Chryseobacterium bovis sp. nov., isolated from raw cow's milk

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Three Gram-negative, rod-shaped, oxidase-positive, aerobic, non-motile bacterial strains, designated H9^T, H10 and H15, were isolated during a study on the diversity of culturable psychrotolerant bacteria in raw cow's milk. Comparisons of 16S rRNA gene sequences showed that the three strains were very closely related to each other (sequence similarities of 99.6–99.8%). A polyphasic taxonomic study of the isolates resulted in their identification as members of the genus *Chryseobacterium* (family *Flavobacteriaceae*, phylum *Bacteroidetes*). The three strains showed ≤96.9% sequence similarity with respect to the type strains of described *Chryseobacterium* species, indicating that H9^T, H10 and H15 represent a novel species of the genus *Chryseobacterium*. The three strains grew at 7–37 °C (strain H10 grew at up to 41 °C), with 0–2.5% NaCl and at pH 5.0–9.8. The dominant cellular fatty acids of strain H9^T were 15:0 iso (38.9%), 15:0 anteiso (15.6%) and 17:0 iso 3-OH (12.7%). Strain H10 also possessed 17:1 iso ω9c (14.8%) as a major fatty acid. On the basis of phenotypic properties and phylogenetic distinctiveness, the three milk isolates represent a novel species in the genus *Chryseobacterium*, for which the name *Chryseobacterium bovis* sp. nov. is proposed. The type strain is H9^T (=LMG 24227^T =DSM 19482^T).

The genus *Chryseobacterium* (Vandamme *et al.*, 1994) is a member of the family *Flavobacteriaceae* (phylum *Bacteroidetes*) and currently consists of 21 species, with *Chryseobacterium gleum* as the type species. Two additional species were recently transferred to the novel genus *Elizabethkingia* (Kim *et al.*, 2005b). *Chryseobacterium* species inhabit water, soil and the clinical environment (Bernardet *et al.*, 2002, 2005, 2006). They are also a part of the psychrotolerant and proteolytic bacterial population that causes a variety of defects in food products such as milk, meat, poultry and fish (González *et al.*, 2000; Hugo *et al.*, 1999, 2003; de Beer *et al.*, 2005, 2006).

Strains H9^T, H10 and H15 were isolated in the course of a study on the diversity of culturable psychrotolerant bacteria in raw milk. Raw milk from four farms in Israel was sampled every other month from April 2004 to January 2005. The samples were serially diluted and plated on sterile standard plate-count agar (SPC; Oxoid CM0463). Plates were incubated at 7 °C for 10 days and colonies displaying distinct morphologies were subcultured to

obtain pure cultures. Of about 300 pure cultures isolated in this study, strains H9^T, H10 and H15 were isolated (at the same farm) on SPC agar during the sampling of April 2004. A comparative analysis of the 16S rRNA gene sequences indicated that these strains were members of the genus *Chryseobacterium*. In order to determine their exact taxonomic positions, they were investigated using a polyphasic taxonomic approach.

Universal bacterial primers 8f and 1512r (based on *Escherichia coli* positions) were used to amplify internal fragments of the 16S rRNA gene according to Felske *et al.* (1997). The amplified PCR product (approx. 1.5 kb) was purified using a Wizard PCR product purification kit (Promega). Purified PCR products were sequenced (at Technion Medical School, Haifa, Israel) directly by using the dideoxynucleotide chain-termination method with an ABI PRISM 3100 DNA sequencer with BigDye terminator reagents (Applied Biosystems) according to the manufacturer's instructions. Sequencing was performed using primers 8f (5'-GGATCCAGACTTTGATYMTGGCTCAG-3'), 534r (5'-ATTACCGCGGCTGCTGG-3'), 968f (5'-AACGCGAAGAACCTTAC-3') and 1512f (5'-GTGAAGC-TTACGGYTAGCTTGTACGACTT-3'). The newly determined sequences were compared with those available in GenBank/EMBL (<http://www.ebi.ac.uk>) by using the WU

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain H9^T is EF204446.

An electron micrograph of strain H9^T is available as a supplementary figure with the online version of this paper.

BLAST program (Washington University Basic Local Alignment Search Tool, version 2.0). Sequence alignment was performed using the CLUSTAL W program; a phylogenetic tree (Fig. 1) was generated using the neighbour-joining method in the MEGA3 software (Kumar *et al.*, 2004).

The three strains shared 99.6–99.8% 16S rRNA gene sequence similarity (Fig. 1). The 1485 bp sequence of strain H9^T showed the highest sequence similarity (96.8%) to *Chryseobacterium hispanicum* JCM 13554^T and showed <96.1% sequence similarity with respect to the type strains of all other *Chryseobacterium* species (Fig. 1).

For electron microscopy, bacteria grown on SPC agar (48 h, 32 °C) were suspended in saline, fixed to a carbon-coated grid, stained with 2% uranyl acetate and then photographed using a JEM-1200EX electron microscope (JEOL). Electron microscopy showed that the cells were non-flagellated rods, 0.5–0.9 µm wide and 1.1–2.3 µm long (see Supplementary Fig. S1 in IJSEM Online).

For phenotypic characterization, SPC agar was used as the basal growth medium. Salt tolerance was determined at 30 °C on SPC agar containing 0–5% (w/v) NaCl (using increments of 0.5%). Growth at 4, 7, 18, 22, 25, 30, 32, 37, 40, 42, 43 and 45 °C was measured using SPC agar. The pH range for growth was determined with SPC adjusted to pH 3.5–10.5 (in increments of 0.5 pH units). The pH was adjusted prior to sterilization by the addition of HCl or NaOH and was measured again after sterilization. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on SPC agar supplemented with 0.5% (w/v) glucose or 0.1% (w/v) potassium nitrate. Biochemical tests were performed by using the API 20E, API 20NE, API 50 CH and API ZYM identification systems

(bioMérieux): these tests were carried out according to the manufacturer's instructions except that the incubation temperature was 32 °C. Catalase activity was evidenced by bubble production in a 3% (v/v) hydrogen peroxide solution. Oxidase activity was determined using 1% *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride (T3134; Sigma-Aldrich). Growth was tested on MacConkey agar (Difco) and cetrimide agar (Himedia). Haemolytic activity was determined using blood agar base supplemented with 5% sheep blood (Hylabs). Hydrolysis of casein and tributyrin were determined using 1% skimmed-milk agar supplemented with 0.5% yeast extract and tributyrin agar (Hylabs), respectively. Phenotypic traits of the three isolates are given in the species description and in Table 1.

Cell mass for H9^T, H10 and H15 was subjected to the KOH test as described by Fautz & Reichenbach (1980) and Bernardet *et al.* (2002). In each case, a very weak colour change was observed, demonstrating that minor amounts of flexirubin-type pigments are produced. The yellow pigments of strain H9^T were extracted and investigated according to Peterson *et al.* (1954). Briefly, the isolate was grown on Luria–Bertani agar at room temperature under light and dark conditions for 48 h. Cells were transferred to acetone, vortexed for 1 min, allowed to stand for 10 min and then centrifuged for 3 min at 13 000 r.p.m. Spectral analysis from 400 to 700 nm was performed on the acetone extract. A broad peak, with two shoulder peaks at 454 and 481 nm that are typical of carotenoids, was observed (Peterson *et al.*, 1954). Hence, the yellow colour of strain H9^T mostly results from the production of carotenoid-type pigments under light conditions.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strains H9^T, H10 and H15, members of the genus *Chryseobacterium* and representatives of related taxa. Bootstrap percentages (>50%) resulting from 1000 replicates are indicated at branching nodes. Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential characteristics of *Chryseobacterium* species

Taxa: 1, strains H9^T, H10 and H15 (*C. bovis* sp. nov.); 2, *C. haifense* LMG 24029^T; 3, *C. gleum* (n=12); 4, *C. balustinum* LMG 8329^T; 5, *C. indologenes* (n=13); 6, *C. indoltheticum* LMG 4025^T; 7, *C. scopthalmum* (n=7); 8, *C. defluvii* DSM 14219^T; 9, *C. joostei* (n=11); 10, *C. daecheongense* DSM 15235^T; 11, *C. formosense* CCUG 49271^T; 12, *C. taichungense* CCUG 50001^T; 13, *C. shigense* DSM 17126^T; 14, *C. vrystaatense* (n=36); 15, *C. soldanellicola* KCTC 12382^T; 16, *C. taeanense* KCTC 12381^T; 17, *C. piscium* (n=4); 18, *C. hispanicum* CECT 7129^T; 19, *C. wanjuense* DSM 17724^T; 20, *C. taiwanense* BCRC 17412^T; 21, *C. caeni* DSM 17710^T; 22, *C. daeguense* JCM 14362^T. The number of strains examined, if greater than one, is shown in parentheses. Data are from Yabuuchi *et al.* (1983), Holmes *et al.* (1984), Hugo *et al.* (2003), Kämpfer *et al.* (2003), de Beer *et al.* (2005, 2006), Kim *et al.* (2005a), Shen *et al.* (2005), Shimomura *et al.* (2005), Young *et al.* (2005), Gallego *et al.* (2006), Park *et al.* (2006), Tai *et al.* (2006), Weon *et al.* (2006), Quan *et al.* (2007), Yoon *et al.* (2007), Hantsis-Zacharov & Halpern (2007a) and this study. +, Positive; w, weakly positive; –, negative; v, variable; NA, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Type of pigment*	F (w), c†	F (w), c†	F	F	F	F	F	NA	F	F	F	F	F	F	NA	NA	F	F (w)	F	F	F	NA
Growth at/on:																						
3 % NaCl	–	–	NA	w	+	w	w	–	NA	NA	NA	NA	NA	–	+	+	+	–	–	+	+	–
Cetrimide agar	–	–	–	–	+	–	–	–	+	NA	NA	NA	NA	+	NA	NA	–	NA	NA	NA	NA	NA
MacConkey agar	–	–	+	+	+	+	–	–	+	–	–	–	–	–	–	–	–	–	w	NA	–	–
5 °C	–	+	–	–	–	+	+	–	+	–	–	–	+	+	+	+	+	+	+	+	+	–
37 °C	+	+	+	+	+	–	–	+	–	+	+	+	–	–	+	+	–	–	+	+	+	+
42 °C	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Nitrate reduction	v‡	–	NA	+	v	–	–	–	–	+	–	–	–	–	–	–	+	+	–	–	–	–
Urease activity	–	–	v	–	–	–	+	–	v	–	–	–	–	+	–	–	+	–	–	–	+	–
β-Galactosidase activity	v§	+	v	–	–	–	+	NA	–	–	–	w	NA	NA	–	–	NA	+	+	–	+	–
Production of:																						
H ₂ S	–	–	–	–	–	+	–	–	–	–	–	–	–	v	–	–	–	–	NA	–	–	–
Indole	–	+	+	+	+	+	–	+	+	–	+	w	+	+	–	–	+	w	NA	+	–	–
Acid production from:																						
Glucose	v§	+	+	+	+	NA	–	+	+	–	w	w	+	NA	NA	NA	NA	+	+	–	–	+
L-Arabinose	–	–	+	NA	v	–	–	–	–	–	–	–	–	NA	–	–	NA	NA	–	+	–	w
D-Fructose	–	+	+	+	+	–	–	NA	+	+	NA	NA	+	NA	–	–	NA	+	–	v	–	+
Lactose	v§	+	–	–	–	–	–	NA	–	–	NA	–	–	NA	–	–	NA	–	–	–	–	+
Maltose	v§	+	+	–	+	+	–	+	+	–	–	+	–	NA	–	–	NA	+	–	–	–	+
D-Mannitol	–	–	–	–	v	NA	–	–	v	–	–	–	–	NA	–	–	NA	–	–	–	–	–
Trehalose	–	–	+	–	+	–	–	+	+	+	+	+	–	NA	–	–	NA	–	+	–	–	+
D-Xylose	–	–	v	–	–	–	–	–	–	+	w	+	–	NA	–	–	NA	+	–	NA	–	w
DNA G+C content (mol%)	38.6	37.8	37.6	33.1	38.5	33.8	34.2	38.8	36.8	36.6	NA	NA	36.6	37.1	28.8	32.1	33.6	34.3	37.8	36.8	38.2	36.8

*c, Carotenoid-type; F, flexirubin-type.

†Yellow pigments are produced only when bacteria are grown under light conditions.

‡Strains H9^T and H15 reduce nitrate to nitrite; strain H10 reduces nitrate to N₂.

§Strains H9^T and H15 are positive; strain H10 is negative.

For cellular fatty acid analysis, the three strains were cultured on tryptic soy agar (Difco) for 24 h at 28 °C and the fatty acids extracted and methylated (Ben-Ze'ev *et al.*, 2005). The fatty acid methyl esters were analysed by gas chromatography using the MIDI/Hewlett Packard microbial identification system (Analytical Services). The major fatty acids (>10 %) of strain H9^T were 15:0 iso, 15:0 anteiso and 17:0 iso 3-OH. Strains H10 and H15 possessed the same major fatty acids except that strain H10 also possessed 17:1 iso ω9c as a major fatty acid.

For determination of the DNA G+C content, genomic DNA of strain H9^T was prepared according to a modification of the procedure of Wilson (1987). The DNA G+C content (mol%) was determined using HPLC analysis of hydrolysed DNA according to Mesbah *et al.* (1989). The analysis was performed by the BCCM/LMG Bacteria Collection Identification Service (Laboratory of Microbiology, Ghent University, Ghent, Belgium). The DNA G+C content of strain H9^T was 38.6 mol%.

Strains H9^T, H10 and H15 were isolated when psychrotolerant bacterial communities in raw milk, and their proteolytic and lipolytic traits, were studied (Hantsis-Zacharov & Halpern, 2007b); 5 % of the isolates were members of the genus *Chryseobacterium* (GenBank accession numbers EF204446–EF204459). The three strains exhibited lipolytic activity, and strains H9^T and H15 also showed proteolytic activity. Heat-stable proteolytic and lipolytic enzymes originating from psychrotolerant bacteria are a limiting factor in the maintenance of the flavour quality of fluid milk and its products. However, the impact of chryseobacterial isolates and, in particular, strains H9^T, H10 and H15 on milk quality remains to be determined.

The main characteristics of the novel isolates are the same as those of the known *Chryseobacterium* strains, i.e. they are non-motile rods, they are catalase- and oxidase-positive, they contain flexirubin-type pigments and they contain 15:0 iso and 17:0 iso 3-OH as the predominant fatty acids. However, the novel isolates differed from the described *Chryseobacterium* species in terms of some phenotypic traits (listed in Table 1) and in having large amounts of the fatty acid 15:0 anteiso (16.6 %) (Bernardet *et al.*, 2006). In addition to containing small amounts of flexirubin-type pigments, the three isolates produced carotenoid-type pigments when grown under light conditions. While flexirubin-type pigments have been found in all *Chryseobacterium* species described to date, the presence of carotenoid-type pigments has not been investigated in these species. Although basically aerobic, the isolates were also able to grow under anaerobic conditions when nitrate was provided, as already reported for some *C. gleum* and *Chryseobacterium indologenes* strains (Holmes *et al.*, 1984; Bernardet *et al.*, 2006).

On the basis of the data from our phenotypic and phylogenetic analysis of the three milk isolates, strains H9^T, H10 and H15 represent a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium bovis* sp. nov. is proposed.

Description of *Chryseobacterium bovis* sp. nov.

Chryseobacterium bovis (bo'vis. L. gen. n. *bovis* of a cow, referring to the isolation from raw cow's milk).

Cells are Gram-negative, non-motile rods, 0.5–0.9 µm wide and 1.1–2.3 µm long. After 48 h incubation on sterile SPC agar at 30 °C in the dark, colonies are circular with entire edges, opaque, smooth and cream-coloured. When light is provided during growth, colonies are yellow-coloured because of the production of carotenoid-type pigments (absorbance peaks at 454 and 481 nm). Weakly positive in the KOH test, indicating the presence of small amounts of flexirubin-type pigments. Good growth occurs under aerobic conditions. Growth is also observed after 5 days under anaerobic conditions on SPC agar containing 0.1 % (w/v) potassium nitrate, but not on SPC agar with 0.5 % glucose. Grows at 7–37 °C (strain H10 grows at temperatures up to 41 °C) (optimum, 30–32 °C), with 0–2.5 %

NaCl (optimum, 0–1.75 % NaCl) and at pH 5.0–9.8 (optimum, pH 6.5–8.5). Good growth occurs on SPC and tryptic soy agars, but not on MacConkey or cetrimide agar. No haemolysis occurs on 5 % sheep-blood agar. Catalase and oxidase activities are present. Casein (except for strain H10) and tributyrin are hydrolysed. In API 20NE tests after 48 h incubation at 32 °C, all three strains hydrolyse aesculin and assimilate glucose, mannose and maltose, but only H9^T and H15 assimilate arabinose, mannitol, *N*-acetylglucosamine, gluconate and adipic and malic acids. The following results are obtained from API 20E strips after 24 h incubation at 32 °C: acetoin is produced; β-galactosidase activity is present (except for strain H10); gelatin is hydrolysed (except for strain H10); H₂S and indole are not produced; urea is not hydrolysed; citrate is not utilized; and arginine dihydrolase, lysine and ornithine decarboxylases and tryptophan deaminase activities are absent. In API 50 CH strips incubated for 48 h at 32 °C, acid is produced from D-glucose, maltose, D-lactose and D-mannose (except for strain H10). In API ZYM strips incubated for 4.5 h, alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase and α-glucosidase activities are present in all the three strains. Cystine arylamidase and β-galactosidase activities are present in strains H9^T and H15 and trypsin activity is present only in strain H10. The predominant cellular fatty acids of the type strain are 15:0 iso (38.9 %), 15:0 anteiso (15.6 %) and 17:0 iso 3-OH (12.7 %). The following fatty acids are present as minor components: summed feature 3 (comprising 15:0 iso 2-OH and/or 16:1ω7c; 8.7 %), 13:0 iso (4.8 %), 16:0 3-OH (3.9 %), 15:0 iso 3-OH (2.6 %), 17:1 iso ω9c (2.5 %), 16:0 (2.1 %), unknown 16.582 (1.7 %), 16:0 iso 3-OH (1.6 %) and 17:0-2-OH (1.3 %). Traces (<1 %) of 13:0 anteiso, 14:0, 14:0 iso, 15:0, 15:0 2-OH and 18:1ω5c are also present. In strain H10, 17:1 iso ω9c is also a major fatty acid (14.8 %). The DNA G+C content of the type strain is 38.6 mol%.

The type strain, H9^T (=LMG 24227^T =DSM 19482^T), was isolated from raw milk in Israel. Strains H15 (=LMG 24229 =DSM 19481) and H10 (=LMG 24228 =DSM 19483) are reference strains.

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References

- Ben-Ze'ev, I. S., Levy, E., Eilam, T. & Anikster, Y. (2005). Whole-cell fatty acid profiles – a tool for species and subspecies classification in the *Puccinia recondita* complex. *J Plant Pathol* **87**, 187–197.
- Bernardet, J.-F., Nakagawa, Y. & Holmes, B. (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.

- Bernardet, J. F., Vancanneyt, M., Matte-Tailliez, O., Grisez, L., Tailliez, P., Bizet, C., Nowakowski, M., Kerouault, B. & Swings, J. (2005).** Polyphasic study of *Chryseobacterium* strains isolated from diseased aquatic animals. *Syst Appl Microbiol* **28**, 640–660.
- Bernardet, J.-F., Hugo, C. & Bruun, B. (2006).** The genera *Chryseobacterium* and *Elizabethkingia*. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 7, pp. 638–676. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.
- de Beer, H., Hugo, C. J., Jooste, P. J., Willems, A., Vancanneyt, M., Coenye, T. & Vandamme, P. A. R. (2005).** *Chryseobacterium vrystaatense* sp. nov., isolated from raw chicken in a chicken processing plant. *Int J Syst Evol Microbiol* **55**, 2149–2153.
- de Beer, H., Hugo, C. J., Jooste, P. J., Vancanneyt, M., Coenye, T. & Vandamme, P. (2006).** *Chryseobacterium piscium* sp. nov., isolated from fish of the South Atlantic Ocean off South Africa. *Int J Syst Evol Microbiol* **56**, 1317–1322.
- Fautz, E. & Reichenbach, H. (1980).** A simple test for flexirubin-type pigments. *FEMS Microbiol Lett* **8**, 87–91.
- Felske, A., Rheims, H., Wolterink, A., Stackebrandt, E. & Akkermans, A. D. (1997).** Ribosome analysis reveals prominent activity of an uncultured member of the class *Actinobacteria* in grassland soils. *Microbiology* **143**, 2983–2989.
- Gallego, V., Garcia, M. T. & Ventosa, A. (2006).** *Chryseobacterium hispanicum* sp. nov., isolated from the drinking water distribution system of Sevilla, Spain. *Int J Syst Evol Microbiol* **56**, 1589–1592.
- González, C. J., Santos, J. A., Garcia-Lopez, M. L. & Otero, A. (2000).** Psychrobacters and related bacteria in freshwater fish. *J Food Prot* **63**, 315–321.
- Hantsis-Zacharov, E. & Halpern, M. (2007a).** *Chryseobacterium haifense* sp. nov., a psychrotolerant bacterium isolated from raw milk. *Int J Syst Evol Microbiol* **57**, 2344–2348.
- Hantsis-Zacharov, E. & Halpern, M. (2007b).** Culturable psychrotrophic bacterial communities in raw milk and their proteolytic and lipolytic traits. *Appl Environ Microbiol* **73**, 7162–7168.
- Holmes, B., Owen, R. J., Steigerwalt, A. G. & Brenner, D. J. (1984).** *Flavobacterium gleum*, a new species found in human clinical specimens. *Int J Syst Bacteriol* **34**, 21–25.
- Hugo, C. J., Jooste, P. J., Segers, P., Vancanneyt, M. & Kersters, K. (1999).** A polyphasic taxonomic study of *Chryseobacterium* strains isolated from dairy sources. *Syst Appl Microbiol* **22**, 586–595.
- Hugo, C. J., Segers, P., Hoste, B., Vancanneyt, M. & Kersters, K. (2003).** *Chryseobacterium joostei* sp. nov., isolated from the dairy environment. *Int J Syst Evol Microbiol* **53**, 771–777.
- Kämpfer, P., Dreyer, U., Neef, A., Dott, W. & Busse, H.-J. (2003).** *Chryseobacterium defluvii* sp. nov., isolated from wastewater. *Int J Syst Evol Microbiol* **53**, 93–97.
- Kim, K. K., Bae, H.-S., Schumann, P. & Lee, S.-T. (2005a).** *Chryseobacterium daecheongense* sp. nov., isolated from freshwater lake sediment. *Int J Syst Evol Microbiol* **55**, 133–138.
- Kim, K. K., Kim, M. K., Lim, J. H., Park, H. Y. & Lee, S. T. (2005b).** Transfer of *Chryseobacterium meningosepticum* and *Chryseobacterium miricola* to *Elizabethkingia* gen. nov. as *Elizabethkingia meningoseptica* comb. nov. and *Elizabethkingia miricola* comb. nov. *Int J Syst Evol Microbiol* **55**, 1287–1293.
- Kumar, S., Tamura, K. & Nei, M. (2004).** MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Park, M. S., Jung, S. R., Lee, K. H., Lee, M. S., Do, J. O., Kim, S. B. & Bae, K. S. (2006).** *Chryseobacterium soldanellicola* sp. nov. and *Chryseobacterium taeanense* sp. nov., isolated from roots of sand-dune plants. *Int J Syst Evol Microbiol* **56**, 433–438.
- Peterson, W. J., Bell, T. A., Etchells, J. L. & Smart, W. W. G., Jr (1954).** A procedure for demonstrating the presence of carotenoid pigments in yeasts. *J Bacteriol* **67**, 708–713.
- Quan, Z. X., Kim, K. K., Kim, M. K., Jin, L. & Lee, S. T. (2007).** *Chryseobacterium caeni* sp. nov., isolated from bioreactor sludge. *Int J Syst Evol Microbiol* **57**, 141–145.
- Shen, F.-T., Kämpfer, P., Young, C.-C., Lai, W.-A. & Arun, A. B. (2005).** *Chryseobacterium taichungense* sp. nov., isolated from contaminated soil. *Int J Syst Evol Microbiol* **55**, 1301–1304.
- Shimomura, K., Kaji, S. & Hiraishi, A. (2005).** *Chryseobacterium shigense* sp. nov., a yellow-pigmented, aerobic bacterium isolated from a lactic acid beverage. *Int J Syst Evol Microbiol* **55**, 1903–1906.
- Tai, C. J., Kuo, H. P., Lee, F. L., Chen, H. K., Yokota, A. & Lo, C. C. (2006).** *Chryseobacterium taiwanense* sp. nov., isolated from soil in Taiwan. *Int J Syst Evol Microbiol* **56**, 1771–1776.
- Vandamme, P., Bernardet, J.-F., Segers, P., Kersters, K. & Holmes, B. (1994).** New perspectives in the classification of the flavobacteria: description of *Chryseobacterium* gen. nov., *Bergeyella* gen. nov., and *Empedobacter* nom. rev. *Int J Syst Bacteriol* **44**, 827–831.
- Weon, H. Y., Kim, B. Y., Yoo, S. H., Kwon, S. W., Cho, Y. H., Go, S. J. & Stackebrandt, E. (2006).** *Chryseobacterium wanjuense* sp. nov., isolated from greenhouse soil in Korea. *Int J Syst Evol Microbiol* **56**, 1501–1504.
- Wilson, K. (1987).** Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 2.4.1–2.4.5. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Green Publishing & Wiley-Interscience.
- Yabuuchi, E., Kaneko, T., Yano, I., Moss, C. W. & Miyoshi, N. (1983).** *Sphingobacterium* gen. nov., *Sphingobacterium spiritivorum* comb. nov., *Sphingobacterium multivorum* comb. nov., *Sphingobacterium mizutae* sp. nov., and *Flavobacterium indologenes* sp. nov.: glucose nonfermenting Gram-negative rods in CDC groups IIC-2 and IIB. *Int J Syst Bacteriol* **33**, 580–598.
- Yoon, J. H., Kang, S. J. & Oh, T. K. (2007).** *Chryseobacterium daeguense* sp. nov., isolated from wastewater of a textile dye works. *Int J Syst Evol Microbiol* **57**, 1355–1359.
- Young, C. C., Kämpfer, P., Shen, F. T., Lai, W. A. & Arun, A. B. (2005).** *Chryseobacterium formosense* sp. nov., isolated from the rhizosphere of *Lactuca sativa* L. (garden lettuce). *Int J Syst Evol Microbiol* **55**, 423–426.