Chryseobacterium wanjuense sp. nov., isolated from greenhouse soil in Korea

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A taxonomic study was performed on strain R2A10-2^T, isolated from greenhouse soil cultivated with lettuce (*Lactuca sativa* L.), collected in Wanju Province, Korea. The bacterial cells were Gram-negative, aerobic, short rods. The growth temperature and pH were 5–35 °C and 5.0–9.0, respectively. Phylogenetic analysis based on 16S rRNA gene sequences revealed that this isolate had 93.3–97.7 % similarity to *Chryseobacterium* species: the highest sequence similarities were to the type strains of *Chryseobacterium daecheongense* (97.7 %), *Chryseobacterium formosense* (97.1 %) and *Chryseobacterium defluvii* (96.9 %). Low levels of DNA–DNA relatedness were found between strain R2A10-2^T and the type strains of these three species (< 28 %). Differences in phenotypic properties were found with respect to *Chryseobacterium* species with validly published names. The predominant cellular fatty acids were iso-15:0 (40.0 %), iso-17:0 3-OH (21.9 %), iso-17:1 ω9c (11.7 %) and summed feature 4 (iso-15:0 2-OH and/or 16:1 ω7c/t, 11.0 %). Menaquinone MK-6 was detected as the sole respiratory quinone. The G+C content of the genomic DNA was 37.8 mol%. On the basis of the genomic and phenotypic evidence, this isolate represents a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium wanjuense* sp. nov. is proposed. The type strain is R2A10-2^T (=KACC 11468^T=DSM 17724^T).

As a result of a thorough study of the genus *Flavobacterium*, the genus *Chryseobacterium* was proposed by Vandamme *et al.* (1994). Recently, *Chryseobacterium meningosepticum* and *Chryseobacterium miricola* were transferred to the novel genus *Elizabethkingia* on the basis of a polyphasic taxonomic approach (Kim *et al.*, 2005b). Hence, 12 species with validly published names are currently classified in the genus *Chryseobacterium*. The type species is *Chryseobacterium gleum*. All members of the genus are characterized by the presence of MK-6 as the major respiratory quinone and by the presence of large amounts of iso-15:0, iso-17:1 ω9c and iso-17:0 3-OH fatty acids.

Strain R2A10-2^T was isolated from greenhouse soil cultivated with lettuce (*Lactuca sativa* L.) in the Wanju region of Korea. A diluted soil sample was spread on R2A medium (Difco) at 28 °C. Colonial properties were observed after

48 h incubation on R2A medium. Phenotypic tests such as Gram staining, catalase and oxidase activities, indole production and hydrolysis of agar, casein, DNA, gelatin and starch were performed according to the methods of Gerhardt *et al.* (1994). The presence of flexirubin-type pigments was determined by flooding the cell mass (taken from agar plates) with 20 % (w/v) KOH (Bernardet *et al.*, 2002). Hydrolysis of carboxymethylcellulose (Sigma) (0.1 %), alginic acid (0.5 % w/v), chitin from crab shells (1 %, w/v), pectin (0.5 % w/v) and tyrosine (0.5 % w/v) was tested according to the procedures of Gerhardt *et al.* (1994). Temperature and salinity tolerances were assessed at 5, 10, 15, 20, 25, 30, 33, 35, 37 and 40 °C and NaCl concentrations of 0, 1, 2, 3, 5 and 7 % (w/v) in R2A agar and broth, respectively. R2A broth adjusted to initial pH values of 4, 5, 6, 7, 8, 9 and 10 with citrate/phosphate buffer or Tris/HCl buffer (Breznak & Costilow, 1994) was used to assess the ability of strains to grow at different pH values. Enzyme activities were determined by using the API ZYM system (bioMérieux). Additional physiological and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain R2A10-2^T is DQ256729.

biochemical tests were performed with the API 20NE, API 50 CH and API ID 32 GN systems (bioMérieux).

The 16S rRNA gene was amplified by using a PCR with two universal primers, as described by Kwon *et al.* (2003). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Kwon *et al.* (2003). Neighbour-joining and maximum-parsimony methods were carried out using MEGA, version 2.1 (Kumar *et al.*, 2001). The resulting trees and topology were evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Isoprenoid quinones were extracted and analysed as described by Groth *et al.* (1996). DNA–DNA hybridization was carried out as described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG High Prime system (Roche); hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad). For fatty acid methyl ester analysis, cell mass was harvested from trypticase soy agar (Difco) after cultivation for 24 h at 28 °C. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The G+C content of the DNA was determined according to Mesbah *et al.* (1989), using a reverse-phase column.

Comparison of the almost complete 16S rRNA gene sequence (1462 nt) of strain R2A10-2^T with sequences deposited in the public databases showed the organism to be a member of the genus *Chryseobacterium*. The isolate shared sequence similarities of 97.7 and 96.9% with *Chryseobacterium daecheongense* DSM 15235^T and *Chryseobacterium defluvii* DSM 14219^T, respectively. The organism also showed a high level of 16S rRNA gene sequence similarity (97.1%) with *Chryseobacterium formosense* KCTC 12435^T. The type strains of all other *Chryseobacterium* species showed sequence similarities of less than 96% with respect to strain R2A10-2^T. On the basis of neighbour-joining and maximum-parsimony analyses, strain R2A10-2^T forms a clade with *C. daecheongense* DSM 15235^T and *C. defluvii* DSM 14219^T. The intracade branching, as well as the branching point with a sister clade, was supported by high bootstrap values (see Fig. 1 for the neighbour-joining analysis). Because of the relatively high 16S rRNA gene sequence similarity values, DNA–DNA hybridization was carried out between the novel isolate and *C. daecheongense* DSM 15235^T, *C. defluvii* DSM 14219^T and *C. formosense* KCTC 12435^T. Low levels of DNA–DNA relatedness were found between strain R2A10-2^T and DSM 15235^T (28%), DSM 14219^T (38%) and KCTC 12435^T (16%), supporting the presence of a novel genospecies.

The phenotypic characteristics of strain R2A10-2^T are indicated in the species description and in Table 1. The predominant cellular fatty acids observed in strain R2A10-2^T were iso-15:0 (40.0%), iso-17:0 3-OH (21.9%),

Fig. 1. Unrooted neighbour-joining tree, based on almost-complete 16S rRNA gene sequences, showing the phylogenetic relationships of strain R2A10-2^T within the genus *Chryseobacterium*. Bootstrap values (>50%) based on 1000 replications are shown at the nodes of the tree. Bar, 0.005 substitutions per nucleotide position.

iso-17:1ω9c (11.7%) and summed feature 4 (iso-15:0 2-OH and/or 16:1ω7c/t, 11.0%). Table 2 shows the detailed fatty acid composition of strain R2A10-2^T in comparison with those of all other *Chryseobacterium* species. Menaquinone MK-6 was detected as the sole respiratory quinone. The G+C content of the genomic DNA was 37.8 mol%. Although the phenotypic record is poor for a range of *Chryseobacterium* type strains, the data available indicate that strain R2A10-2^T differs in terms of several properties, which, in combination, allow its differentiation from other members of the genus *Chryseobacterium*.

On the basis of the results of DNA–DNA hybridization, phenotypic characterization and 16S rRNA gene sequencing, we propose that strain R2A10-2^T represents a novel species within the genus *Chryseobacterium*, for which we propose the name *Chryseobacterium wanjuense* sp. nov.

Description of *Chryseobacterium wanjuense* sp. nov.

Chryseobacterium wanjuense (wan.ju.en'se. N.L. neut. adj. *wanjuense* pertaining to Wanju, a province in Korea).

Cells are Gram-negative rods (0.7–0.8 × 2.0–3.5 μm). Yellow colonies with entire edges are formed on R2A agar plates. Grows at 5–37 °C (optimum 28 °C), with 0–2% NaCl (optimum 0–1% NaCl) and at pH 5–9 (optimum pH 7). Growth occurs on R2A, trypticase soy agar and nutrient agar (Difco), but weak growth is observed on MacConkey agar. Degrades casein, gelatin, starch and tyrosine, and weakly degrades DNA. Urea, chitin and carboxymethylcellulose are not degraded. Utilizes malonate. The type strain shows positive reactions for aesculin and gelatin hydrolysis and for β-galactosidase activity, but shows negative reactions for nitrate reduction, indole production, glucose fermentation and for arginine dihydrolase and urease activities (API 20NE). Assimilates D-glucose, D-mannose, D-maltose,

Table 1. Phenotypic comparisons among type strains of *Chryseobacterium* species

Strains: 1, strain R2A10-2^T; 2, *C. daecheongense* DSM 15235^T; 3, *C. defluvii* DSM 14219^T; 4, *C. formosense* KCTC 12435^T; 5, *C. balustinum* LMG 8329^T; 6, *C. gleum* LMG 8334^T; 7, *C. indologenes* LMG 8337^T; 8, *C. indoltheticum* LMG 4025^T; 9, *C. joostei* LMG 18212^T; 10, *C. scopthalmum* LMG 13028^T; 11, *C. shigense* DSM 17126^T; 12, *C. taichungense* CCUG 50001^T; 13, *C. vrystaatense* LMG 22846^T. Data are from Yabuuchi *et al.* (1983), Holmes *et al.* (1984a, b), Mudarris *et al.* (1994), Vandamme *et al.* (1994), Hugo *et al.* (2003), Kämpfer *et al.* (2003), de Beer *et al.* (2005), Kim *et al.* (2005a), Shen *et al.* (2005), Young *et al.* (2005) and this study. All strains tested were positive for the presence of flexirubin-type pigments and for the hydrolysis of aesculin, casein and gelatin. +, Positive; w, weak; –, negative; NA, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Growth on/at:													
MacConkey agar	w	–	–	–	+	+	–	+	+	–	–	–	–
5 °C	+	–	–	–	+	–	–	+	+	+	+	–	+
37 °C	+	+	+	–	–	+	+	+	–	–	–	+	–
42 °C	–	–	+	NA	–	+	+	–	–	–	–	–	–
3 % NaCl	–	NA	–	NA	w	+	+	w	w	w	NA	NA	–
Nitrate reduction	–	+	–	–	+	–	–	–	–	–	–	–	–
Indole production	–	–	+	+	+	+	+	+	+	–	+	w	+
β-Galactosidase	+	–	NA	–	–	+	–	–	–	+	NA	w	NA
Malonate utilization	+	–	–	–	–	–	+	–	–	–	NA	–	NA
Hydrolysis of:													
DNA	w	NA	NA	NA	+	+	+	+	+	+	NA	NA	+
Starch	+	+	+	NA	–	+	+	–	+	–	+	NA	–
Tyrosine	+	NA	NA	NA	w	+	–	+	+	+	NA	NA	–
Urea	–	–	–	–	–	+	+	–	+	+	–	–	+
Acid production from:													
Glucose	+	–	+	w	+	+	+	+	+	–	+	w	NA
Maltose	–	–	+	–	–	+	+	+	+	–	–	+	NA
Trehalose	+	+	+	+	–	+	+	–	+	+	–	+	NA
DNA G+C content (mol%)	37.8	36.6	38.8	NA	34.7	37.0	37.6	33.8	36.7	34.1	36.6	NA	37.1

Table 2. Fatty acid composition of strain R2A10-2^T in comparison with other members of the genus *Chryseobacterium*

Taxa: 1, strain R2A10-2^T; 2, *C. daecheongense* (*n*=1); 3, *C. defluvii* (*n*=1); 4, *C. formosense* (*n*=1); 5, *C. balustinum* (*n*=1); 6, *C. gleum* (*n*=5); 7, *C. indologenes* (*n*=45); 8, *C. indoltheticum* (*n*=1); 9, *C. joostei* (*n*=11); 10, *C. scopthalmum* (*n*=2); 11, *C. taichungense* (*n*=1); 12, *C. vrystaatense* (*n*=7). Data are from Hugo *et al.* (2003), Kämpfer *et al.* (2003), de Beer *et al.* (2005), Kim *et al.* (2005a), Shen *et al.* (2005), Young *et al.* (2005) and this study. Values are mean percentages of total fatty acids; fatty acids amounting to less than 2% of the total fatty acids in all strains were not included. Major fatty acids are in bold. tr, Trace amount (less than 2%); –, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12
iso-13:0	tr	tr	2.8	3.6	tr	tr	tr	–	tr	tr	tr	tr
Unknown 13:566*	2.9	tr	tr	tr	tr	tr	2.1	tr	tr	2.9	6.7	tr
iso-15:0	40.0	51.2	58.5	52.2	32.3	35.4	34.3	29.4	34.6	35.0	35.4	41.8
iso-15:0 3-OH	3.7	2.0	2.6	tr	2.7	2.5	2.6	2.3	2.9	2.7	4.3	2.7
anteiso-15:0	tr	tr	3.2	2.1	tr	tr	tr	5.9	tr	tr	tr	tr
16:0 3-OH	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	2.6	tr
17:0 2-OH	–	–	–	–	tr	–	–	3.0	–	tr	–	–
iso-17:0	2.9	3.0	2.0	2.3	tr	tr	tr	tr	tr	tr	tr	tr
iso-17:0 3-OH	21.9	15.7	14.1	10.9	16.8	21.8	19.2	14.0	20.1	16.3	22.4	15.4
iso-17:1ω9c	11.7	7.6	4.8	4.3	27.1	20.2	24.2	25.6	22.9	24.8	8.9	19.7
Summed feature 3†	–	–	–	–	–	–	–	–	–	–	–	9.1
Summed feature 4†	11.0	10.3	8.4	6.5	9.2	11.8	11.1	11.2	12.1	11.5	13.8	tr

*Unknown fatty acid; the number indicates the equivalent chain length.

†Summed feature 3 comprises iso-15:0 2-OH and/or 16:1ω7c and summed feature 4 comprises iso-15:0 2-OH and/or 16:1ω7d/t.

sodium acetate, glycogen and L-proline (API 20NE and API ID 32 GN). Does not assimilate L-arabinose, D-mannitol, N-acetylglucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, inositol, D-sucrose, itaconic acid, suberic acid, sodium malonate, lactic acid, L-alanine, potassium 5-ketogluconate, 3-hydroxybenzoic acid, L-serine, salicin, D-melibiose, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid or 4-hydroxybenzoic acid (API 20NE and API ID 32 GN). Activity is observed for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl- β -glucosaminidase, weak activity is observed for esterase (C4), cystine arylamidase, α -chymotrypsin and α -glucosidase and no activity is observed for lipase (C14), trypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, α -mannosidase and α -fucosidase (API ZYM). Acids are produced only from D-glucose, D-mannose, D-trehalose and gentiobiose (API 50 CH). The predominant cellular fatty acids are iso-15:0, iso-17:0 3-OH, iso-17:1 ω 9c and summed feature 4. Menaquinone MK-6 is the sole respiratory quinone. The G + C content of the genomic DNA of the type strain is 37.8 mol%. The closest phylogenetic relative is *C. daecheongense*.

The type strain, R2A10-2^T (=KACC 11468^T=DSM 17724^T), was isolated from greenhouse soil cultivated with lettuce (*L. sativa* L.), collected from Wanju Province, Korea.

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