Caulobacter flavus sp. nov., a stalked bacterium isolated from rhizosphere soil

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A Gram-stain-negative, aerobic, yellow-pigmented and rod-shaped bacterium with a single polar flagellum or a stalk, designated strain RHGG3^T, was isolated from rhizosphere soil of cultivated watermelon (*Citrullus lanatus*) collected from Hefei, China. Optimal growth of strain RHGG3^T was observed at pH 7.0 and 28–30 °C. Cells were catalase-positive and oxidase-negative. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain RHGG3^T belonged to the genus *Caulobacter* and showed the highest 16S rRNA gene sequence similarities to *Caulobacter segnis* ATCC 21756^T (98.6 %), *Caulobacter vibrioides* CB51^T (98.3 %) and *Caulobacter henricii* ATCC 15253^T (97.2 %). The G+C content of the genomic DNA was 70 mol%. Strain RHGG3^T contained Q-10 as the sole ubiquinone and the major fatty acids (>8 %) were 11-methyl $C_{18:1}\omega7c$, $C_{18:1}\omega7c$, $C_{16:0}$, $C_{15:0}$ and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH). The polar lipids were various unknown glycolipids, phosphatidylglycerol and phosphoglycolipids. DNA–DNA relatedness of strain RHGG3^T to type strains of the most closely related species (*Caulobacter segnis* ATCC 21756^T, *Caulobacter vibrioides* DSM 4738 and *Caulobacter henricii* ATCC 15253^T) was 32.4–40.9 %. Based on polyphasic taxonomy analysis (phylogenetic, unique phenotypic traits, chemotaxonomic and DNA–DNA hybridizations), strain RHGG3^T represents a novel species of the genus *Caulobacter*, for which the name *Caulobacter flavus* sp. nov. is proposed. The type strain is RHGG3^T (=CGMCC 1.15093^T=KCTC 42581^T=JCM 30763^T).

The genus *Caulobacter* belongs phylogenetically to the family *Caulobacteraceae*, which includes four genera: *Caulobacter*, *Asticcacaulis*, *Brevundimonas* and *Phenylobacterium*. The genus *Caulobacter* was first described by Henrici & Johnson (1935) with *Caulobacter vibrioides* as the type species and the genus description was subsequently emended by Bowers *et al.* (1954), Poindexter (1964) and Abraham *et al.* (1999). In recent years, there have been some changes on the taxonomic status of members of genus *Caulobacter*. For example, *Mycoplana segnis* was reclassified as *Caulobacter segnis* comb. nov. (Urakami *et al.*, 1990; Abraham *et al.*, 1999), and some species or subspecies of genus *Caulobacter* have been reclassified to the genera *Brevundimonas*, *Maricaulis* and *Sphingomonas* (Abraham *et al.*, 1999; Chen *et al.*, 2012). A psychrotolerant *Caulobacter* sp. was isolated from Russian polar tundra soil by Berestovskaya *et al.* (2006). At the time of writing, the genus *Caulobacter* comprises eight recognized species, which were isolated from a variety of environments, such as soil, water, rhizosphere soil, activated sludge system, deep freshwater sediment: *Caulobacter segnis* (Urakami *et al.*, 1990; Abraham *et al.*, 1999), *Caulobacter vibrioides* (Henrici & Johnson, 1935; Poindexter, 1964), *Caulobacter henricii* (Poindexter, 1964), *Caulobacter fusiformis* (Poindexter, 1964; Poindexter & Lewis, 1966), *Caulobacter mirabilis* (Abraham *et al.*, 2008), *Caulobacter ginsengisoli* (Liu *et al.*, 2010), *Caulobacter profundus* (Jin *et al.*, 2014) and *Caulobacter daechungensis* (Jin *et al.*, 2013). Cell division of the genus *Caulobacter* is mostly asymmetrical with two different cell morphologies: one with a stalk and another with a single polar flagellum, except *C. segnis*. Members of the genus *Caulobacter* have certain characteristics in common, such as Q-10 as the main ubiquinone, the presence of phosphoglycolipid, and the major fatty acids $C_{16:0}$, summed feature 7 ($C_{18:1}\omega7c$), 11 methyl $C_{18:1}\omega7c$ [equivalent chain-length (ECL) 18.081] and $C_{16:1}\omega7c$. The presence of significant amounts of $C_{14:0}$, $C_{12:1}$ 3-OH and ECL 11.798, and the absence of ECL 17.897 are important characteristics that differentiated members of the genus *Caulobacter* from members of the other three genera of the family *Caulobacteraceae*

Abbreviation: ECL, equivalent chain-length.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RHGG3^T is KR086403.

Two supplementary figures are available with the online Supplementary Material.

(Abraham *et al.*, 1999, 2008; Liu *et al.*, 2010; Jin *et al.*, 2013). In this study, we describe the taxonomic position of strain RHGG3^T using a polyphasic approach, and suggest that strain RHGG3^T represents a novel species of the genus *Caulobacter*.

Strain RHGG3^T was isolated from rhizosphere soil of a cultivated watermelon (*Citrullus lanatus*) in Hefei, China, with one-fifth-strength nutrient agar (1/5 NA) by using the serial dilution method. A culture of the isolated strain was purified and stored in a glycerol solution (40 %, v/v) at −70 °C. Cell morphology and the presence of stalk (or prostheca) were determined by transmission electron microscope (HT-7700; Hitachi) using exponential-phase cells negatively stained with phosphotungstic acid. The Gram reaction was determined by using the standard Gram staining method. Growth on nutrient agar (NA), Caulobacter medium (CM) agar, Luria–Bertani (LB) agar, trypticase soy agar (TSA) and R2A agar was evaluated at 30 °C after incubation for 4 days. CM agar was prepared according to the instructions from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany) (http://www.dsmz.de/micro-organisms/medium). Growth on glucose mineral salts basal (GMSB) medium [glucose 2 g, $(NH_4)_2SO_4$ 2 g, $MgSO_4 . 7H_2O$ 0.2 g, $NaH_2PO_4 . H_2O$ 0.5 g, K_2HPO_4 0.5 g, $CaCl_2 . 2H_2O$ 0.1 g, distilled water 1000 ml, pH 7.0] was evaluated to test growth factor demand. For NaCl tolerance, pH range and temperature range tests, growth was examined using Caulobacter medium after incubation for 4 days according to Huang *et al.* (2012). Nitrate reduction, indole production, D-glucose fermentation, arginine dihydrolase and urease activities, hydrolysis of gelatin and aesculin, and assimilation of 12 substrates were tested by using API 20NE strips (bioMérieux) according to the manufacturer's instructions. Acid production from 49 carbohydrates and the activities of various enzymes were determined by using API50 CH strips and API ZYM strips (bioMérieux) respectively, according to the manufacturer's instructions. Catalase activity was examined by bubble production after the application of 3 % (v/v) H_2O_2 solution to isolated colonies. Oxidase activity was examined using oxidase reagent (bioMérieux) according to the manufacturer's instructions. H_2S production, Voges–Proskauer and methyl red reactions were performed as described by Lányí (1987). H_2S production was determined by blackening of the medium during growth after stabbing inoculation. A positive Voges–Proskauer reaction was determined by the observation of a strong red colour after the addition of 5 % (w/v) α-naphthol solution. A positive methyl red reaction was determined by the observation of a bright red colour after the addition of methyl red solution. *C. segnis* DSM 7131^T (=ATCC 21756^T), *C. vibrioides* DSM 4738 and *C. henricii* DSM 4730^T (=ATCC 15253^T) were obtained from the DSMZ and used as reference strains under the same conditions for comparative taxonomic analysis.

Colonies of strain RHGG3^T on CM agar were yellow, convex and circular with a smooth surface and a diameter of 1.0–3.0 mm after incubation for 48 h at 30 °C. Strain RHGG3^T grew well on many media including NA, CM, R2A, TSA, LB agar and GMSB agar. Cells of strain RHGG3^T were Gram-stain-negative, aerobic, motile and rod-shaped. Cells were dimorphic, and able to divide by unequal binary fission to form two daughter cells: one with a stalk (non-motile) and the other with a single polar flagellum (motile) at the end opposite the stalk (Fig. S1, available in the online Supplementary Material). Strain RHGG3^T was able to grow at 10–37 °C, but not at 4 °C or 42 °C. Growth was observed at pH 5.0–10.0, with optimum growth at pH 7.0. Growth occurred with 0–2 % (w/v) NaCl, but was inhibited by the presence of >2 % (w/v) NaCl. Strain RHGG3^T was positive for catalase, but negative for oxidase, H_2S production, nitrate reduction, indole production, D-glucose fermentation and gelatin hydrolysis. The physiological and biochemical properties that differentiate strain RHGG3^T from the closest related species of the genus *Caulobacter* are shown in the Table 1.

Bacterial genomic DNA was extracted using a merion DNA bacteria kit (Qiagen). The 16S rRNA gene of strain RHGG3^T was amplified by PCR using the eubacterial universal primers 27F and 1492R. The PCR product was cloned into vector pMD18-T and sequenced. The 16S rRNA gene sequence of strain RHGG3^T was identified using the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon; Kim *et al.*, 2012), and 16S rRNA gene sequences of closely related species of the genus *Caulobacter* were obtained from the GenBank/EMBL/DDBJ databases. Multiple alignments of the sequences were made using the CLUSTAL X program (Thompson *et al.*, 1997). Evolutionary distances were calculated by using distance options with Kimura's two-parameter model (Kimura, 1980). Phylogenetic trees were reconstructed by using three different methods, neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981), within MEGA software (version 5.0) (Tamura *et al.*, 2011). Bootstrap values were determined based on 1000 replications. For the measurement of DNA G+C content, genomic DNA of strain RHGG3^T was degraded enzymically into nucleosides and the G+C content was determined as described by Mesbah *et al.* (1989) via reversed-phase HPLC (Agilent 1200). DNA–DNA hybridization was carried out using the reassociation-rate method as described by Dong *et al.* (2000) and De Ley *et al.* (1970).

An almost-complete 16S rRNA gene sequence of strain RHGG3^T (1404 bp) was obtained and subjected to comparative analysis. The phylogenetic tree reconstructed by neighbour-joining method based on 16S rRNA gene sequences indicated that strain RHGG3^T was most closely affiliated to the genus *Caulobacter*, and constituted a distinct subclade with *C. segnis* ATCC 21756^T and *C. vibrioides* CB51^T (Fig. 1). These results were consistent with those obtained using the maximum-likelihood and

Table 1. Differential phenotypic and physiological characteristics of strain RHGG3^T and type strains of closely related species of the genus *Caulobacter*

Strains: 1, RHGG3^T; 2, *C. segnis* DSM 7131^T (=ATCC 21756^T); 3, *C. vibrioides* DSM 4738; 4, *C. henricii* DSM 4730^T (=ATCC 15253^T). All data are from this study unless otherwise indicated. All strains were negative for acid production from glycerol, erythritol, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-fructose, L-sorbose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, arbutin, salicin, inulin, melezitose, raffinose, glycogen, xylitol, turanose, D-tagatose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate (API 50CH). All strains were negative for assimilation of D-mannitol, potassium gluconate, capric acid, adipic acid and phenylacetic acid (API 20NE). All strains were negative for nitrate reduction, indole production, Voges–Proskauer test, H_2S production, D-glucose fermentation, gelatin hydrolysis, and for activities of arginine dihydrolase, urease, lipase (C14), cystine arylamidase, α-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase. All strains showed positive reactions for alkaline phosphatase, leucine arylamidase, trypsin, acid phosphatase and β-glucosidase. +, Positive; −, negative; W, weakly positive.

Characteristic	1	2	3	4
Pigmentation of Colonies	Yellow	Colourless	Colourless	Yellow
Cell size (μm)	0.9–1.3 × 2.0–3.3	0.9–1.2 × 1.8–3.0	1.0–1.3 × 2.6–3.6	0.7–0.9 × 2.8–4.7
Prostheca	+	−	+	+
Motility	+	−	+	+
Oxidase	−	+	+	+
Catalase	+	+	W	+
Growth at/with:				
4 °C	−	+	+	−
1 % (w/v) NaCl	+	+	+	−
2 % (w/v) NaCl	+	+	−	−
pH range for growth	5–10	5–10	5–10	6–9
Growth on GMSB agar	+	−	−	−
Methyl red	−	+	−	−
Assimilation of:				
D-Glucose	+	+	−	−
L-Arabinose	+	+	−	−
D-Mannose	+	−	−	−
N-Acetylglucosamine	+	+	−	−
Maltose	+	−	−	−
Malic acid	+	−	−	−
Acid production from:				
D-Arabinose	W	−	−	−
L-Arabinose	+	+	W	−
D-Ribose	W	−	−	−
D-Xylose	+	+	W	−
D-Galactose	+	+	−	−
D-Glucose	+	W	W	−
D-Mannose	+	W	−	−
L-Rhamnose	+	+	−	−
N-Acetylglucosamine	W	W	−	−
Amygdalin	+	W	W	−
Cellobiose	+	W	W	−
Maltose	+	W	W	−
Lactose	+	W	−	−
Melibiose	W	−	W	−
Sucrose	−	W	−	−
Trehalose	+	−	−	−
Starch	+	−	−	−
Gentiobiose	W	W	W	−
D-Lyxose	W	W	W	−
D-Fucose	+	W	−	−
L-Fucose	+	−	−	−
Enzyme activities				
Esterase (C4)	−	W	−	−
Esterase lipase (C8)	−	W	−	−

Table 1. cont.

Characteristic	1	2	3	4
Valine arylamidase	w	w	+	−
α-Chymotrypsin	−	w	−	−
Naphthol-AS-BI-phosphohydrolase	+	w	w	+
β-Galactosidase	+	w	w	−
α-Glucosidase	+	−	−	−
N-Acetyl-β-glucosaminidase	+	−	w	+
DNA G+C content (mol%)*	69.6	67–68[a]	64–65[b]	62–65[b]

*Data from: *a*, Urakami *et al.* (1990); *b*, Liu *et al.* (2010).

maximum-parsimony algorithms. Strain RHGG3^T showed the highest 16S rRNA gene sequence similarities to *C. segnis* ATCC 21756^T (98.6 %), *C. vibrioides* CB51^T (98.3 %), *C. henricii* ATCC 15253^T (97.2 %), *C. fusiformis* ATCC 15257^T (96.9 %), *C. mirabilis* FWC 38^T (96.6 %), *C. ginsengisoli* Gsoil 317^T (96.2 %), *C. daechungensis* H-E3-2^T (95.8 %) and *C. profundus* DS48-5-2^T (95.5 %). The DNA G+C content of strain RHGG3^T was 70 mol%, which is slightly higher than those of type strains of recognized species of the genus *Caulobacter* (62–68 %) (Urakami *et al.*, 1990; Abraham *et al.*, 2008; Liu *et al.*, 2010; Jin *et al.*, 2013, 2014). Strain RHGG3^T showed relatively low DNA–DNA relatedness with *C. segnis* ATCC 21756^T (33.3±0 %), *C. vibrioides* DSM 4738 (40.9±0.5 %) and *C. henricii* ATCC 15253^T(32.4± 1.5 %). These values are far below the threshold value of 70 % that is commonly accepted for a decision on the species status of novel strains (Wayne *et al.*, 1987), thereby indicating that strain RHGG3^T represents a separate species.

For fatty acid methyl ester analysis, 40 mg bacterial cells were harvested from TSA plates after incubation for 2 days at 28 °C, and fatty acid methyl esters were extracted and prepared according to the protocol of Miller (1982) with minor modifications from Kuykendall *et al.* (1988). The fatty acid methyl ester mixtures were separated using a gas chromatograph (6890N; Agilent). Peaks were automatically integrated and fatty acid names and percentages were determined using the Sherlock Microbial Identification System (MIS) standard software (TSBA6 library). Analysis of fatty acids, respiratory quinones and polar lipids were carried out by the Identification Service of the DSMZ. For respiratory quinone and polar lipid analyses, cell mass of strain RHGG3^T, incubated in TSA broth for 48 h at 28 °C, was harvested by centrifugation, washed with sterile distilled water and freeze-dried. Respiratory lipoquinones were extracted from 100 mg freeze-dried cell material using the two-stage method as described by Tindall (1990a; 1990b), separated into their different classes by thin-layer chromatography on silica gel and further analysed by HPLC with a reverse-phase column (Macherey-Nagel, RP18). Polar lipids were extracted, separated by two-dimensional silica gel thin-layer chromatography (Art. No. 818 135; Macherey-Nagel) and identified by spraying with specific detection reagents.

The cellular fatty acid compositions of strain RHGG3^T and type strains of closely related species of the genus *Caulobacter* are shown in Table 2. The major cellular fatty acids of strain RHGG3^T included $C_{18:1}\omega7c$ (22.4 %), $C_{16:0}$ (20.3 %), $C_{15:0}$ (11.9 %) and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 8.1 %), which were found to be dominant in type strains of other related species of the genus *Caulobacter* (Abraham *et al.*, 1999, 2008; Jin *et al.*, 2013, 2014). The fatty acid 11-methyl $C_{18:1}\omega7c$ (ECL 18.081; 24.0 %) was also found in abundance in strain RHGG3^T, while the amount of $C_{18:1}\omega7c$ was relatively reduced, compared with members of the genus *Caulobacter*. This result was consistent with that of the research performed by Abraham *et al.* (2008), who reported large amounts of the fatty acid ECL 18.080 present in *Caulobacter mirabilis* FWC 38^T. The name annotation of fatty acid 11-methyl $C_{18:1}\omega7c$ (ECL 18.081) may be different from that reported by some researchers (Jin *et al.*, 2013, 2014), but was confirmed by Albuquerque *et al.* (2002) with electron ionization mass spectrum. In addition, strain RHGG3^T contained the 3-hydroxy fatty acid $C_{12:1}$ 3-OH, the saturated straight-chain fatty acid $C_{14:0}$ and a trace amount of ECL 11.798, which are the common unique characteristic fatty acids in type strains of members of the genus *Caulobacter* (Abraham *et al.*, 1999, 2008). Strain RHGG3^T contained ubiquinone Q-10 (100 %) as the only isoprenoid quinone. The presence of Q-10 as the major respiratory quinone is in agreement with the results obtained previously for species of the genus *Caulobacter* (Abraham *et al.*, 1999, 2008; Jin *et al.*, 2013, 2014). Strain RHGG3^T exhibited a polar lipid profile consisting of various unknown glycolipids (GL1–GL8), phosphatidylglycerol and phosphoglycolipids (Fig. S2).

On the basis of the results of this taxonomic study using a polyphasic approach that contained phenotypic, physiological and biochemical characteristics, fatty acid profiles, respiratory quinones, polar lipids, phylogenetic analysis and DNA–DNA hybridizations, strain RHGG3^T is proposed to be assigned to the genus *Caulobacter* as a representative of a novel species, for which the name *Caulobacter flavus* sp. nov. is proposed.

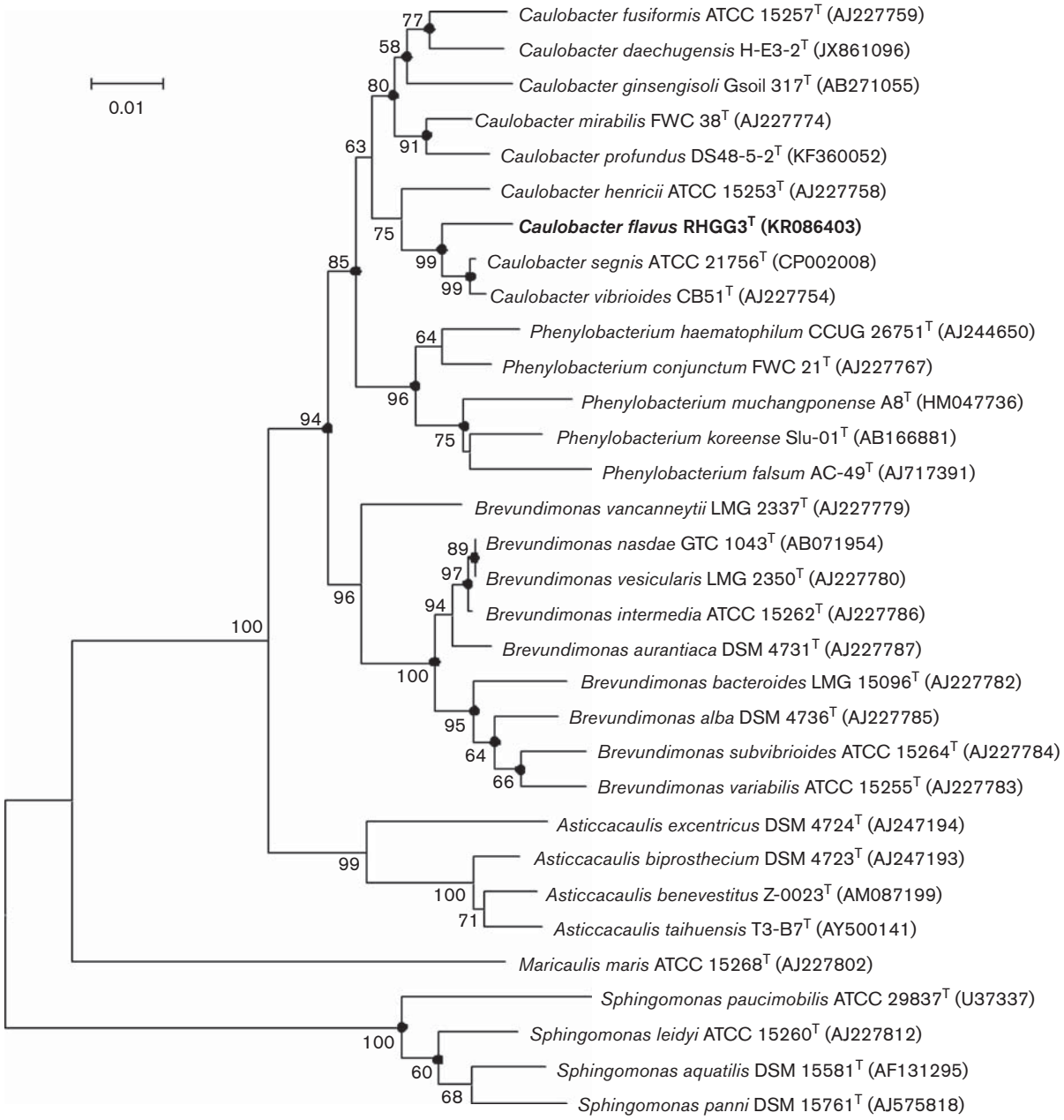

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the positions of strain RHGG3^T, species of the genus *Caulobacter* and some other related taxa. Filled circles indicate that the corresponding nodes (branches) were recovered in the neighbour-joining, maximum-parsimony and maximum-likelihood trees. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branch points. GenBank accession numbers are given in parentheses. Bar, 0.01 substitutions per nucleotide position.

Description of *Caulobacter flavus* sp. nov.

Caulobacter flavus (fla′vus. L. masc. adj. *flavus* yellow, referring to the colour of the colonies).

Cells are Gram-stain-negative, aerobic, non-spore-forming and rod-shaped with a single polar flagellum or a stalk in the end of the cell. Cells are 0.9–1.3 μm in width and 2.0–3.3 μm in length. Colonies on CM agar are yellow, convex, circular and 1.0–3.0 mm in diameter after incubation for 48 h at 30 °C. Growth occurs at 10–37 °C and pH 5.0–10.0, with optimal growth at 28–30 °C and pH 7.0. Growth occurs in the presence of 0–2 % NaCl, but is inhibited in the presence of >2 % (w/v) NaCl. Catalase-positive but oxidase-negative. Voges–Proskauer

Table 2. Cellular fatty acid compositions of strain RHGG3^T and type strains of closely related species of the genus *Caulobacter*

Strains: 1, RHGG3^T; 2, *C. segnis* DSM 7131^T (=ATCC 21756^T); 3, *C. vibrioides* DSM 4738; 4, *C. henricii* DSM 4730^T (=ATCC 15253^T). All data were taken from this study after incubation on TSA at 28 °C for 48 h. Values are percentages of total fatty acids. ECL, Equivalent chain-length; TR, trace amounts (<0.5 %); –, not detected.

Fatty acid	1	2	3	4
$C_{11:0}$	TR	–	–	–
$C_{12:0}$	1.7	1.5	TR	–
$C_{13:0}$	TR	–	–	–
$C_{12:1}$ 3-OH	0.8	0.9	1.2	1.0
$C_{12:0}$ 3-OH	0.5	0.6	TR	0.5
$C_{14:0}$	1.4	1.6	1.1	2.5
iso-$C_{13:0}$ 3-OH	–	–	TR	–
iso-$C_{15:0}$	TR	0.9	8.9	TR
anteiso-$C_{15:0}$	–	–	TR	–
$C_{15:1}\omega8c$	TR	–	–	TR
$C_{15:0}$	11.9	3.4	1.0	9.8
$C_{14:0}$ 2-OH	–	TR	–	–
iso-$C_{16:0}$	–	TR	0.5	–
$C_{16:0}$	20.3	22.7	16.7	15.5
iso-$C_{17:1}\omega9c$	–	TR	2.3	–
iso-$C_{17:0}$	–	0.6	11.2	TR
anteiso-$C_{17:0}$	–	TR	1.9	–
$C_{17:1}\omega8c$	1.4	0.9	TR	3.3
$C_{17:1}\omega6c$	1.9	0.6	TR	1.8
$C_{17:0}$	4.5	0.9	TR	1.7
$C_{16:1}$ 2-OH	–	2.3	–	–
$C_{18:1}\omega9c$	–	–	TR	–
$C_{18:1}\omega7c$	22.4	40.2	35.5	42.9
$C_{18:0}$	0.5	TR	0.6	TR
11-methyl $C_{18:1}\omega7c$	24.0	7.8	6.2	0.9
Summed feature 3*	8.1	13.9	9.0	17.2
Unknown fatty acids				
ECL 9.531	–	–	TR	–
ECL 11.798	TR	TR	2.0	1.9

*Summed features represent groups of one or two fatty acids that could not be separated by GLC using the Microbial Identification System (MIDI). Summed feature 3 contained $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

and methyl red reactions are negative. H_2S is not produced. No vitamins or other growth factors are required. Negative result in tests for nitrate reduction, indole production, D-glucose fermentation and gelatin hydrolysis (API 20NE). Assimilates D-glucose, L-arabinose, D-mannose, *N*-acetylglucosamine, maltose and malic acid, but not D-mannitol, potassium gluconate, capric acid, adipic acid, citrate or phenylacetic acid (API 20NE). Acid is produced from L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose, L-rhamnose, amygdalin, aesculin ferric citrate, cellobiose, maltose, lactose, trehalose, starch, D-fucose and L-fucose, and weakly produced from D-arabinose, D-ribose, *N*-acetylglucosamine, melibiose, gentiobiose and D-lyxose (API 50CH). Acid is not produced from glycerol, erythritol, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-fructose, L-sorbose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, arbutin, salicin, sucrose, inulin, melezitose, raffinose, glycogen, xylitol, turanose, D-tagatose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate (API 50CH). Positive for alkaline phosphatase, leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase activities, weakly positive for valine arylamidase activity, but negative for esterase (C4), esterase lipase (C8), lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase, α-fucosidase, arginine dihydrolase and urease activities (according to API ZYM and API 20NE test strips). The predominant isoprenoid quinone is ubiquinone Q-10. The polar lipid profile contains eight glycolipids (GL1–GL8), phosphatidylglycerol and phosphoglycolipids. The major cellular fatty acids (>8 %) are 11- methyl $C_{18:1}\omega7c$, $C_{18:1}\omega7c$, $C_{16:0}$, $C_{15:0}$ and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH).

The type strain, RHGG3^T (=CGMCC 1.15093^T=KCTC 42581^T=JCM 30763^T), was isolated from rhizosphere soil of a cultivated watermelon (*Citrullus lanatus*) in Hefei, PR China. The DNA G+C content of the type strain is 70 mol%.

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