Chryseobacterium treverense sp. nov., isolated from a human clinical source

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A yellow-pigmented, Gram-reaction-negative bacterium isolated from a human clinical source was investigated using a polyphasic approach in order to clarify its taxonomic status. Comparative 16S rRNA gene sequence analysis showed that the new isolate constituted a distinct phyletic line within the genus *Chryseobacterium*, displaying >2.8% sequence divergence with recognized species of this genus. The generic assignment was confirmed by chemotaxonomic data which revealed a fatty acid profile consisting of straight-chain saturated, monounsaturated and branched-chain fatty acids of iso-/anteiso-types as well as 3-hydroxylated fatty acids; a menaquinone with six isoprene units (MK-6) as the predominant respiratory quinone and sym-homospermidine as the predominant polyamine. The novel isolate could be distinguished from other members of the genus *Chryseobacterium* by a set of distinct biochemical properties. On the basis of phenotypic and phylogenetic evidence, it is proposed that the new isolate represents a novel species of the genus *Chryseobacterium* for which the name *Chryseobacterium treverense* sp. nov. is proposed. The type strain is IMMIB L-1519^T (=DSM 22251^T=CCUG 57657^T).

The genus *Chryseobacterium* Vandamme *et al.* 1994 emend. Kämpfer *et al.* 2009 was originally proposed to comprise the generically misclassified organisms *Flavobacterium balustinum*, *Flavobacterium gleum*, *Flavobacterium indologenes*, *Flavobacterium indoltheticum*, *Flavobacterium meningosepticum* and *Flavobacterium scophthalmum* with *Chryseobacterium gleum* as the type species. Members of the genus *Chryseobacterium* are aerobic, catalase- and oxidase-positive, chemo-organotrophic, non-motile, non-spore-forming, Gram-reaction-negative and rod-shaped bacteria. These include typically pigmented (yellow to orange) species as well as non-pigmented ones. Members of the genus can be characterized chemotaxonomically by having a fatty acid profile that contains iso-, anteiso- and 3-hydroxy fatty acids (Segers *et al.*, 1993; Vandamme *et al.*, 1994; Kämpfer *et al.*, 2009b). They contain menaquinone MK-6 as the only respiratory quinone and sym-homospermidine as the major polyamine. The number of recognized species of the genus *Chryseobacterium* has increased rapidly. Some of the newly described species have resulted from the taxonomic revision of existing genera, e.g. *Kaistella* and *Sejongia* (Kämpfer *et al.*,

2009a, b). At the time of writing, the genus *Chryseobacterium* comprises 42 recognized species (<http://www.bacterio.cict.fr/>). These include species isolated from a variety of environmental sources, e.g. soil, water, sludge, plants, food products such as fish, meat, poultry, milk and lactic acid beverages, and human clinical specimens (Bernardet *et al.*, 2005, 2006; Quan *et al.*, 2007; Vaneechoutte *et al.*, 2007). During the course of characterization of bacterial isolates encountered from clinical sources, a polyphasic approach was used to characterize a Gram-reaction-negative, yellow-pigmented, rod-shaped bacterium. Based on the results of this study, we describe a novel species of the genus *Chryseobacterium*.

Strain IMMIB L-1519^T was isolated from an aerobic BACTEC bottle (BD) inoculated with a blood sample from an apparently healthy man in Trier, Germany. The strain was grown aerobically at 37 °C on Columbia agar (Oxoid) supplemented with 5% sheep blood and preserved in fetal calf serum by lyophilization. Anaerobic growth was investigated using incubation in the GasPak anaerobic system (BBL) for 15 days at 27 °C on Columbia agar. *Chryseobacterium jeonii* DSM 17048^T and *Chryseobacterium anthropi* NF 1366^T were used as reference strains. The three strains were characterized biochemically using the API 20E, API 20NE, API 50CH and API ZYM identification systems

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMMIB L-1519^T is FN297836.

A figure showing the TLC of the polar lipids of strain IMMIB L-1519^T is available as supplementary material with the online version of this paper.

according to the manufacturer's instructions (bioMérieux). Growth was tested on cetrimide agar and MacConkey agar (Oxoid). Growth temperature (tested at 5, 10, 22, 30, 37 and 42 °C) was determined on Columbia agar supplemented with 5 % sheep blood. Tolerance of NaCl (0, 0.5, 1.5, 3, 6, 8 and 10 %; w/v) was determined in nutrient broth. Motility was observed by the hanging drop technique for cells in the exponential phase in nutrient broth. The KOH test was used to detect flexirubin pigments (Bernardet *et al.*, 2002). Oxidase was determined using two different methods: the conventional method based on using *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (Smibert & Krieg, 1994) and the alternative method based on the DrySlide Oxidase Test (BD BBL System). The presence of catalase was assessed using 3 % (v/v) H₂O₂. DNase test agar (Difco) was used for the DNase assay. Citrate utilization was tested on Simmons' citrate agar. Indole production was determined with Kovács' indole reagent in 1 % tryptone broth. H₂S production was determined on Kligler's iron agar. Hydrolysis of starch was examined on nutrient agar containing 0.2 % soluble starch (Barrow & Feltham, 1993). Hippurate hydrolysis was determined according to the method of Hwang & Ederer (1975). Tween 80 hydrolysis was detected using Tween 80 medium according to the method of Sierra (1957). Hydrolysis of adenine, casein, elastin, guanine, xanthine, hypoxanthine, testosterone and tyrosine was investigated by using previously described methods (Gordon, 1966, 1967; Gordon & Mihm, 1957). For chemotaxonomic studies, the three strains were cultivated in shake flasks containing BHI broth (BD) for 1 week at 22 °C. After checking for purity at maximum growth, the organisms were killed with formaldehyde (1 %, v/v), harvested by centrifugation, washed with distilled water and freeze-dried. Standard procedures were used to determine the fatty acid profile (Minnikin *et al.*, 1980; Yassin *et al.*, 2007), phospholipid composition (Yassin *et al.*, 1993) and isoprenoid quinones (Collins *et al.*, 1977; Yassin & Hupfer, 2006). The biomass of strain IMMIB L-1915^T used for polyamine analysis was grown on PYE medium (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2), harvested at the late exponential growth phase and lyophilized. Extraction of polyamines was performed as described by Busse & Auling (1988) and analysis was conducted using the HPLC equipment described by Stolz *et al.* (2007).

For phylogenetic analysis, the 16S rRNA gene of strain IMMIB L-1519^T was amplified by PCR using the procedure of Rainey *et al.* (1996) then directly sequenced using a *Taq* dye-deoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 310; Applied Biosystems). The closest relatives of the new isolate were determined by performing BLAST database searches. The 16S rRNA gene sequence of isolate IMMIB L-1519^T, as well as closely related sequences retrieved from GenBank, were added to the ARB database (Ludwig *et al.*, 2004) and aligned using the appropriate tool from the ARB package. Phylogenetic trees were reconstructed according to the neighbour-joining (Saitou & Nei, 1987), maximum-

parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. The stability of the groupings was estimated by bootstrap analysis (Felsenstein, 1985) of the neighbour-joining method based on 1000 replications.

To establish the phylogenetic position of strain IMMIB L-1519^T, its 16S rRNA gene sequence was determined in this study (1523 nt). Sequence database searches revealed that strain IMMIB L-1519^T was most closely related to species of the genus *Chryseobacterium* (data not shown). Phylogenetic analysis confirmed the association of strain IMMIB L-1519^T with the genus *Chryseobacterium*. A tree constructed using the neighbour-joining method showing the phylogenetic position of strain IMMIB L-1519^T is shown in Fig. 1. It is evident from the tree that strain IMMIB L-1519^T represents a hitherto unknown subline that clusters with *Chryseobacterium antarcticum*, *Chryseobacterium jeonii* and *Chryseobacterium marinum*, although bootstrap resampling analysis showed that this association was not particularly significant. Comparative 16S rRNA gene sequence analysis demonstrated that strain IMMIB L-1519^T displayed sequence similarity values of less than 97.2 % to the type strains of recognized species of the genus *Chryseobacterium*. The highest sequence similarity was shown with *C. jeonii* JCM 12382^T (97.2 %), *C. anthropi* NF 1366^T (96.7 %) and *Chryseobacterium haifense* DSM 19056^T (96.6 %). Other species of the genus *Chryseobacterium* displayed substantially lower levels of similarity (data not shown). Although it is not possible to delineate species solely on the basis of 16S rRNA gene sequence similarities, it is clear that the observed ≥2.8 % divergence between the unidentified organism and the currently recognized species of the genus *Chryseobacterium* is consistent with separate species status. It is now recognized that the genus *Chryseobacterium* contains a number of species that share 16S rRNA gene sequence similarities of significantly higher than 97 % while showing DNA–DNA relatedness values well below the 70 % cut-off point recommended by Wayne *et al.* (1987) for the delineation of genomic species. For instance, *Chryseobacterium daecheongense* and *Chryseobacterium defluvii* share 97.9 % 16S rRNA gene sequence similarity while their genomic DNA exhibits only 33.9 % relatedness (Kim *et al.*, 2005). Similarly, the 16S rRNA gene sequence of *Chryseobacterium aquifrigidense* is 98.4 % and 97.8 % similar to those of *Chryseobacterium gleum* and *Chryseobacterium indologenes*, respectively, but the corresponding DNA–DNA relatedness values are 39 % and 17 % (Park *et al.*, 2008). A similar situation has also been reported for the following pairs of type strains: *C. ureilyticum*/*C. joostei* (Herzog *et al.*, 2008), *C. luteum*/*C. shigense* (Behrendt *et al.*, 2007) and *C. piscium*/*C. balustinum* and *C. scopthalmum* (de Beer *et al.*, 2006). Thus the 97.2 % 16S rRNA gene sequence similarity value between strain IMMIB L-1519^T and its closest neighbour *C. jeonii* JCM 12382^T demonstrates that they belong to different genospecies (Stackebrandt & Ebers, 2006). We therefore did not determine the level of DNA–DNA relatedness between the two strains. Moreover, the distinctive phenotype of strain IMMIB L-1519^T (Table 1) confirmed that it represents a novel species.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain IMMIB L-1519^T within the radiation of species of the genus *Chryseobacterium*. The numbers at the nodes indicate the level of bootstrap support >70% based on neighbour-joining analyses of 1000 resampled datasets; solid circles indicate that the corresponding nodes (groupings) were also recovered in the maximum-likelihood and maximum-parsimony trees. Bar, 1.0% sequence divergence. The tree was rooted using *Bergeyella zoohelcum* ATCC 43767^T as an outgroup.

Strain IMMIB L-1519^T exhibited chemical markers characteristic for the genus *Chryseobacterium*. Cellular fatty acid analysis (Table 2) revealed the presence of saturated and monounsaturated fatty acid with straight-chain, iso-/anteiso-branched-chain and 3-OH fatty acids. The predominant fatty acids were C_{15:0} anteiso (65.3%), C_{15:0} iso (16.8%) and C_{17:0} anteiso 3-OH (55.9%). The new isolate could be distinguished from its two closest phylogenetic neighbours by clear differences in the proportions of C_{15:0} iso, C_{17:0} iso 3-OH and C_{17:0} anteiso 3-OH. Strain IMMIB L-1519^T contained phosphatidylethanolamine as the only identified phospholipid. Mass spectral analysis of the main isoprenoid quinone isolated from strain IMMIB L-1519^T showed a strong peak at *m/z* 581.37 attributable to [M+H]⁺ in the high mass region. This corresponds to a menaquinone with six unsaturated isoprene units (MK-6).

The polyamine pattern of strain IMMIB L-1519^T consisted of the predominant compound *sym*-homospermidine (17.4 μmol g⁻¹ dry weight), spermidine (3.5 μmol g⁻¹ dry weight), spermine (1.4 μmol g⁻¹ dry weight) and traces of putrescine (<0.1 μmol g⁻¹ dry weight). The results of the chemotaxonomic studies and the polyamine pattern were in excellent agreement with those of other species of the genus *Chryseobacterium* (Kämpfer *et al.*, 2009b).

Strain IMMIB L-1519^T could be distinguished from its phylogenetic neighbours *C. jeonii* DSM 17048^T and *C. anthropi* NF 1366^T by its ability to grow in the presence of 6% NaCl, to produce flexirubin type pigments, to assimilate adipate and to hydrolyse starch (in 2 days) and by its inability to hydrolyse DNA, hippurate and Tween 80 (Table 1). Using two different methods, the oxidase reaction

Table 1. Differentiating phenotypic characteristics of strain IMMIB L-1519^T and closely related species of the genus *Chryseobacterium*

Strains: 1, IMMIB L-1519^T; 2, *C. jeonii* DSM 17048^T; 3, *C. anthropi* NF 1366^T. All data are from this study. All strains are positive for: growth at 5, 10 and 22 °C; assimilation of D-glucose and malate; hydrolysis of casein and tyrosine; and catalase, acid phosphatase, alkaline phosphatase, leucine arylamidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase activities. All strains are negative for: growth at 42 °C and on MacConkey and cetrimide agars; production of indole, acetoin, H₂S and L-phenylalanine deaminase; citrate alkalization; assimilation of L-arabinose, capric acid and D-mannitol; hydrolysis of elastin, guanine, hypoxanthine, testosterone and xanthine; and α-chymotrypsin, cystine arylamidase, esterase C₄, ester lipase C₈, lecithinase, lipase C₁₄, β-galactosidase, nitrate reductase and urease activities. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3
Assimilation of:			
N-Acetylglucosamine	+	+	–
Adipate	+	–	–
Citrate	+	+	–
Gluconate	+	+	–
D-Mannose	+	–	+
Phenylacetic acid	+	+	–
Growth at:			
30 °C	+	–	+
37 °C	+*	–	+
Growth with 6% NaCl	+	–	–
Hydrolysis of			
Adenine (21 days)	–	+	–
Hippurate	–	+	+
DNA (3 days)	–	w	+
Starch (3 days)	+	–	–
Tween 80 (16 days)	–	+	+
Oxidase activity	+	– (+)†	+
Production of flexirubin pigments	+	–	–

*The strain lost its viability after 4 days of incubation at this temperature.

†Data from Yi *et al.* (2005).

of *C. jeonii* DSM 17048^T was negative in our hands, although a positive reaction was originally reported by Yi *et al.* (2005). The detailed phenotypic characteristics of strain IMMIB L-1519^T are given in the species description and in Table 1.

Therefore, based on both phenotypic and molecular-genetic considerations, we consider that strain IMMIB L-1519^T represents a novel species of the genus *Chryseobacterium*, for which the name *Chryseobacterium treverense* sp. nov. is proposed.

Description of *Chryseobacterium treverense* sp. nov.

Chryseobacterium treverense [tre.ve.ren'se. L. neut. adj. *treverense* pertaining to Augusta Trevirorum, the Latin

Table 2. Cellular fatty acid composition (%) of strain IMMIB L-1519^T and the most closely related species of the genus *Chryseobacterium*

Strains: 1, IMMIB L-1519^T; 2, *C. jeonii* DSM 17048^T; 3, *C. anthropi* NF 1366^T. All data from this study. Fatty acids amounting to <0.5% of the total fatty acids are not shown. tr, traces (<0.5%); ND, not detected. Values do not add up to 100% as the hydroxylated and non-hydroxylated fatty acids were firstly separated by TLC and then analysed separately with GC-MS (see text for further details).

Fatty acid	1	2	3
Saturated			
C _{14:0}	tr	tr	0.7
C _{16:0}	tr	0.8	1.6
C _{18:0}	ND	tr	1.0
Unsaturated			
C _{16:1ω5c}	ND	2.8	0.6
C _{16:1ω7c}	tr	0.7	0.6
C _{18:1ω5c}	ND	1.3	0.5
Branched-chain			
C _{13:0} iso	ND	3.1	1.8
C _{13:0} anteiso	tr	3.0	tr
C _{14:0} iso	ND	0.5	ND
C _{15:0} iso	16.8	24.8	ND
C _{15:0} anteiso	65.3	50.4	59.7
C _{16:1} iso ω7c	tr	0.7	ND
C _{16:0} iso	1.7	tr	3.4
C _{17:1} iso ω9c	8.6	8.6	13.3
C _{17:0} iso	0.8	ND	8.3
C _{17:0} anteiso	2.6	tr	4.5
Hydroxy			
C _{13:0} iso 3-OH	0.7	4.8	5.3
C _{13:0} anteiso 3-OH	4.1	9.5	6.0
C _{15:0} iso 3-OH	4.5	9.1	1.4
C _{15:0} anteiso 3-OH	0.6	2.8	tr
C _{17:0} iso 3-OH	31.6	34.3	73.2
C _{17:0} anteiso 3-OH	55.9	22.6	13.9

name of Treves (Trier, West Germany), the city from which the strain was sent for identification].

Cells are approximately 1.4–2.7 × 0.5–0.6 µm. Gram-reaction-negative, non-spore-forming, non-motile, strictly aerobic, oxidase- and catalase-positive rods. Grows at 5–30 °C (optimum, 20 °C). Growth also occurs at 37 °C but the organism loses its viability after 4 days of incubation. No growth on MacConkey agar, cetrimide agar or with 8% NaCl. On Columbia agar, colonies are yellow-pigmented, mucous, circular with regular edges and smooth with a diameter of 2–3 mm. Grows on BHI agar and nutrient agar. Tolerates 0–6% (w/v) NaCl with optimum growth at 6% NaCl. Flexirubin pigments are produced. N-Acetylglucosamine, adipate, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, glycogen, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, D-lyxose, D-tagatose, and turanose are assimilated. D-Adonitol, amygdalin, D-arabinose, L-arabinose, arbutin, capric acid, cellobiose, trisodium citrate, gentiobiose,

D-galactose, potassium gluconate, D-glucose, glycerol, D-fructose, inositol, inulin, D-lactose, D-mannose, D-mannitol, maltose, malate, melezitose, melibiose, phenylacetate, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, sucrose, trehalose, xylitol, D-xylose and L-xylose are not assimilated. Acid is not produced from L-arabinose, glycogen, D-glucose, inulin, D-lactose, maltose, D-mannitol, raffinose, D-ribose, D-sorbitol, starch, sucrose, trehalose or D-xylose. Aesculin and casein are hydrolysed. Tyrosine is weakly hydrolysed after 3 weeks of incubation. Adenine, elastin, gelatin, guanine, hippurate, hypoxanthine, testosterone and xanthine are not hydrolysed. Activity is detected for acid phosphatase, alkaline phosphatase, ester lipase C8, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase and α -glucosidase. No activity is detected for arginine dihydrolase, α -chymotrypsin, cystine arylamidase, esterase C4, α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, N-acetyl- β -glucosaminidase, lipase C14, α -mannosidase, trypsin or urease. Nitrate is not reduced. Tests for indole, acetoin, H₂S, lecithinase and L-phenylalanine deaminase production are negative. Menaquinone MK-6 is the major isoprenoid quinone. The polyamine pattern consists of the major compound sym-homospermidine, minor amounts of spermidine and spermine and traces of putrescine. The only identified phospholipid is phosphatidylethanolamine. The fatty acids C_{15:0} anteiso, C_{15:0} iso and C_{17:1} iso ω 9c are the major non-hydroxylated fatty acids and C_{17:0} iso 3-OH and C_{17:0} anteiso 3-OH are the major hydroxylated fatty acids.

The type strain, IMMIB L-1519^T (=DSM 22251^T=CCUG 57657^T), was isolated from human blood in Trier, Germany.

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