# *Chryseobacterium gallinarum* sp. nov., isolated from a chicken, and *Chryseobacterium contaminans* sp. nov., isolated as a contaminant from a rhizosphere sample

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Two yellow-pigmented bacterial strains (100$^T$ and C26$^T$), showing 98.4 % 16S rRNA gene sequence similarity to each other and isolated from a chicken in Germany and as a contaminant from an agar plate of a rhizosphere sample in Alabama, were studied by using a polyphasic taxonomic approach. Cells of both isolates were rod-shaped and stained Gram-negative. A comparison of the 16S rRNA gene sequences of the two organisms with the sequences of the type strains of the most closely related species of the genus *Chryseobacterium* showed the highest sequence similarities of strains 100$^T$ and C26$^T$ to the type strains of *Chryseobacterium joostei* (respectively 97.5 and 98.2 %), *C. viscerum* (96.6, 97.8 %), *C. gleum* (97.1, 97.7 %), *C. arthrosphaerae* (97.3%, 97.7 %), *C. indologenes* (97.2, 97.7 %), *C. tructae* (96.6, 97.6 %), *C. jejuense* (97.0, 97.6 %) and *C. oncorhynchi* (96.3, 97.5 %); 16S rRNA gene sequence similarities to members of all other species of the genus *Chryseobacterium* were below 97.5 %. The fatty acid profiles of both strains consisted of the major fatty acids iso-$C_{15:0}$, summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), iso-$C_{17:1}\omega9c$ and iso-$C_{17:0}$ 3-OH, but also showed slight differences (absence or presence of $C_{16:0}$ 3-OH and iso-$C_{15:1}$ F). DNA–DNA hybridizations between the two strains and between the novel strains and the type strains of *C. joostei*, *C. indologenes*, *C. jejuense*, *C. tructae* and *C. viscerum* resulted in relatedness values clearly below 70 %. These DNA–DNA hybridization results and the differentiating biochemical and chemotaxonomic properties showed that both strains 100$^T$ and C26$^T$ represent novel species, for which the names *Chryseobacterium gallinarum* sp. nov. (type strain 100$^T$=LMG 27808$^T$=CCM 8493$^T$) and *Chryseobacterium contaminans* sp. nov. (type strain C26$^T$=LMG 27810$^T$=CCM 8492$^T$) are proposed.

The genus *Chryseobacterium*, described by Vandamme *et al.* (1994), comprises many species from very different sources. In the last few years, the number of species of the genus has increased to more than 70 (e.g. Hugo *et al.*, 2003; Kämpfer *et al.*, 2003, 2009, 2010a, b, 2011; Li *et al.*, 2003; de Beer *et al.*, 2005; Kim *et al.*, 2005, 2008; Shen *et al.*, 2005; Shimomura *et al.*, 2005; Young *et al.*, 2005; Park *et al.*, 2006; Tai *et al.*, 2006; Behrendt *et al.*, 2007, 2008; Hantsis-Zacharov *et al.*, 2008; Herzog *et al.*, 2008; Szoboszlay *et al.*, 2008; Weon *et al.*, 2008; Cho *et al.*, 2010), with more than 10 species proposed in 2013 (Bajerski *et al.*, 2013; Charimba *et al.*, 2013; Hoang *et al.*, 2013; Holmes *et al.*, 2013; Kirk *et al.*, 2013; Montero-Calasanz *et al.*, 2013; Nguyen *et al.*, 2013; Park *et al.*, 2013; Sang *et al.*, 2013; Wu *et al.*, 2013). They have been isolated from a wide variety of samples, including environmental and also clinical sources (Bernardet *et al.*, 2006; Kämpfer *et al.*, 2009; Holmes *et al.*, 2013).

Two yellow-pigmented strains, one isolated from a pharyngeal scrape sample obtained from a living and apparently

Abbreviations: DDH, DNA–DNA hybridization; pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 100$^T$ and C26$^T$ are KC494697 and KF652079.

healthy chicken in Saxony-Anhalt, Germany (100$^T$), and another as a contaminant on an agar plate from a rhizosphere sample (C26$^T$), isolated on CHROMagar Acinetobacter (CHROMagar, Paris, France) and tryptic soy agar (TSA; Oxoid), respectively, showed some morphological similarities and were studied comparatively. Strain C26$^T$ appeared on about a dozen different culture plates after transferring cultures. The origin of the contaminant was never determined, but it appeared to be part of a batch of TSA that was not sterilized thoroughly in the autoclave. The source of the organism was Auburn University, Auburn, AL, USA.

Strains 100$^T$ and C26$^T$ were maintained and subcultivated on nutrient agar (NA; Oxoid) at 30 °C for 48 h and subsequently analysed for their 16S rRNA gene sequence, fatty acid methyl ester composition of whole-cell hydrolysates, further phenotypic features and DNA–DNA relatedness to those type strains most closely related on the basis of 16S rRNA gene sequence similarity. Reference strains for physiological and biochemical tests and fatty acid analysis (*C. joostei* CCUG 46665$^T$, *C. viscerum* 687B-08$^T$ and *C. tructae* 1084-08$^T$) and further reference strains for DNA–DNA relatedness analysis were grown under the same conditions as the novel strains.

Cultural and morphological characteristics were determined from cultures grown on NA. The Gram reaction was tested on the basis of a modified method of Gerhardt *et al.* (1994) and the motility test was done under a light microscope on cells grown for 3 days in nutrient broth (Oxoid) at 30 °C. Oxidase activity was tested using the bioMérieux oxidase reagent according to the instructions of the manufacturer. Catalase activity was tested by gas formation after dropping $H_2O_2$ on fresh biomass grown for 48 h on NA (Oxoid). Growth was investigated at 4, 8 or 10, 30, 37, 45 and 50 °C on NA. NaCl tolerance was investigated in tryptic soy broth (TSB; Oxoid) supplemented with 0.5–8.0 % (w/v) NaCl. The dependence of growth on pH was also tested in TSB adjusted to pH 4.5–12.5 (in increments of 1.0 pH unit) by the addition of HCl or NaOH.

Both strains showed Gram-negative staining behaviour and formed visible (diameter about 2 mm) yellowish colonies within 48 h at 30 °C. Colonies of the two strains were quite similar, translucent and glistening with entire edges. A bright yellow pigment of the flexirubin type [KOH method according to Reichenbach (1989)] was produced by both strains on NA. Strains 100$^T$ and C26$^T$ were positive for oxidase activity. Cells of both strains were non-motile, non-spore-forming rods (approx. 1 μm wide and 2 μm long). Both strains grew well on NA, brain heart infusion agar, R2A agar and TSA but not on MacConkey agar (Oxoid). For strain C26$^T$, no growth was observed below 8 °C or above 37 °C; for strain 100$^T$, no growth was observed below 8 °C or above 45 °C. Strain C26$^T$ grew in TSB at 28 °C in the presence of 0–2 % (w/v) NaCl, but not 3 %; strain 100$^T$ also grew with 0–2 % NaCl and grew weakly in the presence of 3 % NaCl but did not grow in the presence of 4 % NaCl. Both strains grew in TSB adjusted to pH 5.5–12.5, but not pH 4.5.

Detailed physiological characterization and biochemical tests were performed to assess the carbon source utilization pattern and hydrolysis of chromogenic substrates as described by Kämpfer *et al.* (1991). Additional biochemical tests were performed: production of hydrogen sulphide, using the lead acetate paper and triple-sugar-iron methods, the indole reaction with Ehrlich's and Kovacs' reagents, activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and DNase (Oxoid CM321; supplemented with 0.01 % toluidine blue), β-galactosidase (ONPG) and urease on Christensen's urea agar and hydrolysis of casein, gelatin (plate method), starch and tyrosine (Smibert & Krieg, 1994). Both strains utilized only very few carbon sources, similar to all species of the genus *Chryseobacterium*, but were able to hydrolyse many chromogenic substrates. Biochemical and physiological data are given in Table 1 and in the species descriptions.

Phylogenetic analyses based on nearly full-length 16S rRNA gene sequences were performed in ARB release 5.2 (Ludwig *et al.*, 2004) using the All-Species Living Tree Project (LTP; Yarza *et al.*, 2008) database release LTPs111 (February 2013). Sequences not included in the LTP database were aligned with SINA (version 1.2.11; Pruesse *et al.*, 2012) and implemented in the LTP database. The alignment including all sequences used for tree reconstruction was checked manually based on secondary structure information. Pairwise 16S rRNA gene sequence similarities were calculated using the ARB neighbour-joining tool without an evolutionary substitution model. Phylogenetic trees were reconstructed with the maximum-likelihood method using RAxML version 7.04 (Stamatakis, 2006) with GTR-Gamma and rapid bootstrap analysis (100 resamplings) and based on 16S rRNA gene sequences between sequence positions 82 and 1394 (according to the *Escherichia coli* numbering; Brosius *et al.*, 1978).

The sequenced 16S rRNA gene fragments from strains 100$^T$ and C26$^T$ were respectively continuous stretches of 1394 (positions 20–1430) and 1397 (positions 43–1469) unambiguous nucleotides. Strains 100$^T$ and C26$^T$ shared 98.1 % 16S rRNA gene sequence similarity. The 16S rRNA gene sequence similarities to type strains of closest related species of the genus *Chryseobacterium* were respectively 97.5 and 98.2 % to *C. joostei*, 96.6 and 97.8 % to *C. viscerum*, 97.1 and 97.7 % to *C. gleum*, 97.3 and 97.7 % to *C. arthrosphaerae*, 97.2 and 97.7 % to *C. indologenes*, 96.6 and 97.6 % to *C. tructae*, 97.0 and 97.6 % to *C. jejuense*, 96.3 and 97.5 % to *C. oncorhynchi*, 97.4 and 97.1 % to *C. aquifrigidense*, 96.3 and 97.3 % to *C. ureilyticum* and 96.9 and 97.1 % to *C. culicis*. Sequence similarities to members of all other species of the genus *Chryseobacterium* were below 97.5 %. The reconstruction of a phylogenetic tree using the maximum-likelihood method showed that the two strains formed a distinct cluster (bootstrap support of 99 %) and grouped together with the type strains of *C. joostei* and *C. oncorhynchi*, but without high bootstrap support (Fig. 1).

**Table 1.** Comparison of characteristics of strains C26$^T$ and 100$^T$ with the type strains of the most closely related species of the genus *Chryseobacterium*

Strains/species: 1, C26$^T$; 2, 100$^T$; 3, *C. joostei* CCUG 46665$^T$; 4, *C. viscerum* 687B-08$^T$; 5, *C. tructae* 1084-08$^T$; 6, *C. culicis* R4-1A$^T$; 7, *C. jejuense* KACC 12501$^T$; 8, *C. indologenes* CCUG 14556$^T$; 9, *C. aquifrigidense* KCTC 12894$^T$; 10, *C. arthrosphaerae* CC-VM7$^T$; 11, *C. gleum* (two strains). Data were obtained in this study (columns 1–5) or were taken from Kämpfer *et al.* (2010a, b) (columns 6–10) or Hugo *et al.* (2003) (column 11). Analyses by Kämpfer *et al.* (2010 a, b) were performed under the same conditions as in the present study. +, Positive; (+), weakly positive; −, negative; ND, no data available. All strains hydrolysed aesculin and produced indole from tryptophan. None of the strains produced acid from lactose.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Growth on MacConkey agar | − | − | − | − | − | − | + | + | + | − | ND |
| Acid production from: | | | | | | | | | | | |
| L-Arabinose | + | − | − | − | − | − | − | − | − | − | + |
| D-Glucose | + | (+) | + | + | + | + | + | + | + | + | + |
| Maltose | + | − | + | + | + | + | + | + | + | + | + |
| D-Mannitol | − | − | − | − | − | + | − | − | − | (+) | − |
| Trehalose | + | − | + | + | + | + | + | + | + | − | + |
| Sucrose | − | − | − | + | − | − | − | − | − | − | ND |
| D-Fructose | − | − | − | − | − | − | − | − | − | − | + |
| D-Xylose | − | − | − | − | − | − | − | − | − | − | + |
| Nitrate reduction | − | − | − | − | − | − | − | + | − | + | + |
| Urease activity | + | + | + | + | + | + | + | − | + | + | − |

DNA–DNA hybridization (DDH) experiments were performed between strains 100$^T$ and C26$^T$ and the type strains of the five most closely related species of the genus *Chryseobacterium* (at least those showing >97.5 % 16S rRNA gene sequence similarity) according to the method of Ziemke *et al.* (1998) (except that, for nick translation, 2 μg DNA was labelled for 3 h of incubation at 15 °C). Strains 100$^T$ and C26$^T$ showed low DDH (36 %, reciprocal 22.4 %). Strain 100$^T$ showed low to moderate DDH to *C. joostei* CCUG 46665$^T$ (38.4 %, reciprocal 54.3 %), *C. aquifrigidense* KCTC 12894$^T$ (49.4 %, reciprocal 47.9 %), *C. arthrosphaerae* CC-VM-7$^T$ (17.3 %), *C. indologenes* CCUG 14556$^T$ (19.8 %, reciprocal 54.6 %), *C. gleum* CCUG 14555$^T$ (60.4 %, reciprocal 30.2 %), *C. jejuense* KACC 12501$^T$ (59.4 %, reciprocal 46.6 %) and *C. tructae* 1084-08$^T$ (15.1 %, reciprocal 65.3 %). Strain C26$^T$ also showed low to moderate DDH to the type strains of the most closely related species of the genus *Chryseobacterium*, *C. joostei* CCUG 46665$^T$ (20.4 %, reciprocal 22.5 %), *C. viscerum* 687B-08$^T$ (41.3 %, reciprocal 50.2 %), *C. indologenes* CCUG 14556$^T$ (67.5 %, reciprocal 53.7 %), *C. jejuense* KACC 12501$^T$ (19.8 %, reciprocal 30.5 %) and *C. tructae* 1084-08$^T$ (54.1 %, reciprocal 62.2 %).

Genomic fingerprint analysis based on three rep-PCR techniques [BOX, $(GTG)_5$ and ERIC PCRs] were also performed to differentiate strains 100$^T$ and C26$^T$ at the genomic level. Analysis was performed as described previously (Glaeser *et al.*, 2013) using the following primers: BOXA1R (5′-CTACGGCAAGGCGACGCTGACG-3′) for BOX PCR, $(GTG)_5$ (5′-GTGGTGGTGGTGGTG-3′) for $(GTG)_5$ PCR (both from Versalovic *et al.*, 1994) and ERIC1R (5′-ATGTAAGCTCCTGGGGATTCA-3′) and ERIC2 (5′-AAGTAAGTGACTGGGGTGAGC-3′) for ERIC PCR (Versalovic *et al.*, 1991). All three fingerprint patterns showed clear differences between the two strains at the genomic level (Fig. 2) and therefore supported the distinction of the two strains into separate species, as indicated by DDH analysis.

Analysis of the cellular fatty acid profiles of whole-cell hydrolysates was done as described previously (Kämpfer & Kroppenstedt, 1996) and revealed the following most abundant fatty acids for both strains: iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{17:1}\omega9c$ and summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$). The complete fatty acid patterns of strains 100$^T$ and C26$^T$ also showed slight differences and are shown in Table 2 in comparison with those of the type strains of the most closely related species of the genus *Chryseobacterium*.

Polyamines, quinones and polar lipids of the two isolates were extracted from biomass grown on PYE medium (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2). Biomass subjected to polyamine extraction was harvested at the late exponential growth phase, whereas biomass subjected to quinone and polar lipid extraction was harvested at stationary growth phase. Polyamine analysis was carried out as described previously (Busse & Auling, 1988; Busse *et al.*, 1997). Quinones and polar lipids were extracted as described by Tindall (1990a, b) and Altenburger *et al.* (1996). HPLC analyses were carried out using the equipment described by Stolz *et al.* (2007). The polyamine patterns of strains 100$^T$ and C26$^T$, with the major compound *sym*-homospermidine, are consistent with the emended description of the genus *Chryseobacterium* (Kämpfer *et al.*, 2009). The polyamine pattern of strain 100$^T$ contained (per g dry weight) 21.0 μmol

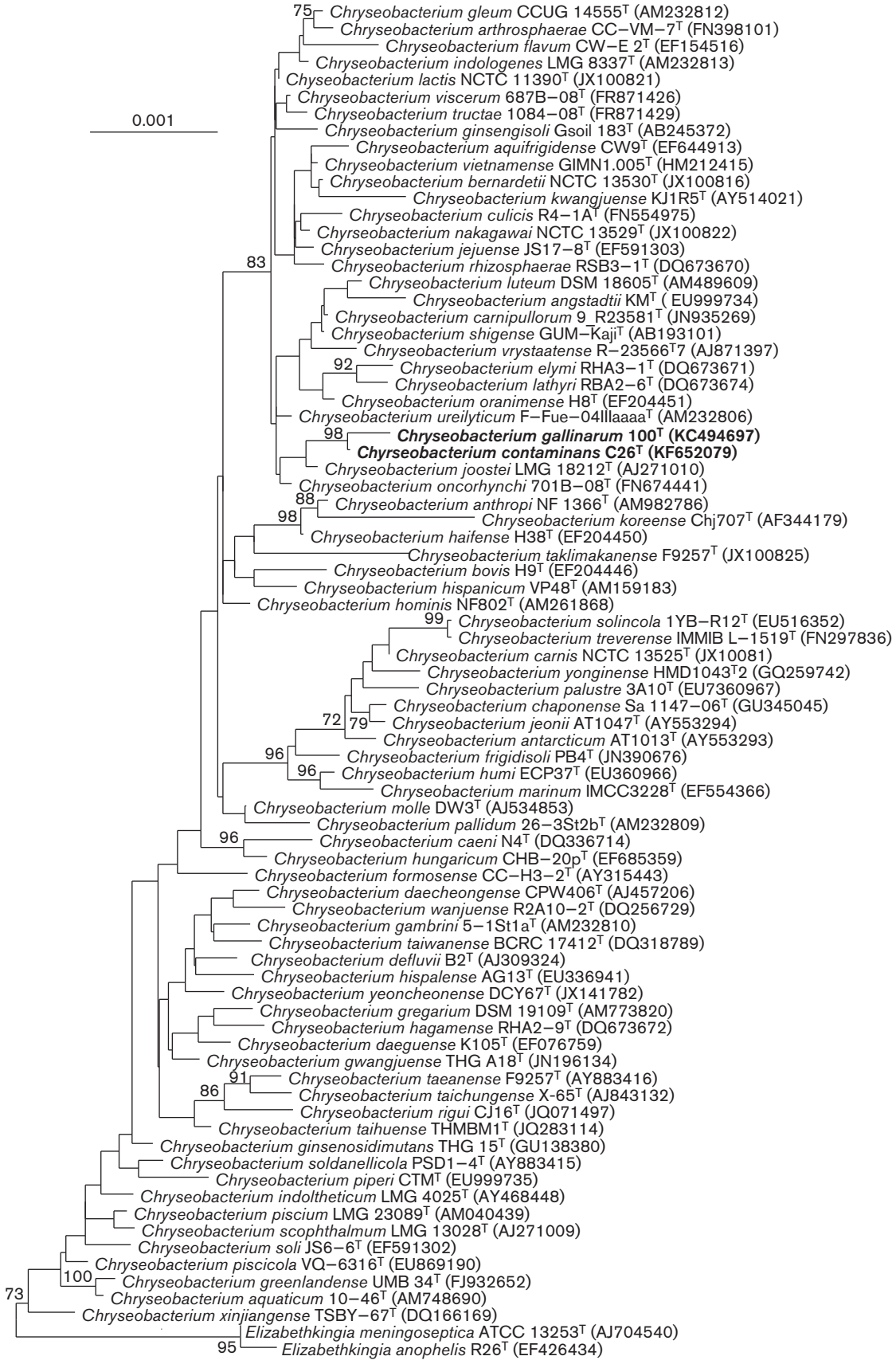
0.001
Chryseobacterium gleum CCUG 14555T (AM232812)
Chryseobacterium arthrosphaerae CC–VM–7T (FN398101)
Chryseobacterium flavum CW–E 2T (EF154516)
Chryseobacterium indologenes LMG 8337T (AM232813)
Chyseobacterium lactis NCTC 11390T (JX100821)
Chryseobacterium viscerum 687B–08T (FR871426)
Chryseobacterium tructae 1084–08T (FR871429)
Chryseobacterium ginsengisoli Gsoil 183T (AB245372)
Chryseobacterium aquifrigidense CW9T (EF644913)
Chryseobacterium vietnamense GIMN1.005T (HM212415)
Chryseobacterium bernardetii NCTC 13530T (JX100816)
Chryseobacterium kwangjuense KJ1R5T (AY514021)
Chryseobacterium culicis R4–1AT (FN554975)
Chyrseobacterium nakagawai NCTC 13529T (JX100822)
Chryseobacterium jejuense JS17–8T (EF591303)
Chryseobacterium rhizosphaerae RSB3–1T (DQ673670)
Chryseobacterium luteum DSM 18605T (AM489609)
Chryseobacterium angstadtii KMT ( EU999734)
Chryseobacterium carnipullorum 9_R23581T (JN935269)
Chryseobacterium shigense GUM–KajiT (AB193101)
Chryseobacterium vrystaatense R–23566T7 (AJ871397)
Chryseobacterium elymi RHA3–1T (DQ673671)
Chryseobacterium lathyri RBA2–6T (DQ673674)
Chryseobacterium oranimense H8T (EF204451)
Chryseobacterium ureilyticum F–Fue–04IIIaaaaT (AM232806)
Chryseobacterium gallinarum 100T (KC494697)
Chyrseobacterium contaminans C26T (KF652079)
Chryseobacterium joostei LMG 18212T (AJ271010)
Chryseobacterium oncorhynchi 701B–08T (FN674441)
Chryseobacterium anthropi NF 1366T (AM982786)
Chryseobacterium koreense Chj707T (AF344179)
Chryseobacterium haifense H38T (EF204450)
Chryseobacterium taklimakanense F9257T (JX100825)
Chryseobacterium bovis H9T (EF204446)
Chryseobacterium hispanicum VP48T (AM159183)
Chryseobacterium hominis NF802T (AM261868)
Chryseobacterium solincola 1YB–R12T (EU516352)
Chryseobacterium treverense IMMIB L–1519T (FN297836)
Chryseobacterium carnis NCTC 13525T (JX10081)
Chryseobacterium yonginense HMD1043T2 (GQ259742)
Chryseobacterium palustre 3A10T (EU7360967)
Chryseobacterium chaponense Sa 1147–06T (GU345045)
Chryseobacterium jeonii AT1047T (AY553294)
Chryseobacterium antarcticum AT1013T (AY553293)
Chryseobacterium frigidisoli PB4T (JN390676)
Chryseobacterium humi ECP37T (EU360966)
Chryseobacterium marinum IMCC3228T (EF554366)
Chryseobacterium molle DW3T (AJ534853)
Chryseobacterium pallidum 26–3St2bT (AM232809)
Chryseobacterium caeni N4T (DQ336714)
Chryseobacterium hungaricum CHB–20pT (EF685359)
Chryseobacterium formosense CC–H3–2T (AY315443)
Chryseobacterium daecheongense CPW406T (AJ457206)
Chryseobacterium wanjuense R2A10–2T (DQ256729)
Chryseobacterium gambrini 5–1St1aT (AM232810)
Chryseobacterium taiwanense BCRC 17412T (DQ318789)
Chryseobacterium defluvii B2T (AJ309324)
Chryseobacterium hispalense AG13T (EU336941)
Chryseobacterium yeoncheonense DCY67T (JX141782)
Chryseobacterium gregarium DSM 19109T (AM773820)
Chryseobacterium hagamense RHA2–9T (DQ673672)
Chryseobacterium daeguense K105T (EF076759)
Chryseobacterium gwangjuense THG A18T (JN196134)
Chryseobacterium taeanense F9257T (AY883416)
Chryseobacterium taichungense X-65T (AJ843132)
Chryseobacterium rigui CJ16T (JQ071497)
Chryseobacterium taihuense THMBM1T (JQ283114)
Chryseobacterium ginsenosidimutans THG 15T (GU138380)
Chryseobacterium soldanellicola PSD1–4T (AY883415)
Chryseobacterium piperi CTMT (EU999735)
Chryseobacterium indoltheticum LMG 4025T (AY468448)
Chryseobacterium piscium LMG 23089T (AM040439)
Chryseobacterium scophthalmum LMG 13028T (AJ271009)
Chryseobacterium soli JS6–6T (EF591302)
Chryseobacterium piscicola VQ–6316T (EU869190)
Chryseobacterium greenlandense UMB 34T (FJ932652)
Chryseobacterium aquaticum 10–46T (AM748690)
Chryseobacterium xinjiangense TSBY–67T (DQ166169)
Elizabethkingia meningoseptica ATCC 13253T (AJ704540)
Elizabethkingia anophelis R26T (EF426434)
75
83
92
98
88
98
99
72
79
96
96
96
91
86
100
73
95

**Fig. 1.** Maximum-likelihood tree based on nearly full-length 16S rRNA gene sequences showing the phylogenetic position of strains 100$^T$ and C26$^T$ among type strains of the genus *Chryseobacterium*. The tree was calculated in ARB using RAxML with rapid bootstrap analysis and 100 replications. Nucleotides between positions 82 and 1394 (*E. coli* numbering; Brosius *et al.*, 1978) were included in the analysis. Bootstrap values above 70 % are shown at branch nodes. Two type strains of the genus *Elizabethkingia* were used as an outgroup. Bar, 0.001 nucleotide substitutions per site.

*sym*-homospermidine, 0.5 μmol spermidine, 0.4 μmol spermine, 0.1 μmol putrescine and cadaverine and traces (<0.1 μmol) of 1,3-diaminopropane and *sym*-norspermidine. The polyamine pattern of strain C26$^T$ contained (per g dry weight) 32.4 μmol *sym*-homospermidine, 0.5 μmol spermine, 0.2 μmol spermidine, 0.1 μmol *sym*-norspermidine and traces (<0.1 μmol) of putrescine and cadaverine. The quinone system of strain C26$^T$ contained predominantly menaquinone MK-6 (96 %), MK-5 (4 %) and traces of MK-7 (<0.1) and that of strain 100$^T$ contained exclusively MK-6. The only identified lipid in the polar lipid profile of strain C26$^T$ was phosphatidylethanolamine, which was present as a major component. Additional major compounds detected were unidentified lipids, including four glycolipids, GL1, GL3, GL4 and GL6, three aminolipids, AL1, AL3 and AL4, one aminophospholipid (APL1) and two polar lipids not containing a sugar moiety, an amino group or a phosphate group (L3, L8). Furthermore, moderate to minor amounts of one aminolipid (AL2), two glycolipids (GL2, GL5) and six polar lipids (L1, L2, L4–L7) were detected (Fig. 3a). Strain 100$^T$ showed an almost identical polar lipid profile, differing only in the presence of an additional lipid L9 and the absence of lipid L6 (Fig. 3b). Detection of phosphatidylethanolamine as the only identifiable lipid is a common trait of chryseobacteria and also other species of the phylum *Bacteroidetes*. The presence and distribution of unidentified aminolipids and polar lipids, not stainable with any of the specific reagents that detect glycolipids, aminolipids and phospholipids, is quite similar to the polar lipid profiles reported for other members of the genus *Chryseobacterium* such as *C. defluvii* B2$^T$, *C. gambrini* 5-1Sta$^T$, *C. molle* DW3$^T$, *C. ginsengisoli* DCY 63$^T$, *C. indoltheticum* LMG 4025$^T$ and *C. gleum* ATCC 35910$^T$ (Kämpfer *et al.*, 2003; Herzog *et al.*, 2008; Nguyen *et al.*, 2013). However, the presence of the six glycolipids with their specific chromatographic motility clearly distinguishes strains C26$^T$ and 100$^T$ from all species of the genus *Chryseobacterium* described so far and reflects their close phylogenetic relatedness.

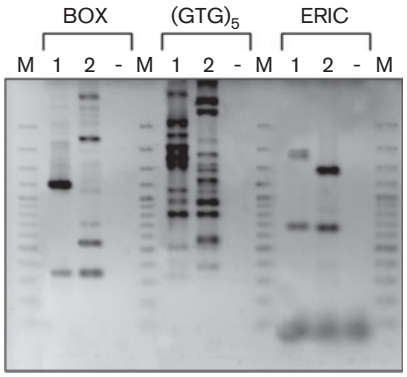


**Fig. 2.** Comparative genomic fingerprint analysis of strains C26$^T$ and 100$^T$ using BOX, $(GTG)_5$ and ERIC-PCR. Strains: 1, C26$^T$; 2, 100$^T$. Agarose gel (1.5 % in 1× TBE) stained with ethidium bromide. M, 100 bp Genomic DNA Ladder Plus (Fermentas); –, negative controls.

On the basis of the results of this polyphasic study, it is obvious that strains 100$^T$ and C26$^T$ represent two novel species of the genus *Chryseobacterium*, for which the names *Chryseobacterium gallinarum* sp. nov. and *Chryseobacterium contaminans* sp. nov. are respectively proposed.

## Description of *Chryseobacterium gallinarum* sp. nov.

*Chryseobacterium gallinarum* (gal.li.na′rum. L. gen. fem. pl. n. *gallinarum* from/of chickens or hens).

Cells are Gram-negative staining, non-motile, non-spore-forming rods, approx. 1 μm wide and 2 μm long. Aerobic and oxidase- and catalase-positive. Good growth occurs after 48 h on NA, brain heart infusion agar, TSA and R2A agar (all Oxoid), but not on MacConkey agar (Oxoid) at 28 °C. Growth occurs on NA at 10–45 °C, but not at 4 or 50 °C. Grows at 28 °C in the presence of 1.0–2.0 % NaCl (weak growth at 3 %, no growth at 4 %) as an additional ingredient of TSB and at pH 5.5–12.5 but not at pH 4.5. Colonies on NA are smooth, yellowish, circular, translucent and glistening with entire edges. Colonies become mucoid and cannot be identified as single entities after prolonged incubation. The yellow pigment of the flexirubin type is non-diffusible and non-fluorescent. Produces acid weakly from D-glucose. No acid is produced from adonitol, L-arabinose, D-arabitol, dulcitol, erythritol, *myo*-inositol, lactose, maltose, trehalose, D-mannitol, melibiose, methyl α-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose or D-xylose. Urease activity, indole production and hydrolysis of casein, gelatin, starch, DNA and tyrosine are positive, while production of brown diffusible pigments on tyrosine agar, hydrogen sulphide production and activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and β-galactosidase (ONPG) are negative. The following compounds are not utilized as sole sources of carbon: acetate, propionate, *N*-acetylgalactosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, cellobiose, D-galactose, gluconate, D-glucose, maltose, D-mannose, D-fructose, trehalose, glycerol, D-mannitol, maltitol, melibiose, L-rhamnose, D-ribose, sucrose, salicin, D-xylose,

**Table 2.** Long-chain fatty acid compositions of strains C26$^T$ and 100$^T$ and type strains of related species of the genus *Chryseobacterium*

Strains/species: 1, C26$^T$; 2, 100$^T$; 3, *C. joostei* CCUG 46665$^T$; 4, *C. viscerum* 687B-08$^T$; 5, *C. tructae* 1084-08$^T$; 6, *C. culicis* R4-1A$^T$; 7, *C. jejuense* KACC 12501$^T$; 8, *C. indologenes* CCUG 14556$^T$; 9, *C. aquifrigidense* KCTC 12894$^T$; 10, *C. arthrosphaerae* CC-VM7$^T$; 11, *C. gleum*. Data were obtained from this study (columns 1–5) or from Kämpfer *et al.* (2010a, b) (columns 6–10); data in parentheses and in column 11 (obtained from cells grown on TSA at 20 °C for 24 h) are means ± SD taken from a survey of 45 *C. indologenes* strains and five *C. gleum* strains by Hugo *et al.* (2003). Fatty acids amounting to less than 1 % of the total fatty acids in all strains were not included. TR, Trace (less than 1.0 %); −, not detected.

| Fatty acid | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| iso-$C_{13:0}$ | − | − | − | − | 0.8 | TR | 1.0 | − (TR) | − | TR | TR |
| Unknown 13.565* | 4.6 | 18.1 | 8.4 | 7.5 | 8.3 | 8.7 | 8.5 | 13.1 (21 ± 0.7) | 4.2 | 20.5 | 1.2 ± 0.4 |
| iso-$C_{15:0}$ | 34.2 | 30.3 | 34.9 | 34.3 | 32.9 | 33.9 | 31.2 | 28.5 (34.3 ± 4.9) | 36.2 | 32.3 | 35.4 ± 2.9 |
| anteiso-$C_{15:0}$ | − | − | 1.2 | − | − | TR | TR | − | − | − | TR |
| iso-$C_{15:0}$ 3-OH | 2.5 | 2.9 | 3.7 | 3.4 | 3.1 | 2.5 | 2.5 | 2.1 (2.6 ± 0.2) | 3.0 | 1.0 | 2.5 ± 0.1 |
| iso-$C_{15:1}$ F | − | 2.8 | 1.7 | 2.1 | 2.0 | TR | TR | 3.4 (−) | − | 4.6 | − |
| $C_{16:0}$ | 1.5 | 2.1 | − | 1.0 | 1.7 | 1.8 | 1.7 | 1.2 (TR) | 1.8 | 1.6 | 1.3 ± 0.1 |
| $C_{15:0}$ 3-OH | − | − | − | − | − | 1.0 | − | − | − | − | − |
| $C_{16:0}$ 3-OH | 1.8 | − | 1.9 | 1.8 | 1.9 | 1.3 | 1.3 | TR (1.0 ± 0.2) | 2.3 | − | 1.1 ± 0.1 |
| iso-$C_{16:0}$ 3-OH | − | − | − | 1.6 | 1.2 | − | − | 0.8 | − | − | − |
| Unknown 16.582* | 1.2 | − | 1.2 | 1.3 | 1.4 | 1.3 | 1.4 | 1.3 (1.7 ± 0.2) | 1.2 | 1.7 | 1.6 ± 0.1 |
| $C_{17:0}$ 2-OH | − | − | − | − | − | − | − | − | − | 2.0 | − |
| iso-$C_{17:0}$ | 1.4 | 1.6 | − | 0.9 | 1.3 | 1.3 | 1.5 | 1.1 (TR) | 1.0 | 2.0 | 1.6 ± 0.6 |
| iso-$C_{17:0}$ 3-OH | 22.0 | 14.2 | 21.3 | 20.2 | 20.1 | 15.5 | 15.4 | 14.4 (19.2 ± 1.8) | 18.2 | 3.5 | 21.8 ± 0.3 |
| iso-$C_{17:1}\omega 9c$ | 18.2 | 12.1 | 13.5 | 14.5 | 15.6 | 20.7 | 21.9 | 23.4 (24.2 ± 3.1) | 17.3 | 18.9 | 20.2 ± 3.9 |
| Summed feature 3† | 12.4 | 15.8 | 12.3 | 11.2 | 9.8 | 10.0 | 11.0 | 9.8 (11.1 ± 1.3) | 13.8 | 5.5 | 11.8 ± 0.8 |
| Summed feature 5† | − | − | − | − | − | − | − | TR (TR) | − | 1.0 | TR |

*Unknown fatty acids; numbers indicate the equivalent chain-length (ECL). Values for ECL 13.565/13.566 and 16.580/16.582 are respectively combined in single rows.

†Fatty acids that could not be separated by GC using the Microbial Identification System (Microbial ID) software were considered summed features. Summed feature 3 contains $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH; summed feature 5 contains iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.

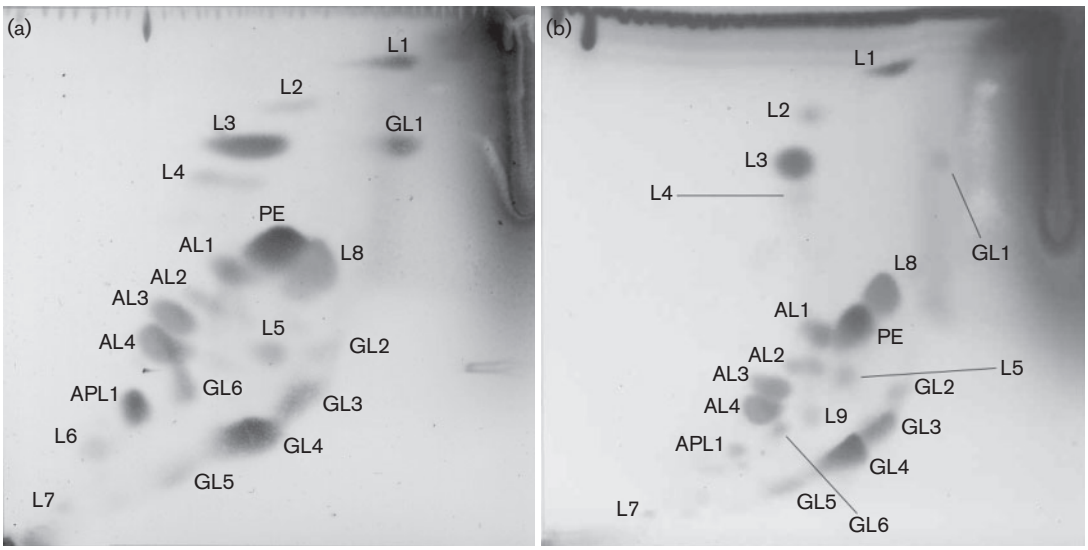


**Fig. 3.** Polar lipid profiles of strains C26$^T$ (a) and 100$^T$ (b) after separation by two-dimensional TLC and detection using 5 % ethanolic molybdatophosphoric acid. PE, Phosphatidylethanolamine; AL1–4, unidentified aminolipids; APL1, unidentified aminophospholipid; GL1–6, unidentified glycolipids; L1–9, unidentified polar lipids not detectable with any of the spray reagents specific for lipids containing a phosphate group, an amino group or a sugar moiety.

adonitol, *myo*-inositol, D-sorbitol, putrescine, *cis*- and *trans*-aconitate, 4-aminobutyrate, adipate, azelate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, 2-oxoglutarate, pyruvate, suberate, citrate, mesaconate, L-alanine, $\beta$-alanine, L-ornithine, L-phenylalanine, L-serine, L-aspartate, L-histidine, L-leucine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. The following chromogenic substrates are hydrolysed: *p*-nitrophenyl (pNP) $\alpha$-D-glucopyranoside, pNP $\beta$-D-glucopyranoside (weakly), pNP $\beta$-D-galactopyranoside, bis-pNP phosphate, bis-pNP phenylphosphonate, bis-pNP phosphorylcholine, 2-deoxythymidine-2′-pNP phosphate, L-alanine *p*-nitroanilide (pNA), $\gamma$-L-glutamate pNA and L-proline pNA. pNP $\beta$-D-xylopyranoside and pNP $\beta$-D-glucuronide are not hydrolysed. The major cellular fatty acids (>15 %) are iso-$C_{15:0}$, summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), iso-$C_{17:1}\omega9c$ and iso-$C_{17:0}$ 3-OH. The polyamine pattern contains the major compound *sym*-homospermidine and small amounts of 1,3-diaminopropane, putrescine, cadaverine, spermidine and spermine. The quinone system is exclusively MK-6. The polar lipid profile is composed of the major lipids phosphatidylethanolamine, unknown aminolipids AL1, AL3 and AL4, unknown glycolipids GL1, GL3, GL4 and GL6 and unknown lipids L3 and L8. Additionally, minor amounts of one aminophospholipid (APL1), one aminolipid (AL2), two glycolipids (GL2 and GL5) and six lipids (L1, L2, L4, L5, L7 and L9) not containing a sugar residue, an amino group or a phosphate group are present.

The type strain is $100^T$ (=LMG $27808^T$=CCM $8493^T$), isolated from a pharyngeal scrape sample obtained from a living and apparently healthy chicken in Saxony-Anhalt, Germany.

### Description of *Chryseobacterium contaminans* sp. nov.

*Chryseobacterium contaminans* (con.ta′mi.nans. L. part. adj. *contaminans* contaminating, polluting, referring to the isolation of the type strain as a contaminant of an agar plate from a rhizosphere sample).

Cells are Gram-negative staining, non-motile, non-spore-forming rods, approx. 1 μm wide and 2 μm long. Aerobic and oxidase- and catalase-positive. Good growth occurs after 48 h on NA, brain heart infusion agar, TSA and R2A agar (all Oxoid) but not on MacConkey agar (Oxoid) at 28 °C. Growth occurs on NA at 8–37 °C, but not at 4 or 45 °C. Grows at 28 °C in the presence of 1.0–2.0 % NaCl (not at 3 %) as an additional ingredient of TSB and at pH 5.5–12.5, but not at pH 4.5. Colonies on NA are smooth, yellowish, circular, translucent and glistening with entire edges. Colonies become mucoid and cannot be identified as single entities after prolonged incubation. The yellow pigment of the flexirubin type is non-diffusible and non-fluorescent. Produces acid from D-glucose, L-arabinose, maltose and trehalose. No acid is produced from adonitol, D-arabitol, dulcitol, erythritol, *myo*-inositol, lactose, D-mannitol, melibiose, methyl $\alpha$-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose or D-xylose. Urease activity, indole production and hydrolysis of casein, gelatin, starch, DNA and tyrosine are positive, while production of brown diffusible pigments on tyrosine agar, hydrogen sulphide production and activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and $\beta$-galactosidase (ONPG) are negative. The following compounds are not utilized as sole sources of carbon: acetate, propionate, *N*-acetylgalactosamine, *N*-acetylglucosamine, L-arabinose, L-arbutin, cellobiose, D-galactose, gluconate, D-glucose, maltose, D-mannose, D-fructose, trehalose, glycerol, D-mannitol, maltitol, melibiose, L-rhamnose, D-ribose, sucrose, salicin, D-xylose, adonitol, *myo*-inositol, D-sorbitol, putrescine, *cis*- and *trans*-aconitate, 4-aminobutyrate, adipate, azelate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, 2-oxoglutarate, pyruvate, suberate, citrate, mesaconate, L-alanine, $\beta$-alanine, L-ornithine, L-phenylalanine, L-serine, L-aspartate, L-histidine, L-leucine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. The following chromogenic substrates are hydrolysed: pNP $\alpha$-D-glucopyranoside, pNP $\beta$-D-glucopyranoside (weakly), pNP $\beta$-D-galactopyranoside, bis-pNP phosphate, bis-pNP phenylphosphonate, bis-pNP phosphorylcholine, 2-deoxythymidine-2′-pNP phosphate, L-alanine pNA, $\gamma$-L-glutamate pNA and L-proline pNA. pNP $\beta$-D-xylopyranoside and pNP $\beta$-D-glucuronide are not hydrolysed. The major cellular fatty acids (>15 %) are iso-$C_{15:0}$, summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), iso-$C_{17:1}\omega9c$ and iso-$C_{17:0}$ 3-OH. The polyamine pattern contains the major compound *sym*-homospermidine and small amounts of 1,3-diaminopropane, putrescine, cadaverine, spermidine and spermine. The quinone system is composed predominantly of menaquinone MK-6. The polar lipid profile is composed of the major lipids phosphatidylethanolamine, unknown aminolipids AL1, AL3 and AL4, unknown aminophospholipid APL1, unknown glycolipids GL3 and GL4 and unknown lipids L3 and L8. Additionally, minor amounts of one aminolipid (AL2), four glycolipids (GL1, GL2, GL5 and GL6) and six lipids that do not contain a sugar residue, an amino group or a phosphate group are present.

The type strain is $C26^T$ (=LMG $27810^T$=CCM $8492^T$), isolated from an agar plate of a rhizosphere sample in Auburn, AL, USA.

## Acknowledgements

We thank Dr S.-W. Kwon for kindly providing the type strain of *C. jejuense*. We thank Gundula Will, Maria Sowinsky and Evelyn Skiebe for excellent technical assistance and Dr Karin Böhland and Dr Christiane Cuny for providing chicken samples.

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