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Chryseobacterium kwangjuense sp. nov., isolated from pepper (*Capsicum annuum* L.) root

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The yellow-pigmented, Gram-stain-negative, rod-shaped bacterium KJ1R5^T was isolated from the root of a pepper plant grown in a field in Kwangju, Korea. Strain KJ1R5^T was characterized by physiological, biochemical, and molecular genetic analyses. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain KJ1R5^T was most closely related to members of the genus *Chryseobacterium*, and that the strain exhibited the highest similarities with type strains of *Chryseobacterium vrystaatense* (97.0 %) and *Chryseobacterium rhizosphaerae* (97.1 %). DNA–DNA hybridization reassociation values between strain KJ1R5^T and type strains of *C. vrystaatense* KACC 11675^T and *C. rhizosphaerae* KACC 14918^T were 46.9 and 38.4 %, respectively. The DNA G+C content of KJ1R5^T is 40.2 mol%. The predominant respiratory quinone of KJ1R5^T was menaquinone MK-6; major cellular fatty acids were iso-$C_{15:0}$, summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), iso-$C_{17:1}\omega9c$, and iso-$C_{17:0}$ 3-OH. On the basis of these phenotypic and genotypic characteristics, the strain significantly differed from representative strains belonging to the genus *Chryseobacterium*. Thus, we propose that strain KJ1R5^T represents a novel species of the genus *Chryseobacterium*, named *Chryseobacterium kwangjuense* sp. nov. The type strain is KJ1R5^T (=KACC 13029^T=JCM 15904^T).

The genus *Chryseobacterium* is type genus of the family *Flavobacteriaceae* (Bernardet *et al.*, 1996). Vandamme *et al.* (1994) suggested a novel genus *Chryseobacterium* for some species of the genus *Flavobacterium* based on genotypic, biochemical, and phenotypic characteristics. Recently, the genus *Chryseobacterium* has been reported to comprise 62 species (Bernardet *et al.*, 2011; http://www.bacterio.cict.fr/c/chryseobacterium.html); these species are typically yellow rods containing non-motile, non-spore-forming cells (Vandamme *et al.*, 1994), which are abundant in various environments such as freshwater, soil, raw chicken, decaying plant material and fish (Bergey *et al.*, 1923; Bernardet *et al.*, 1996; de Beer *et al.*, 2005; Weon *et al.*, 2006; Behrendt *et al.*, 2008; Szoboszlay *et al.*, 2008; Zamora *et al.*, 2012a). In addition, some species of the genus *Chryseobacterium* have been obtained from clinical samples and some have been shown to be pathogenic to fish (Bernardet & Bowman, 2006; Ilardi *et al.*, 2009; Zamora *et al.*, 2012b). However, in plants, several species of the genus *Chryseobacterium* are known to play a role in growth promotion and biological control of pathogens (Domenech *et al.*, 2006; Lucas *et al.*, 2009; Kim *et al.*, 2012). In our earlier studies (Kim *et al.*, 2008a, 2008b), we reported the bacterial strain KJ1R5 as a potential biocontrol agent against Phytophthora blight of pepper (*Capsicum annuum* L.) caused by the oomycete *Phytophthora capsici*, which is one of the most destructive soil-borne plant pathogens known (Hausbeck & Lamour, 2004; Sang & Kim, 2012). To elucidate the taxonomic position of strain

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Abbreviations: FAME, fatty acid methyl ester; TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Chryseobacterium kwangjuense* KJ1R5^T is AY514021.

A supplementary figure and a supplementary table are available with the online version of this paper.

KJ1R5^T, morphological, physiological, and biochemical analyses, as well as Biolog, fatty acid methyl ester (FAME), and 16S rRNA gene sequence analyses, were conducted. On the basis of the results of these analyses, the strain was identified as a novel species in the genus *Chryseobacterium*.

The bacterial strain KJ1R5^T was obtained from a pepper plant grown in a field in Kwangju, Korea in 2001 (Kim *et al.*, 2008a). The strain was isolated from the surface of the plant root and cultured on tryptic soy agar (Difco) in the presence of 50 μg cycloheximide ml^{-1} at 28 °C for 2 days. In this study, for investigation of the phenotypic characteristics of strain KJ1R5^T, *Chryseobacterium vrystaatense* KACC 11675^T (=LMG 22846^T), *Chryseobacterium rhizosphaerae* KACC 14918^T (=NBRC 105248^T), *Chryseobacterium balustinum* KCTC 2903^T (=LMG 8329^T), *Chryseobacterium gleum* KCTC 2904^T (=LMG 8334^T), *Chryseobacterium indologenes* KCTC 2905^T (=LMG 8337^T), *Chryseobacterium indoltheticum* KCTC 2920^T (=LMG 4025^T), *Chryseobacterium scophthalmum* KCTC 2907^T (=LMG 13028^T), *Chryseobacterium jejuense* KACC 12501^T (=DSM 19299^T), *Chryseobacterium flavum* KACC 14205^T (=CCTCC AB 206147^T), *Chryseobacterium daecheongense* KACC 11377^T (=DSM 15235^T), *Chryseobacterium aquifrigidense* KACC 14190^T (=JCM 14756^T), and *Elizabethkingia meningoseptica* KCTC2906^T (=LMG 12279^T) were used as reference strains. These reference strains were obtained from the Korean Agricultural Culture Collection (KACC) of the National Institute of Agricultural Biotechnology (Suwon, Korea) and the Korean Collection for type Culture (KCTC) of the Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea).

The phenotypic characteristics of strain KJ1R5^T and the reference strains were examined using the following tests. Gram staining; hydrolysis of starch, casein and Tween 80; degradation of gelatin; and production of indole were tested by the methods of Gerhardt (1994). Degradation of aesculin and urea was determined by the procedures of Smibert & Krieg (1994) and Bowman *et al.* (1996), respectively. Degradation of malonate and the production of β-galactosidase and phenylalanine deaminase were assessed according to the method of Williams *et al.* (1989). Acid production from L-arabinose, maltose and mannitol was observed according to the protocol of Barrow & Feltham (1993). Colony colour on nutrient agar (NA; Difco); growth in nutrient broth (NB; Difco) at 28 and 37 °C; growth in NB containing 1, 2, 3 and 4 % (w/v) NaCl; and growth on MacConkey (Difco) agar at 5, 37 and 42 °C were also examined. Bacterial motility was determined on motility test medium with 2,3,5-triphenyltetrazolium chloride (TTC) (Ball & Sellers, 1966). Growth of strain KJ1R5^T in trypticase soy broth (TSB; Oxoid), which was adjusted to pH 4.0, 5.0, 6.0, 7.0 and 8.0, at 10, 28, 38 and 45 °C was tested. The pH was adjusted with HCl or NaOH before sterilization and was confirmed after sterilization. The morphological features of the strain KJ1R5^T, including the cell shape and size and surface ornamentation, were examined by transmission electron microscopy (TEM). To prepare the specimen for TEM, the strain was grown on NA for 20 h at 28 °C. The bacterial colonies were serially diluted in sterile triple-distilled H_2O on glass slides; the grids were then soaked in the bacterial suspension. Excess bacterial suspension on the grids was removed using filter paper (Whatman No. 1) by blotting along the margins of the grids. The cells in the grids were stained with 1 % (w/v) uranyl acetate, air-dried, and then observed with a transmission electron microscope (LEO 912AB; LEO Electron Microscopy). Carbon source utilization by strain KJ1R5^T was also characterized using the Biolog GN Microplate system (Biolog), which contains 95 single carbon sources; the data were analysed using the Biolog software (Microlog 3 database, release 4.01A) according to the manufacturer's instructions.

Chromosomal DNA of strain KJ1R5^T was extracted using a Qiagen genomic-tip system according to the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using universal primers fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rP2 (5′-ACGGCTACCTTGTTACGACTT-3′) (Weisburg *et al.*, 1991) and the PCR product was purified using the QIAquick spin PCR purification kit (Qiagen). The purified 16S rRNA gene was sequenced on an ABI Prism model 377 DNA sequencer (Applied Biosystems) using the ABI Prism Dye Primer Cycle Sequencing kit (Perkin Elmer) with the following specific primers: fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′), SP1 (5′-GCCACACTGGAACTGAGACAC-3′), SP2 (5′-TGTAGCGGCCACACTGGAACTGAGACAC-3′), SP3 (5′-GGAGCATGTG-GTTTAAGTGAAATGCGTG-3′), SP4 (5′-CTACACACGTGCTACGGTGG-3′), RSP1 (5′-TTCGCACCTGAGCGTCAGTC-3′), RSP2 (5′-TGACGACAGCCATGCAGCAC-3′), and rP2 (5′-ACGGCTACCTTGTTACGACTT-3′) according to the manufacturer's instructions. The 16S rRNA gene sequence analysis was performed with the BLAST sequence analysis software (http://blast.ncbi.nlm.nih.gov/Blast.cgi), using the National Center for Biotechnology Information (NCBI) server. The 16S rRNA gene sequence (1432 bases) of strain KJ1R5^T was aligned with representative sequences of related species of the genus *Chryseobacterium* from the NCBI's GenBank database. Phylogenetic analysis was performed using the MEGA version 5.1 (Kumar *et al.*, 2004) software package after performing multiple alignment of the sequences using the CLUSTAL W program (Thompson *et al.*, 1997). The phylogenetic tree was constructed with distance options according to the Kimura two-parameter model (Kimura, 1980, 1983), and clustering was performed based on the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods. Bootstrap analysis with 1000 replications was used to evaluate the stability of the groups in the neighbour-joining tree (Felsenstein, 1985).

DNA–DNA hybridization was performed using the DIG High Prime DNA Labelling and Detection Starter kit II (Roche Applied Science); the percentage of DNA–DNA hybridization was determined using a densitometer (Bio-Rad). The DNA–DNA hybridization experiments were performed three times

independently, and the mean value was calculated. The G+C contents of DNA were determined from the mid-point value of the thermal denaturation profile obtained with a UV Vis Lambda 20 spectrophotometer (Perkin-Elmer) at 260 nm (Marmur & Doty, 1962).

Respiratory quinones of strain KJ1R5^T were also determined using reverse phase-high performance liquid chromatography (RP-HPLC) conducted by the Korean Culture Center of Microorganisms (KCCM) (Seoul, Korea) (Komagata & Suzuki, 1987). For cellular FAME analysis of strain KJ1R5^T and the reference strains, bacterial cells (approximately 40 mg) grown on trypticase soy agar (TSA; Oxoid) at 28 °C for 24 h were harvested and analysed by gas chromatography (5898A, GC system; Hewlett Packard) using the MIDI system (Microbial Identification System); the fatty acids were identified using the Aerobe RTSBA (version 6.0 B database) according to the manufacturer's instructions.

Phenotypic characteristics of strain KJ1R5^T and those of other related species of the genus *Chryseobacterium* are described in Table 1. Cells of strain KJ1R5^T are short rods 0.8–1.0 μm wide and 1.5–2.0 μm long, without flagella (Fig. S1, available in IJSEM Online). From the Biolog identification using the Biolog GN Microplate system, the data for utilization of 95 carbon sources by strain KJ1R5^T are

Table 1. Phenotypic characteristics that differentiate strain KJ1R5^T from the type strains of related species of the genus *Chryseobacterium*

Strains: 1, strain KJ1R5^T; 2. *Chryseobacterium vrystaatense* KACC 11675^T (=LMG 22846^T); 3, *C. rhizosphaerae* KACC 14918^T (=NBRC 105248^T); 4, *C. balustinum* KCTC 2903^T (=LMG 8329^T); 5, *C. gleum* KCTC 2904^T (=LMG 8334^T); 6, *C. indologenes* KCTC 2905^T (=LMG 8337^T); 7, *C. indoltheticum* KCTC 2920^T (=LMG 4025^T); 8, *C. scophthalmum* KCTC 2907^T (=LMG 13028^T); 9, *C. jejuense* KACC 12501^T (=DSM 19299^T); 10, *C. flavum* KACC 14205^T (=CCTCC AB 206147^T); 11, *C. daecheongense* KACC 11377^T (=DSM 15235^T); 12, *C. aquifrigidense* KACC 14190^T (=JCM 14756^T); 13, *Elizabethkingia meningoseptica* KCTC2906^T (=LMG 12279^T). All strains are positive for growth in nutrient broth (NB) at 28 °C, grow in NB containing 1 % NaCl and degrade gelatin. All strains are non-motile and Gram-negative. Y, yellow; YW, yellowish-white; +, positive; w, weakly positive; −, negative.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Colour of colonies	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	YW
Growth in NB at 37 °C	+	+	+	−	+	+	−	−	−	+	+	+	+
Growth in NB containing:													
2 % NaCl	+	−	+	+	+	+	+	+	+	+	+	−	+
3 % NaCl	+	−	+	−	+	+	+	+	+	+	−	−	+
4 % NaCl	−	−	−	−	−	−	+	−	−	w	−	−	+
Growth on MacConkey agar at:													
5 °C	−	−	−	−	+	−	−	+	+	−	w	−	+
37 °C	−	−	−	−	+	+	+	−	+	+	+	+	−
42 °C	−	−	−	−	+	+	+	−	−	+	−	−	−
Hydrolysis of:													
Starch	+	−	+	−	−	−	−	−	+	+	+	+	−
Casein	+	+	+	+	+	+	+	−	+	+	+	−	+
Tween 80	+	+	+	+	+	+	+	+	+	+	−	+	+
Acid production from:													
L-Arabinose	w	+	+	−	+	−	−	−	−	+	−	+	−
Maltose	+	−	+	−	+	+	+	−	+	+	−	−	+
Mannitol	+	−	+	−	−	−	+	−	−	−	−	−	+
Degradation of:													
Aesculin	+	+	+	+	+	−	+	+	+	+	+	+	+
Urea	−	+	+	−	+	+	−	+	+	−	−	−	−
Malonate	−	−	−	−	+	+	−	−	−	−	−	−	−
Production of:													
Indole	+	+	−	+	+	+	+	−	+	+	−	−	+
β-Galactosidase	−	−	−	−	+	+	+	+	−	+	−	−	+
Phenylalanine deaminase	−	−	+	−	−	+	+	+	−	+	+	−	+
DNA G+C content (mol%)	40.2	37.7	35.9	34.7	38.0	38.5	33.8	34.1	41.4	37.2	36.6	35.6	37.1

presented in Table S1. In order to compare strain KJ1R5^T with other related species belonging to the genus *Chryseobacterium* at the species level, a phylogenetic tree was constructed using published 16S rRNA gene sequences of 38 taxa in the family *Flavobacteriaceae*, including 31 species of the genus *Chryseobacterium*, one species of the genus *Bergeyella*, two species of the genus *Elizabethkingia*, one species of the genus *Cloacibacterium*, one species of the genus *Epilithonimonas*, and two species of the genus *Riemerella*. According to the maximum-parsimony method and neighbour-joining analysis, strain KJ1R5^T was clustered with *C. vrystaatense* R-23566^T; the sequence of strain KJ1R5^T showed similarities to *C. vrystaatense* R-23566^T (97.0 %) and *C. rhizosphaerae* RSB3-1^T (97.1 %) (Fig. 1). Because of relative high similarity values (⩾97 %) of the 16S rRNA gene sequences, DNA–DNA hybridization was conducted between strain KJ1R5^T and these strains (*C. vrystaatense* KACC 11675^T and *C. rhizosphaerae* KACC 14918^T) as well as other representative species of the genus *Chryseobacterium* [*C. indologenes* KCTC 2905^T (96.5 %), *C. gleum* KCTC 2904^T (96.5 %), *C. indoltheticum* KCTC 2920^T (96.4 %), and *C. balustinum* KCTC 2903^T (95.7 %)]. The data showed relatively low DNA–DNA reassociation values (*C. vrystaatense* KACC 11675^T=46.9 %, *C. rhizosphaerae* KACC 14918^T=

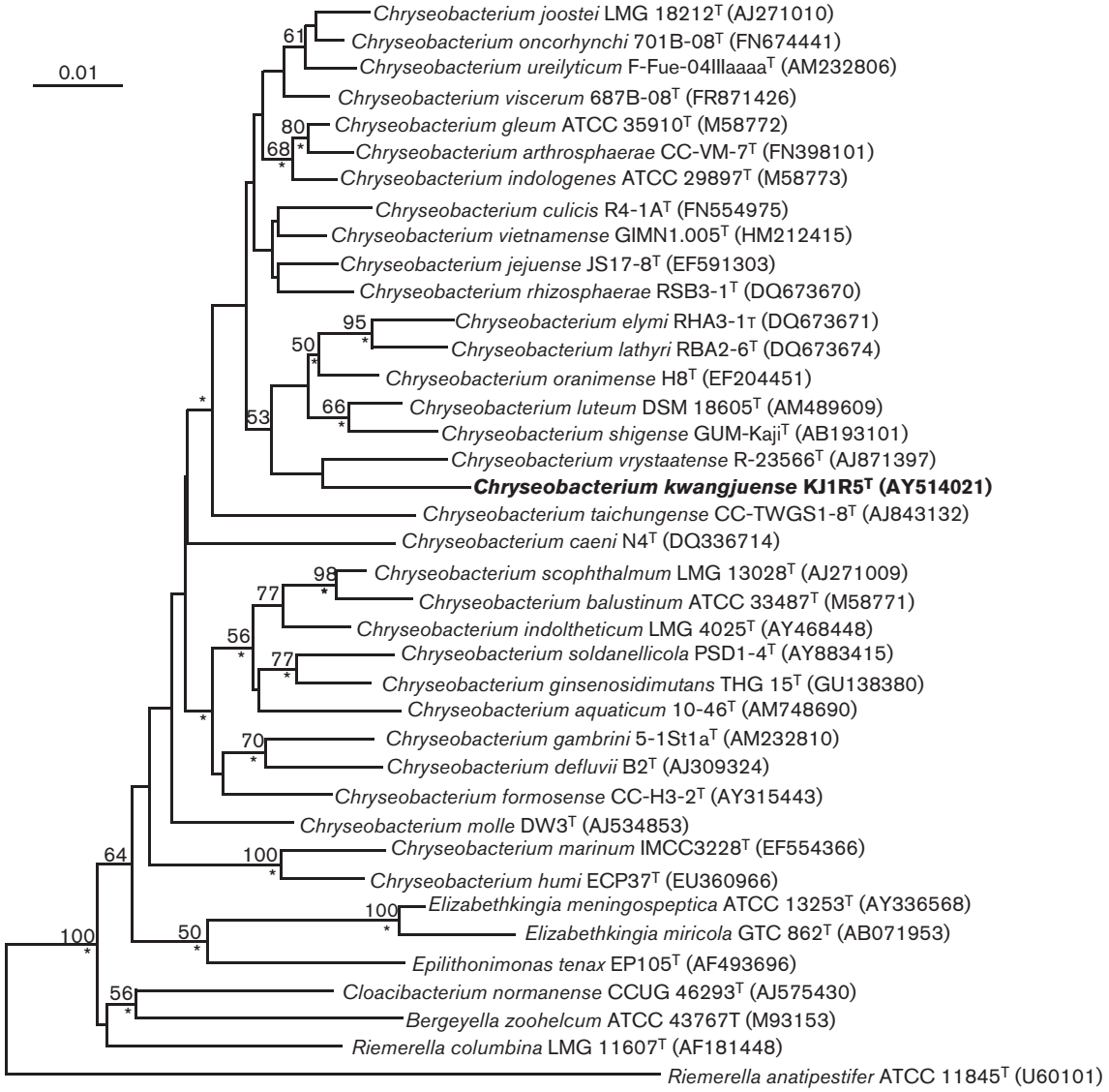

Fig. 1. Phylogenetic neighbour-joining tree showing the relationship of strain KJ1R5^T with other members of the genus *Chryseobacterium* and representative members of related genera. Bootstrap values (>50 %) based on 1000 replications are shown atr the branch points. Asterisks indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony method. *Riemerella anatipestifer* ATCC 11845^T (GenBank accession no. U60101) was used as the outgroup. Bar, 1 nt substitution per 100 nt of the 16S rRNA gene sequence.

Table 2. Cellular fatty acid compositions (%) of strain KJ1R5^T and the type strains of related species of the genus *Chryseobacterium*

Strains: 1, KJ1R5^T; 2. *Chryseobacterium vrystaatense* KACC 11675^T (=LMG 22846^T); 3, *C. rhizosphaerae* KACC 14918^T (=NBRC 105248^T); 4, *C. balustinum* KCTC 2903^T (=LMG 8329^T); 5, *C. gleum* KCTC 2904^T (=LMG 8334^T); 6, *C. indologenes* KCTC 2905^T (=LMG 8337^T); 7, *C. indoltheticum* KCTC 2920^T (=LMG 4025^T); 8, *C. scophthalmum* KCTC 2907^T (=LMG 13028^T); 9, *C. jejuense* KACC 12501^T (=DSM 19299^T); 10, *C. flavum* KACC 14205^T (=CCTCC AB 206147^T); 11, *C. daecheongense* KACC 11377^T (=DSM 15235^T); 12, *C. aquifrigidense* KACC 14190^T (=JCM 14756^T); 13, *Elizabethkingia meningoseptica* KCTC2906^T (=LMG 12279^T). TR, Trace amount (⩽1 %); −, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
iso-$C_{13:0}$	1.7	2.0	2.2	1.2	TR	TR	TR	1.7	2.5	TR	2.2	TR	2.5
iso-$C_{14:0}$	4.6	−	−	−	−	−	−	−	−	−	6.4	−	−
iso-$C_{15:0}$	45.4	48.0	41.9	44.5	41.5	41.6	41.6	39.5	42.4	42.2	57.5	39.1	41.6
iso-$C_{15:0}$ 3-OH	4.2	3.5	4.3	3.3	3.3	3.2	2.9	2.8	4.1	3.8	3.0	3.0	4.2
anteiso-$C_{15:0}$	TR	TR	TR	1.8	TR	TR	7.0	4.3	TR	TR	1.2	TR	3.0
$C_{16:0}$	1.2	TR	1.6	1.2	1.2	1.1	1.2	2.5	1.4	TR	TR	1.7	1.4
$C_{16:0}$ 3-OH	TR	TR	TR	1.1	TR	TR	TR	TR	−	TR	TR	1.5	2.1
iso-$C_{17:0}$	1.4	TR	1.8	TR	1.5	1.0	TR	TR	1.7	1.1	1.3	1.1	TR
iso-$C_{17:0}$ 3-OH	10.8	13.0	15.2	12.2	10.1	11.7	7.8	8.7	10.8	12.8	9.3	12.5	9.9
iso-$C_{17:1}\omega 9c$	11.6	14.6	15.3	21.9	21.1	21.7	22.6	1.3	16.3	16.6	5.2	21.1	4.9
Summed feature 3*	15.4	12.5	13.7	7.0	16.9	13.1	9.7	8.5	16.2	15.9	10.7	13.4	23.8

*Summed features represent groups of two or more fatty acids that could not be resolved by gas chromatography; summed feature 3 contained $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$.

38.4 %, *C. indologenes* KCTC 2905^T=33.4 %), *C. gleum* KCTC 2904^T=32.8 %), *C. indoltheticum* KCTC 2920^T= 33.1 %), and *C. balustinum* KCTC 2903^T=25.5 % (Supplementary Fig. S2). These DNA–DNA relatedness values (25.5−46.9 %) were lower than a threshold value of 70 % as a criterion recommended for the definition of bacterial species (Wayne *et al.*, 1987), clearly supporting the fact that strain KJ1R5^T can be a distinct species differentiated from other species of the genus *Chryseobacterium*.

The G+C content of the DNA of strain KJ1R5^T was 40.2 mol%, which is within the range of the G+C contents of members of the genus *Chryseobacterium* (Bernardet *et al.*, 2011; Li & Zhu, 2012). The predominant respiratory quinone of strain KJ1R5^T was menaquinone MK-6; major cellular fatty acids were iso-$C_{15:0}$ (45.4 %), summed feature 3 ($C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$) (15.4 %), iso-$C_{17:1}\omega 9c$ (11.6 %), and iso-$C_{17:0}$ 3-OH (10.8 %) (Table 2). A comparison of major cellular fatty acids of strain KJ1R5^T with those of other members of the genus *Chryseobacterium* is shown in Table 2. Based on the results of 16S rRNA sequence analysis, DNA–DNA hybridization and phenotypic characterization, we propose strain KJ1R5^T as a novel species in the genus *Chryseobacterium* under the name of *Chryseobacterium kwangjuense* sp. nov.

Description of *Chryseobacterium kwangjuense* sp. nov.

Chryseobacterium kwangjuense (kwang·ju·en′se N.L. neut. adj. *kwangjuense* of or belonging to Kwangju, Jeonnam Province, Korea).

Strain KJ1R5^T is Gram-stain-negative aerobic. Cells are short rods 0.8–1.0 μm in width and 1.5–2.0 μm in length, without flagella or motility. Colonies of strain KJ1R5^T are yellow and this strain grows at 10–38 °C (optimum, 28–38 °C), at pH 6.0–8.0 (optimum, pH 7.0–8.0), and in media containing 1–3 % NaCl. Strain KJ1R5^T could grow on NB at 28 and 37 °C; however, the strain did not grow or grew weakly on MacConkey agar at 5, 37, and 42 °C. Starch, casein, and Tween 80 were hydrolysed, and aesculin and gelatin were degraded by the strain. However, the strain did not degrade urea and malonate. Strain KJ1R5^T produced indole but not β-galactosidase and phenylalanine deaminase. Menaquinone MK-6 is the predominant respiratory quinone. Major fatty acids of strain KJ1R5^T were iso-$C_{15:0}$, summed feature 3 ($C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$), iso-$C_{17:1}\omega 9c$, and iso-$C_{17:0}$ 3-OH.

The type strain, KJ1R5^T (=KACC 13029^T=JCM 15904^T), was isolated from the root of pepper (*Capsicum annuum* L.) in Kwangju, Jeonnam Province, Korea. The DNA G+C content of strain KJ1R5^T is 40.2 mol%.

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