness (strain CJ16T as probe; reciprocal 19%), which is below the 70% cut off recommended for the delineation of genomic species (Wayne et al., 1987). It is evident that strain CJ16T represents a novel species of the genus Chryseobacterium.

Growth temperature and pH were determined in tryptic soy broth (TSB; BD Difco) at 5, 20, 30, 37 and 40 °C and at pH 4–10 (at 1 pH value intervals). Growth with 0–10% (w/v) NaCl (at 1% intervals) was examined in TSB. Anaerobic growth was investigated in an anaerobic chamber on TSA using a BD Gaspack pouch (Becton Dickinson) at 30 °C for 10 days. Growth was tested on various media at 30 °C: TSA, marine agar 2216 (BD Difco), nutrient agar (NA; Conda), R2A agar (BD Difco) and MacConkey agar (BD Difco). Cell morphology was observed by light microscopy (BX40; Olympus) using cells.
Strains: 1, *Chryseobacterium rigui* sp. nov. CJ16<sup>T</sup>; 2, *C. hagamense* KCTC 22545<sup>T</sup>; 3, *C. hominis* KACC 14168<sup>T</sup>; 4, *C. taeanense* KACC 15162<sup>T</sup>; 5, *C. taichungense* KACC 11658<sup>T</sup>. Data were taken from this study unless otherwise indicated. ry, Pale yellow; y, yellow; +, positive; w, weakly positive; −, negative.

Table 1. Differential characteristics of strain CJ16<sup>T</sup> and type strains of closely related species of the genus *Chryseobacterium*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>Colony pigmentation on TSA</td>
<td>Y</td>
<td>Y</td>
<td>PY</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>Growth at 5 °C</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Flexirubin-type pigments</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td>Hydrolysis of:</td>
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<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>−*</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Tween 80</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td>Acid production from (API 50 CH)</td>
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<tr>
<td>D-Arabinose</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
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<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>D-Xylose</td>
<td>+</td>
<td>w†</td>
<td>+</td>
<td>−</td>
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<td>+</td>
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<td>Sucrose</td>
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<td>−</td>
<td>+</td>
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<td>Enzyme activities (API ZYM)</td>
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<td>Cystine arylamidase</td>
<td>w</td>
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<td>w</td>
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<td>β-Galactosidase</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>β-Glucosidase</td>
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<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>w</td>
<td>−</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>37.8</td>
<td>36.9&lt;sup&gt;II&lt;/sup&gt;</td>
<td>36.5&lt;sup&gt;§&lt;/sup&gt;</td>
<td>32.1&lt;sup&gt;II&lt;/sup&gt;</td>
<td>36.4&lt;sup&gt;§&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Opposite result to that reported by Vaneechoutte et al. (2007).*
†Opposite result to that reported by Cho et al. (2010).
§Opposite result to that reported by Park et al. (2006).
Opposite result to that reported by Shen et al. (2005).
II|Data reported by: a, Cho et al. (2010); b, Vaneechoutte et al. (2007); c, Park et al. (2006).

Genomic DNA for the determination of G+C content was extracted and purified using a Wizard Genomic DNA purification kit (Promega). DNA G+C content was determined by HPLC (Varian) with a reversed-phase column (Capcell Pak C18 UG 120; SHISHEIDO) according to the method of Mesbah et al. (1989). The G+C content of genomic DNA of strain CJ16<sup>T</sup> was 37.9 mol%.

Chemo taxonomic features were obtained using cells grown on TSA (fatty acid analysis) or TSB at 30 °C for 2 days. Isoprenoid quinones were extracted as described by Minnikin et al. (1984) and determined by HPLC according to the method of Collins (1985). Polyamines were extracted and analysed as described by Busse & Auling (1988) and Busse et al. (1997). Polar lipids were extracted from freeze-dried cells and determined by 2D silica-gel TLC as described by Tindall (1990a, b). The fatty acid composition was analysed by GLC using the methods described in the instructions of the Microbial Identification System version 6.1 and the RTSBA6 6.10 database (Microbial ID). Strain CJ16<sup>T</sup> was shown to possess MK-6 as the major isoprenoid quinone and sym-homospermidine as the predominant polyamine; these findings correspond with all other members of the genus *Chryseobacterium* (Bernardet et al., 2002, 2011). Phosphatidylethanolamine was shown as the predominant polar lipid and several unknown polar lipids, including unidentified aminolipids, were also detected. The polar lipid pattern of strain CJ16<sup>T</sup> was highly similar to those of the other related type strains (Fig. S1, available in IJSEM Online). The predominant fatty acids of strain CJ16<sup>T</sup> were iso-C<sub>15</sub>:0 (26.9 %), iso-C<sub>17</sub>:0 3-OH (16.9 %) and summed feature 9 (comprising C<sub>16</sub>:0 10-methyl and/or iso-C<sub>17</sub>:1ω9<sub>C</sub> 10.5 %), which is consistent with the reference strains (Table 2).

On the basis of phylogenetic, physiological and chemotaxonomic characteristics, strain CJ16<sup>T</sup> is considered to represent a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium rigui* sp. nov. is proposed.

Description of *Chryseobacterium rigui* sp. nov.

*Chryseobacterium rigui* (ri’gu.i. L. gen. n. rigui of a well-watered place).
Cells are Gram-stain-negative, strictly aerobic, non-spore-forming and non-motile rods (about 2.0 mm long and 0.6–0.7 mm wide). Colonies are circular, smooth and yellow on TSA. Growth occurs at 5–37 °C (optimum 30 °C), at pH 5.0–9.0 (optimum pH 6.0) and with 0–3 % NaCl (optimum 0 %). Abundant growth occurs on TSA and NA, but not on MacConkey agar. Flexirubin pigments are produced on TSA. Oxidase- and catalase-positive. DNA, arbutin, aesculin, casein, gelatin, Tween 80 and starch are hydrolysed. Crystalline cellulose (filter paper) is not degraded. Cystine arylamidase (weakly), leucine arylamidase, valine arylamidase, acid phosphatase, N-acetyl-β-glucosaminidase (weakly), naphthol-AS-BI-phosphohydrolase and α-glucosidase, but negative for lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, urease, lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase. With GN2 MicroPlates, oxidizes α-cyclodextrin, dextrin, glyogen, Tween 40, L-arabinose, D-cellobiose, D-fructose, gentiobiose, α-D-glucose, maltose, D-mannose, methyl β-D-glucoside, D-psicose, succrose, succinic acid monomethyl ester, acetic acid, γ-hydroxybutyric acid, α-ketobutyric acid, α-ketovaleric acid, propionic acid, succinic acid, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycol L-aspartic acid, glycyll L-glutamic acid, L-phenylalanine, L-proline, L-serine, L-threonine (weakly), inosine, uridine, thymidine, 2,3-butanediol (weakly), α-D-glucose 1-phosphate (weakly) and D-glucose 6-phosphate; all other substrates are not oxidized. Acid is produced from D-xylene, D-glucose, D-fructose, D-mannose, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, succrose, starch, glycolgen and gentiobiose. The polar lipids consist of phosphatidylethanolamine, three unidentified aminolipids and three unknown polar lipids. Contains menaquinone MK-6 as the major isoprenoid quinone and sym-homospermidine as the predominant polyamine. The predominant fatty acids are iso-C15:0, iso-C17:0 3-OH and summed feature 9 (comprising C16:0 10-methyl and/or iso-C17:1 ω9c).

The type strain is CJ16T (=KACC 16560T =JCM 18078T), isolated from the sediment of estuarine wetland of Han River, South Korea. The DNA G+C content of the type strain is 37.9 mol%.

**Acknowledgements**

We thank Messrs. S. Choi and J. Kim for their help at the Center for Research Facilities, Chung-Ang University. This study was supported by a project of the National Institute of Biological Resources (NIBR) to survey Korean indigenous species. This work was also funded by the Technology Development Program for Agriculture and Forestry (TDPAF) of the Ministry for Agriculture, Forestry and Fisheries.

**References**


