Chryseobacterium ureilyticum sp. nov., *Chryseobacterium gambrini* sp. nov., *Chryseobacterium pallidum* sp. nov. and *Chryseobacterium molle* sp. nov., isolated from beer-bottling plants

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Four Gram-negative, rod-shaped, non-spore-forming and non-motile bacterial strains were isolated from surfaces and biofilms associated with beer-bottling plants. Based on their 16S rRNA gene sequences these isolates were allocated to the genus *Chryseobacterium*. The sequence similarities of the isolates to the next most closely related type strains of this genus ranged from 96.4 to 98.3 %. The presence of menaquinone MK-6 and predominant fatty acids 15:0 iso, 17:1 iso *cis*9, 15:0 iso 2-OH and 17:0 iso 3-OH supported the affiliation of these strains to the genus. The results of DNA–DNA hybridization, biochemical tests and chemotaxonomic properties allowed genotypic and phenotypic differentiation of the strains from the next most closely related *Chryseobacterium* species with validly published names. Therefore, the isolates represent four novel species for which the names *Chryseobacterium ureilyticum* (type strain F-Fue-04IIlaaaa^T=DSM 18017^T=CCUG 52546^T), *Chryseobacterium gambrini* (type strain 5-1St1a^T=DSM 18014^T=CCUG 52549^T), *Chryseobacterium pallidum* (type strain 26-3St2b^T=DSM 18015^T=CCUG 52548^T) and *Chryseobacterium molle* (type strain DW3^T=DSM 18016^T=CCUG 52547^T) are proposed.

The genus *Chryseobacterium* represents one of the genera with the fastest growing number of species. The genus was described by Vandamme *et al.* (1994) and contained five species, *C. balustinum* (Harrison, 1929), *C. indoltheticum* (Campbell & Williams, 1951), *C. indologenes* (Yabuuchi *et al.*, 1983), *C. gleum* (Holmes *et al.*, 1984) and *C. scophthalmum* (Mudarris *et al.*, 1994). The species number

remained constant until 2003 when *C. defluvii* (Kämpfer *et al.*, 2003) and *C. joostei* (Hugo *et al.*, 2003) were described. In 2005 five more species of the genus were added to the list of approved bacterial names, while six more species were described in 2006. The strains that were isolated and described during the last 4 years were isolated from a broad spectrum of environmental sources, such as activated sludge (Kämpfer *et al.*, 2003), soils and sediments (Kim *et al.*, 2005a; Shen *et al.*, 2005; Tai *et al.*, 2006), rhizosphere (Young *et al.*, 2005; Park *et al.*, 2006) and also from the food processing industry (Hugo *et al.*, 2003; Shimomura *et al.*, 2005; de Beer *et al.*, 2005). This demonstrated that the genus *Chryseobacterium* encompasses a group of organisms that is ubiquitous in nature.

In this report we describe four novel species of this genus, which were isolated from surfaces of beer-bottling plants located in three different breweries in Germany. Strain DW3^T was isolated from a biofilm collected from a bottling plant conveyor (Timke *et al.*, 2004), whereas strains 5-1St1a^T, 26-3St2b^T and F-Fue-04IIlaaaa^T were isolated from cleaned steel coupons that were mounted onto the outer surfaces of the beer-bottling plant and exposed

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Chryseobacterium gleum* CCUG 14555^T, *Chryseobacterium indologenes* LMG 8337^T, strain F-Fue-04IIlaaaa^T, strain 5-1St1a^T, strain 26-3St2b^T and strain DW3^T are AM232812, AM232813, AM232806, AM232810, AM232809 and AJ534853, respectively.

API ZYM profiles, polar lipid patterns, pigment absorption spectra and scanning electron micrographs of cells of the four novel isolates are available as supplementary material with the online version of this paper.

overnight to the airborne microbiota of the filling area. The type strains of *C. defluvii* and *Chryseobacterium formosense* were used as reference strains (Kämpfer *et al.*, 2003; Young *et al.*, 2005). Other reference strains were obtained from the DSMZ, the CCUG and the LMG. All isolates and reference strains were cultivated on trypticase soy agar at 25 °C.

Genomic DNA was isolated using a DNeasy Tissue kit according to the instructions of the manufacturer (Qiagen). The 16S rRNA gene of the purified chromosomal DNA was amplified using the universal bacterial primers GM3F and GM4R (Muyzer *et al.*, 1995). An ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit v2.0 (Applied Biosystems) was used for the sequencing reaction. The sequencing primers used were 518F, 787R and 1099F (Buchholz-Cleven *et al.*, 1997). Sequencing products were purified using a DyeEx Spin kit (Qiagen) and electrophoresis was performed on an ABI377 (Applied Biosystems) sequencer. The sequences, each at a length of more than 1400 bases, were compared with the National Center for Biotechnology Information nucleotide sequence database using the BLAST program (Altschul *et al.*, 1997). BLAST analyses revealed that the 16S rRNA gene sequences of all four isolates shared the highest similarity with type strains of species exclusively of the genus *Chryseobacterium*. The type strain with the most similar sequence to that of strain 5-1St1a^T was *C. formosense* CC-H3-2^T (97.5 % sequence similarity). *C. joostei* LMG 18212^T showed the highest sequence similarities to strains DW3^T and F-Fue-04IIIIaaa^T (96.9 and 98.3 %, respectively). The next most closely related type strain to isolate 26-3St2b^T was *Chryseobacterium caeni* N4^T, with a sequence similarity of 96.4 %. A phylogenetic analysis was performed by using MEGA version 3.1 (Kumar *et al.*, 2004). The phylogenetic reconstruction, based on a neighbour-joining analysis, placed all four isolates within the genus *Chryseobacterium* (Fig. 1). The 16S rRNA genes of the reference strains *C. gleum* CCUG 14555^T and *C. indologenes* LMG 8337^T were also sequenced and included in the analysis because sequences available for these species were of poor quality.

Phenotypic analyses of the isolates were performed in parallel with the reference strains *C. formosense* CC-H3-2^T, *C. joostei* CCUG 46665^T, *Chryseobacterium daecheongense* DSM 15235^T and *C. defluvii* B2^T. Isoprenoid quinones and polar lipids were extracted and analysed using the small-scale integrated procedure of Minnikin *et al.* (1984). Menaquinones (MK) were analysed using a Hewlett Packard series 1050 HPLC equipped with an ODS Hypersil column and a diode-array detector. Methanol and isopropyl ether (9:2, v/v) were used as the mobile phase with a flow rate of 1.0 ml min⁻¹ and a column temperature of 30 °C (Hu *et al.*, 1999). The quinone type of the four strains was identical to those of the reference strains analysed and was therefore identified as MK-6. The polar lipids of the novel isolates were composed of phosphatidylethanolamine, several aminolipids and other polar lipids (see Supplementary Fig. S1, available in IJSEM Online). The two-dimensional patterns of the polar lipids

of the isolates and reference strains analysed (not shown) were highly similar and in accordance with published data (Kämpfer *et al.*, 2003; Kim *et al.*, 2005a).

Fatty acid analysis was performed on the novel isolates and the four reference strains using cells grown on trypticase soy agar for 24 h at 30 °C. Saponification with 15 % NaOH in 50 % methanol, acid methylation with 6 M HCl in 50 % methanol and extraction of fatty acid methyl esters were performed as described by Sasser (1990). The fatty acid methyl esters extracts were analysed by GC-FID using a Hewlett Packard model 6890 gas chromatograph equipped with a 5 % phenyl-methyl-silicone capillary column. The identity of the fatty acids was verified by GC-MS with a Hewlett Packard model 5890 series II gas chromatograph equipped with a 5 % phenyl-methyl-silicone capillary column and a model 5972 mass selective detector. The chromatographic conditions used were as described previously (Lipski & Altendorf, 1997). The positions of double bonds were verified by analysing the dimethyl-disulfide adducts of the fatty acid methyl esters (Nichols *et al.*, 1986). The positions of hydroxy-, methyl- and cyclopropene groups and double bonds were given from the carboxyl group of the fatty acid molecule according to the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (CBN) (1977). The fatty acid profiles of the isolates showed the branched chain fatty acid 15:0 iso as the major compound. Other predominating compounds were 17:1 iso *cis*9 and the hydroxy fatty acids 17:0 iso 3-OH and 15:0 iso 2-OH.

An in-depth analysis of the fatty acid profiles revealed some discrepancies with the profiles published previously for this genus (Mudarris *et al.*, 1994; Bernardet *et al.*, 2005; Kim *et al.*, 2005b; Quan *et al.*, 2007). We identified a compound as 17:1 iso *cis*9 that was previously named by the MIDI system as 17:1 iso ω 9c. The different position of the double bond was clearly located by mass spectrometric analysis of the DMDS adducts of the fatty acid methyl esters. All reference strains analysed showed this compound (Table 1). This indicates that 17:1 iso *cis*9 instead of 17:1 iso ω 9c is the usual compound of the fatty acid profiles of members of this genus. The compounds 15:0 iso 2-OH and 16:1 *cis*9 (identical to 16:1 ω 7c) were not separated by the chromatographic system used. Discrete amounts for both fatty acids were calculated after DMDS derivatization of unsaturated fatty acids. We found that only 15:0 iso 2-OH was present in all isolates and reference strains. The fatty acid 16:1 *cis*9 was detected only in some strains and therefore can be used as a differential feature within the genus.

The DNA G+C content of the novel isolates was determined after degradation of isolated genomic DNA with P1 nuclease and alkaline phosphatase. The resulting nucleosides were separated and quantified by HPLC (Mesbah *et al.*, 1989). Lambda-DNA was used as a reference. The DNA G+C contents of the isolates were 36.4 mol% for strain F-Fue-04IIIIaaa^T, 37.8 mol% for

Fig. 1. Phylogenetic positions of the novel isolates based on a neighbour-joining analysis of 16S rRNA gene sequences. Bootstrap values based on 1000 replications greater than 70 % are indicated at nodes. Bar, 0.01 substitutions per nucleotide position.

strain 5-1St1a^T, 39.2 mol% for strain DW3^T and 38.1 mol% for strain 26-3St2b^T. For the reference strain *C. joostei* CCUG 46665^T, a G+C content of 36.9 mol% was obtained, which conforms to the data published by Hugo *et al.* (2003) for this species.

Flexirubin-type pigments were detected after extraction with acetone by their absorption, with a maximum absorbance at 450 nm and a shift to higher wavelengths after addition of 20 % KOH (Yabuuchi *et al.*, 1983). Pigments were detected in strains F-Fue-04IIlaaaa^T and 5-1St1a^T and the four reference strains (see Supplementary Fig. S2 in IJSEM Online), but not in strains 26-3St2b^T or DW3^T. All isolates stained Gram-negative using the standard method of Gerhardt *et al.* (1981). This result was confirmed by using the non-staining KOH and the

aminopeptidase tests according to Buck (1982) and Cerny (1978), respectively.

Cell morphology and motility were analysed by phase-contrast microscopy (Zeiss axioscope) of cells incubated for 3 h in trypticase soy broth at 30 °C and at room temperature. Cell dimensions were measured with Zeiss KS300 software from phase-contrast micrographs, based on a minimum of 100 cells. Cells of all isolates were non-motile and rod-shaped. For scanning electron microscopy, cells were grown overnight in trypticase soy broth at 30 °C and spread on polylysine-coated slides. The samples were fixed with glutaraldehyde (2.5 %), dehydrated with a graded ethanol series, dried by critical-point drying with liquid carbon dioxide and coated with gold. Preparations were observed using a Zeiss DSM 962 scanning electron

Table 1. Fatty acid compositions of strains F-Fue-04IIlaaaa^T (*C. ureilyticum* sp. nov.), 5-1St1a^T (*C. gambrini* sp. nov.), 26-3St2b^T (*C. pallidum* sp. nov.) and DW3^T (*C. molle* sp. nov.) and of the type strains of related *Chryseobacterium* species

Strains: 1, F-Fue-04IIlaaaa^T; 2, 5-1St1a^T; 3, 26-3St2b^T; 4, DW3^T; 5, *C. formosense* CC-H3-2^T; 6, *C. daecheongense* DSM 15235^T; 7, *C. joostei* CCUG 46665^T; 8, *C. defluvii* B2^T. Values are percentages of total fatty acids; fatty acids amounting to less than 1 % of the total fatty acids in all strains were not included. —, Not detected. Fatty acids 15:0 iso 2-OH and 16:1 *cis*9 could not be separated by the chromatographic system used; the identification and splitting of the peak signal of both compounds was based on mass spectrometric data. All data are from this study.

Fatty acid	1	2	3	4	5	6	7	8
13:0 iso	1.3	2.4	3.7	6.0	2.1	2.0	1.2	4.1
14:0	0.3	0.4	0.7	1.2	0.4	—	0.2	0.3
15:0 iso	40.2	57.4	41.8	32.3	51.7	56.1	43.4	61.8
15:0 anteiso	0.4	0.6	5.2	10.3	2.4	1.0	0.3	1.4
15:0 iso 2-OH	7.6	8.3	5.2	5.6	4.5	9.0	9.2	8.9
16:1 <i>cis</i> 9	1.3	—	12.4	12.2	3.8	—	—	—
16:0	1.2	1.4	4.0	5.6	0.8	1.2	1.0	0.9
15:0 iso 3-OH	3.3	2.7	3.2	2.9	2.9	2.7	2.6	2.9
17:1 iso <i>cis</i> 9	25.4	6.7	4.4	5.7	16.4	6.5	22.8	3.6
17:1 iso <i>cis</i> 11	0.5	—	0.7	1.0	0.7	—	0.3	—
17:0 cyclo	0.8	0.9	0.8	0.8	0.8	1.2	0.9	0.6
17:0 iso	1.0	2.3	—	0.6	0.6	2.3	1.4	1.9
16:0 3-OH	1.0	0.6	4.4	4.1	0.9	0.5	0.7	0.4
17:0 iso 3-OH	14.7	16.2	11.4	10.1	10.6	17.4	14.6	13.3

microscope. No flagella were detected (see Supplementary Fig. S3 in IJSEM Online). All chemotaxonomic and morphological data were in accordance with the genus description (Vandamme *et al.*, 1994) and confirmed the assignment of the novel isolates to the genus *Chryseobacterium*.

The sequence similarities between the novel isolates and the reference type strains were below 98.3 %, indicating that the isolates may represent novel species. Statistical analysis based on Keswani & Whitman (2001) predicted a DNA–DNA hybridization value of less than 70 % from a sequence similarity of 98.6 % with a high level of confidence (99 %). A DNA–DNA hybridization value of 70 % is accepted for species delineation (Wayne *et al.*, 1987). Within the genus *Chryseobacterium* the sequence similarity between closely related but different species can be as high as 99.3 %, as reported for the *C. scophthalmum*–*Chryseobacterium piscium*–*C. balustinum* complex (de Beer *et al.*, 2006). Hybridization experiments were performed with genomic DNA from the novel isolates and DNA from the next most closely related type strains, *C. joostei* LMG 18212^T and *C. formosense* CC-H3-2^T. The method described by Ziemke *et al.* (1998) was used except that, for nick translation, 2 µg DNA was labelled during a 3 h incubation at 15 °C. These experiments revealed hybridization values of 21 % (reciprocal 22 %) between strain F-Fue-04IIlaaaa^T and *C. joostei*

LMG 18212^T, 26 % (reciprocal 35 %) between strain 5-1St1a^T and *C. formosense* CC-H3-2^T, 44 % (reciprocal 45 %) between strain 26-3St2b^T and *C. joostei* LMG 18212^T and 44 % (reciprocal 40 %) between strain DW3^T and *C. joostei* LMG 18212^T. The closely related isolates DW3^T and 26-3St2b^T showed a hybridization value of 25 % (reciprocal 30 %), which supported the separation of these isolates as representing two different species.

Biochemical tests were performed at 25 °C on the isolates and the four reference strains according to MacFaddin (1980) unless indicated otherwise. Growth at 5, 25, 37 and 42 °C and in the presence of 1, 2, 3, 4 and 5 % NaCl was tested on nutrient agar, containing 5 g peptone l⁻¹, 3 g meat extract l⁻¹ and 15 g agar l⁻¹. The susceptibility to 11 antimicrobials was determined by using the disc-diffusion method with susceptibility test discs (Oxoid). API ZYM, API 20E and API 20NE profiles (bioMérieux) were recorded according to the recommendations of the manufacturer. In addition to the carbohydrates included in the API 20E galleries, acid production from mannitol, adonitol, rhamnose, *myo*-inositol, sorbitol and dulcitol was also examined using Hugh and Leifson's O–F medium (MacFaddin, 1980). Haemolytic activity was tested on Columbia agar with 5 % sheep blood (bioMérieux). Phenotypically, strain F-Fue-04IIlaaaa^T could be differentiated from all other strains tested including the next most closely related type strain, *C. joostei* CCUG 46665^T, by a set of biochemical characteristics such as urease activity and resistance to erythromycin and tetracycline (Table 2). Phylogenetically, strain 5-1St1a^T was only distantly related to *C. formosense* CC-H3-2^T, although the two strains showed high 16S rRNA gene sequence similarity. Accordingly, several phenotypic markers differed between both taxa: amounts of the fatty acids 16:1 *cis*9 and 17:1 iso *cis*9, growth at 37 and 4 °C, formation of indole, assimilation of glucose, arabinose and maltose, susceptibility to kanamycin and trypsin, and α - and β -glucosidase activities (Tables 1 and 2 and Supplementary Table S1 in IJSEM Online). In contrast to all isolates and reference strains analysed, strain 5-1St1a^T showed two absorption maxima (455 and 479 nm) when acetone-extracted pigments were treated with 20 % KOH (see Supplementary Fig. S2 in IJSEM Online). Strains DW3^T and 26-3St2b^T differed from all other *Chryseobacterium* strains by the absence of flexirubin-type pigments. This property was also reported for the closely related genus *Sejorgia* (Yi *et al.*, 2005), but the strains of this genus were phylogenetically well separated from strains DW3^T and 26-3St2b^T and also showed different chemotaxonomic properties such as the presence of 16:0 iso and different amounts of 15:0 iso and 16:1 *cis*9 (Table 1 and Yi *et al.*, 2005). Morphologically, strains DW3^T and 26-3St2b^T showed the smallest cell length (about 1.3 µm) of all strains analysed (see Supplementary Fig. S3 in IJSEM Online). Phenotypically, strains DW3^T and 26-3St2b^T could be separated based on growth in the presence of 2 % NaCl, reduction of nitrate, growth on adipate and

Table 2. Biochemical reactions of strains F-Fue-04IIIaaaa^T (*C. ureilyticum* sp. nov.), 5-1St1a^T (*C. gambrini* sp. nov.), 26-3St2b^T (*C. pallidum* sp. nov.) and DW3^T (*C. molle* sp. nov.) and of the type strains of related *Chryseobacterium* species

Strains: 1, F-Fue-04IIIaaaa^T; 2, 5-1St1a^T; 3, 26-3St2b^T; 4, DW3^T; 5, *C. formosense* CC-H3-2^T; 6, *C. daecheongense* DSM 15235^T; 7, *C. joostei* CCUG 46665^T; 8, *C. defluvii* B2^T. All strains are positive for the KOH test; aminopeptidase, oxidase, catalase, gelatinase, and haemolytic activities; growth in the presence of 1% NaCl and at 25 °C; and hydrolysis of aesculin and Tweens 20 and 80. All strains are negative for starch hydrolysis; oxidation of gluconate; motility; fluorescence on King B medium; growth in the presence of 3% NaCl and on MacConkey and Simmons' citrate agars; assimilation of *N*-acetylglucosamine and caprate; methyl red test; production of acetoin; reduction of nitrate and nitrite to N₂; arginine dihydrolase, lysine and ornithine decarboxylases and tryptophan deaminase activities; production of H₂S; acid formation from glucose, lactose, sucrose, arabinose, rhamnose, mannitol, adonitol, *myo*-inositol, sorbitol, dulcitol, melibiose and amygdalin; and resistance to novobiocin (30 µg), nalidixic acid (30 µg), neomycin (30 µg), furazolidone (50 µg) and fusidic acid (10 µg). +, Positive; w, weakly positive; –, negative. All data are from this study.

Characteristic	1	2	3	4	5	6	7	8
Production of flexirubin-type pigments	+	+	–	–	+	+	+	+
Growth at/with or on:								
2% NaCl	+	–	–	+	+	–	+	–
4 °C	–	–	–	–	+	–	+	–
37 °C	–	+	+	+	–	+	–	+
42 °C	–	+	–	–	–	+	–	+
Cetrimide agar	+	+	–	–	+	+	+	+
Nitrate reduction	–	–	+	–	–	+	–	–
Indole formation	+	–	+	+	+	–	+	–
Lecithinase activity	–	–	+	+	–	–	–	–
Urease activity	+	–	–	–	–	–	–	–
Assimilation of:								
Glucose	+	+	+	+	–	+	–	+
Arabinose	–	+	+	+	–	+	–	+
Mannose	–	+	+	+	+	+	+	+
Maltose	+	+	+	+	–	+	+	+
Gluconate	–	w	–	–	–	+	–	w
Adipate	–	+	+	–	–	w	–	–
Malate	–	–	+	+	–	–	–	–
Citrate	–	–	–	–	–	w	–	–
Phenylacetate	–	+	–	–	–	–	–	+
Resistant to:*								
Kanamycin (30 µg)	+	–	–	–	+	+	+	+
Streptomycin (10 µg)	+	–	–	–	–	+	+	–
Erythromycin (10 µg)	+	–	–	–	–	–	–	–
Tetracycline (30 µg)	+	–	–	–	–	–	–	–
Ampicillin (10 µg)	+	+	+	–	+	+	+	+
Gentamicin (10 µg)	+	–	–	–	–	+	+	+

*Given as resistant (+) if radius of inhibition zone was smaller than 2 mm.

susceptibility to ampicillin. Isolate DW3^T was the only strain in our study that showed this susceptibility. This is also in contrast to all other representatives of the genus *Chryseobacterium* (Vandamme *et al.*, 1994; Michel *et al.*, 2005).

None of the novel isolates were able to grow in beer and therefore the strains were not considered as beer-spoiling organisms. However, the isolation from three different breweries shows that species of the genus *Chryseobacterium* are regular members of biofilms on bottling plant surfaces. This is in accordance with the detection of significant numbers of *Chryseobacterium*-related sequences by direct cloning procedures (Timke *et al.*, 2005). Three of the novel strains were isolated from short-term exposed steel coupons. This indicates the involvement of *Chryseobacterium* strains in the rapid recolonization of cleaned plant surfaces and their participation in biofilm formation.

Description of *Chryseobacterium ureilyticum* sp. nov.

Chryseobacterium ureilyticum (u.re.i.ly'ti.cum. N.L. fem. n. *urea urea*; Gr. adj. *lyticus* dissolving; N.L. neut. adj. *ureilyticum urea-dissolving*).

Cells are aerobic, non-spore-forming, non-motile rods, approximately 2.0 µm (SD 0.4 µm) in length and 0.7 µm (SD 0.1 µm) in width. After growth for 2 days at 30 °C on trypticase soy agar, colonies are circular and yellow-pigmented with a diameter of 2–3 mm. Gram-negative and oxidase- and catalase-positive. Yellow flexirubin-type pigments are produced. Grows at 25 °C but not at 4 or 37 °C. Grows on plain nutrient agar and on nutrient agar containing up to 2% NaCl, but not on nutrient agar containing 3% NaCl. Growth occurs on Cetrimide agar but not on MacConkey or Simmons' citrate agars. Positive for urease, gelatinase and haemolytic activities; production of indole; and hydrolysis of aesculin and Tweens 20 and 80. Negative for starch hydrolysis; oxidation of gluconate; fluorescence on King B agar; production of acetoin; lecithinase, arginine dihydrolase, lysine and ornithine decarboxylases and tryptophan deaminase activities; reduction of nitrate and nitrite to N₂; production of H₂S; and acid formation from glucose, lactose, sucrose, arabinose, rhamnose, mannitol, adonitol, *myo*-inositol, sorbitol, dulcitol, melibiose and amygdalin. Glucose and maltose are utilized as sole carbon sources, but arabinose, mannose, *N*-acetylglucosamine, gluconate, adipate, malate, citrate, phenylacetate and caprate are not. Susceptible to novobiocin, nalidixic acid, neomycin, furazolidone and fusidic acid and resistant to kanamycin, streptomycin, erythromycin, tetracycline, ampicillin and gentamicin. Major fatty acids are 15:0 iso, 17:1 iso *cis*9, 17:0 iso 3-OH and 15:0 iso 2-OH. Predominant respiratory quinone type is MK-6. Polar lipids are composed of phosphatidylethanolamine, several aminolipids and other polar lipids. The G+C content of the genomic DNA of the type strain is 36.4 mol%.

The type strain, F-Fue-04IIaaaa^T (=DSM 18017^T=CCUG 52546^T), was isolated from a steel surface of a beer-bottling plant in Germany.

Description of *Chryseobacterium gambrini* sp. nov.

Chryseobacterium gambrini [gam.bri'ni. N.L. gen. masc. n. *gambrini* of Gambrinus, the alleged 'god of beer' of the German students (17th century)].

Cells are aerobic, non-spore-forming, non-motile rods, approximately 3.6 µm (SD 1.1 µm) in length and 1.1 µm (SD 0.3 µm) in width. After growth for 2 days at 30 °C on trypticase soy agar, colonies are circular and yellow-pigmented with a diameter of approximately 2 mm. Gram-negative and oxidase- and catalase-positive. Yellow flexirubin-type pigments are produced. Growth occurs at 25, 37 and 42 °C, but not at 4 °C. Grows on plain nutrient agar and on nutrient agar containing up to 1 % NaCl, but not on nutrient agar containing 2 % NaCl. Growth occurs on Cetrimide agar but not on MacConkey or Simmons' citrate agars. Positive for gelatinase and haemolytic activities and hydrolysis of aesculin and Tweens 20 and 80. Negative for starch hydrolysis; oxidation of gluconate; fluorescence on King B agar; production of acetoin and indole; lecithinase, urease, arginine dihydrolase, lysine and ornithine decarboxylases and tryptophan deaminase activities; reduction of nitrate and nitrite to N₂; production of H₂S; and acid formation from glucose, lactose, sucrose, arabinose, rhamnose, mannitol, adonitol, *myo*-inositol, sorbitol, dulcitol, melibiose and amygdalin. Glucose, arabinose, mannose, maltose, adipate and phenylacetate are utilized as sole carbon sources, but *N*-acetylglucosamine, malate, citrate and caprate are not. Susceptible to kanamycin, streptomycin, erythromycin, tetracycline, gentamicin, novobiocin, nalidixic acid, neomycin, furazolidone and fusidic acid and resistant to ampicillin. Major fatty acids are 15:0 iso, 17:0 iso 3-OH, 15:0 iso 2-OH and 17:1 iso *cis*9. Predominant respiratory quinone type is MK-6. Polar lipids are composed of phosphatidylethanolamine, several aminolipids and other polar lipids. The G+C content of the genomic DNA of the type strain is 37.8 mol%.

The type strain, 5-1St1a^T (=DSM 18014^T=CCUG 52549^T), was isolated from a steel surface of a beer-bottling plant in Germany.

Description of *Chryseobacterium pallidum* sp. nov.

Chryseobacterium pallidum (pal'li.dum. L. neut. adj. *pallidum* pale).

Cells are aerobic, non-spore-forming, non-motile rods, approximately 1.3 µm (SD 0.3 µm) in length and 0.8 µm (SD 0.1 µm) in width. After growth for 2 days at 30 °C on trypticase soy agar, colonies are circular and pale-cream-coloured with a diameter of approximately 1 mm.

Gram-negative and oxidase- and catalase-positive. Does not produce flexirubin-type pigments. Growth occurs at 25 and 37 °C but not at 4 or 42 °C. Grows on plain nutrient agar and on nutrient agar containing up to 1 % NaCl, but not on nutrient agar containing 2 % NaCl. No growth occurs on Cetrimide, MacConkey or Simmons' citrate agars. Positive for gelatinase, lecithinase and haemolytic activities; production of indole; nitrate reduction; and hydrolysis of aesculin and Tweens 20 and 80. Negative for starch hydrolysis; oxidation of gluconate; fluorescence on King B agar; production of acetoin; urease, arginine dihydrolase, lysine and ornithine decarboxylases and tryptophan deaminase activities; production of H₂S; and acid formation from glucose, lactose, sucrose, arabinose, rhamnose, mannitol, adonitol, *myo*-inositol, sorbitol, dulcitol, melibiose and amygdalin. Glucose, arabinose, mannose, maltose, adipate and malate are utilized as sole carbon sources, but *N*-acetylglucosamine, gluconate, phenylacetate, citrate and caprate are not. Susceptible to kanamycin, streptomycin, erythromycin, tetracycline, gentamicin, novobiocin, nalidixic acid, neomycin, furazolidone and fusidic acid and resistant to ampicillin. Major fatty acids are 15:0 iso, 16:1 *cis*9 and 17:0 iso 3-OH. Predominant respiratory quinone type is MK-6. Polar lipids are composed of phosphatidylethanolamine, several aminolipids and other polar lipids. The G+C content of the genomic DNA of the type strain is 38.1 mol%.

The type strain, 26-3St2b^T (=DSM 18015^T=CCUG 52548^T), was isolated from a steel surface of a beer-bottling plant in Germany.

Description of *Chryseobacterium molle* sp. nov.

Chryseobacterium molle (mol'le. L. neut. adj. *molle* pliant, sensitive, referring to the sensitivity to antibiotics).

Cells are aerobic, non-spore-forming, non-motile rods, approximately 1.3 µm (SD 0.8 µm) in length and 0.9 µm (SD 0.1 µm) in width. After growth for 2 days at 30 °C on trypticase soy agar, colonies are circular and pale-cream-coloured with a diameter of approximately 1 mm. Gram-negative and oxidase- and catalase-positive. Does not produce flexirubin-type pigments. Growth occurs at 25 and 37 °C but not at 4 or 42 °C. Grows on plain nutrient agar and on nutrient agar containing up to 2 % NaCl, but not on nutrient agar containing 3 % NaCl. No growth occurs on Cetrimide, MacConkey or Simmons' citrate agars. Positive for gelatinase, lecithinase and haemolytic activities; production of indole; and hydrolysis of aesculin and Tweens 20 and 80. Negative for starch hydrolysis; oxidation of gluconate; fluorescence on King B agar; production of acetoin; urease, arginine dihydrolase, lysine and ornithine decarboxylases and tryptophan deaminase activities; reduction of nitrate and nitrite to N₂; production of H₂S; and acid formation from glucose, lactose, sucrose, arabinose, rhamnose, mannitol, adonitol, *myo*-inositol, sorbitol, dulcitol, melibiose and amygdalin. Glucose, arabinose, mannose, maltose and malate are utilized as sole carbon

sources, but *N*-acetylglucosamine, gluconate, adipate, phenylacetate, citrate and caprate are not. Susceptible to kanamycin, streptomycin, erythromycin, tetracycline, ampicillin, gentamicin, novobiocin, nalidixic acid, neomycin, furazolidone and fusidic acid. Major fatty acids are 15:0 iso, 15:0 anteiso, 16:1 *cis*9 and 17:0 iso 3-OH. Predominant respiratory quinone type is MK-6. Polar lipids are composed of phosphatidylethanolamine, several aminolipids and other polar lipids. The G+C content of the genomic DNA of the type strain is 39.2 mol%.

The type strain, DW3^T (=DSM 18016^T=CCUG 52547^T), was isolated from a biofilm of a beer-bottling plant conveyor of a brewery in Germany.

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References

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- 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.
- Buchholz-Cleven, B. E. E., Rattunde, B. & Straub, K. L. (1997). Screening for genetic diversity of isolates of anaerobic Fe(II)-oxidizing bacteria using DGGE and whole-cell hybridization. *Syst Appl Microbiol* **20**, 301–309.
- Buck, J. D. (1982). Nonstaining (KOH) method for determination of gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.
- Campbell, L. L. & Williams, O. B. (1951). A study of chitin-decomposing micro-organisms of marine origin. *J Gen Microbiol* **5**, 894–905.
- Cerny, G. (1978). Studies on the aminopeptidase test for the distinction of gram-negative from gram-positive bacteria. *Appl Microbiol Biotechnol* **5**, 113–122.
- 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.
- Gerhardt, P., Murray, R. G. E., Costilow, R. N., Nester, E. W., Wood, W. A., Krieg, N. R. & Phillips, G. B. (editors) (1981). *Methods for General Bacteriology*. Washington, DC: American Society for Microbiology.
- Harrison, F. C. (1929). The discoloration of halibut. *Can J Res* **1**, 214–239.
- 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.
- Hu, H.-Y., Fujie, K. & Urano, K. (1999). Development of a novel solid phase extraction method for the analysis of bacterial quinones in activated sludge with a higher reliability. *J Biosci Bioeng* **87**, 378–382.
- 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.
- IUPAC-IUB Commission on Biochemical Nomenclature (CBN) (1977). The nomenclature of lipids. *Eur J Biochem* **79**, 11–21.
- 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.
- Keswani, J. & Whitman, W. B. (2001). Relationship of 16S rRNA sequence similarity to DNA hybridization in prokaryotes. *Int J Syst Evol Microbiol* **51**, 667–678.
- 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.
- Lipski, A. & Altendorf, K. (1997). Identification of heterotrophic bacteria isolated from ammonia-supplied experimental biofilters. *Syst Appl Microbiol* **20**, 448–457.
- MacFaddin, J. F. (1980). *Biochemical Tests for Identification of Medical Bacteria*. Baltimore: Williams & Wilkins.
- 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.
- Michel, C., Matte-Tailliez, O., Kerouault, B. & Bernardet, J.-F. (2005). Resistance pattern and assessment of phenicol agents' minimum inhibitory concentration in multiple drug resistant *Chryseobacterium* isolates from fish and aquatic habitats. *J Appl Microbiol* **99**, 323–332.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Mudarris, M., Austin, B., Segers, P., Vancanneyt, M., Hoste, B. & Bernardet, J.-F. (1994). *Flavobacterium scophthalmum* sp. nov., a pathogen of turbot (*Scophthalmus maximus* L.). *Int J Syst Bacteriol* **44**, 447–453.
- Muyzer, G., Teske, A., Wirsén, C. O. & Jannasch, H. W. (1995). Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis. *Arch Microbiol* **164**, 165–172.
- Nichols, P. D., Guckert, J. B. & White, D. C. (1986). Determination of monounsaturated fatty acid double-bond position and geometry for microbial monocultures and complex consortia by capillary GC-MS of their dimethyl disulphide adducts. *J Microbiol Methods* **5**, 49–55.
- 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.

- 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.
- Sasser, M. (1990). Identification of bacteria through fatty acid analysis. In *Methods in Phytobacteriology*, pp. 199–204. Edited by Z. Klement, K. Rudolph & D. C. Sands. Budapest: Akademiai Kiado.
- 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.
- Timke, M., Wolking, D., Wang-Lieu, N. Q., Altendorf, K. & Lipski, A. (2004). Microbial composition of biofilms in a brewery investigated by fatty acid analysis, fluorescence in situ hybridisation and isolation techniques. *Appl Microbiol Biotechnol* **66**, 100–107.
- Timke, M., Wang-Lieu, N. Q., Altendorf, K. & Lipski, A. (2005). Community structure and diversity of biofilms from a beer bottling plant as revealed using 16S rRNA gene clone libraries. *Appl Environ Microbiol* **71**, 6446–6452.
- 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.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- 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 IIK-2 and IIb. *Int J Syst Bacteriol* **33**, 580–598.
- Yi, H., Yoon, H. I. & Chun, J. (2005). *Sejongia antarctica* gen. nov., sp. nov. and *Sejongia jeonii* sp. nov., isolated from the Antarctic. *Int J Syst Evol Microbiol* **55**, 409–416.
- 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.
- Ziemke, F., Höfle, M. G., Lalucat, J. & Rosselló-Mora, R. (1998). Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* **48**, 179–186.