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- and 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-1519T (=DSM 22251T = CCUG 57657T).

The genus Chryseobacterium Vandamme et al. 1994 emend. Kämpfer et al. 2009 was originally proposed to comprise the generically misclassified organisms Flavobacterium balustri num, 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-spor-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; Vanechoutte 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-1519T 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 17048T and Chryseobacterium anthropi NF 1366T were used as reference strains. The three strains were characterized biochemically using the API 20E, API 20NE, API 50CH and API ZYM identification systems.
For phylogenetic analysis, the 16S rRNA gene of strain IMMIB L-1519T was amplified by PCR using the procedure of Rainey et al. (1996) and 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-1519T, 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 method (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-1519T, its 16S rRNA gene sequence was determined in this study (1523 nt). Sequence database searches revealed that strain IMMIB L-1519T was most closely related to species of the genus Chryseobacterium (data not shown). Phylogenetic analysis confirmed the association of strain IMMIB L-1519T with the genus Chryseobacterium. A tree constructed using the neighbour-joining method showing the phylogenetic position of strain IMMIB L-1519T is shown in Fig. 1. It is evident from the tree that strain IMMIB L-1519T represents a hitherto unknown subline that clusters with Chryseobacterium antarcticum, Chryseobacterium jeonii and Chryseobacterium marism, although bootstrap resampling analysis showed that this association was not particularly significant. Comparative 16S rRNA gene sequence analysis demonstrated that strain IMMIB L-1519T 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 12382T (97.2 %), C. anthrophi NF 1366T (96.7 %) and Chryseobacterium haifense DSM 19056T (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 aquifrigidendes 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. jeoestei (Herzog et al., 2008), C. luteum/C. shigense (Behrendt et al., 2007) and C. piscium/C. balustinum and C. scopithalum (de Beer et al., 2006). Thus the 97.2 % 16S rRNA gene sequence similarity value between strain IMMIB L-1519T and its closest neighbour C. jeonii JCM 12382T 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-1519T (Table 1) confirmed that it represents a novel species.
Strain IMMIB L-1519\textsuperscript{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\textsubscript{15:0} anteiso (65.3\%), C\textsubscript{15:0} iso (16.8\%) and C\textsubscript{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\textsubscript{15:0} iso, C\textsubscript{17:0} iso 3-OH and C\textsubscript{17:0} anteiso 3-OH. Strain IMMIB L-1519\textsuperscript{T} contained phosphatidylethanolamine as the only identified phospholipid. Mass spectral analysis of the main isoprenoid quinone isolated from strain IMMIB L-1519\textsuperscript{T} showed a strong peak at $m/z$ 581.37 attributable to [M + H]\textsuperscript{+} 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\textsuperscript{T} consisted of the predominant compound *sym*-homospermidine (17.4\,µmol\,g\textsuperscript{-1} dry weight), spermidine (3.5\,µmol\,g\textsuperscript{-1} dry weight), spermine (1.4\,µmol\,g\textsuperscript{-1} dry weight) and traces of putrescine (<0.1\,µmol\,g\textsuperscript{-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\textsuperscript{T} could be distinguished from its phylogenetic neighbours *C. jeonii* DSM 17048\textsuperscript{T} and *C. anthropi* NF 1366\textsuperscript{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 IMMB L-1519\(^T\) and closely related species of the genus Chryseobacterium**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Adipate</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Gluconate</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenylacetic acid</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Growth at:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 °C</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>37 °C</td>
<td>+*</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Growth with 6 % NaCl</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
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<tr>
<td>Adenine (21 days)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hippurate</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNA (3 days)</td>
<td>-</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>Starch (3 days)</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80 (16 days)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase activity</td>
<td>+</td>
<td>-</td>
<td>(+)(^t)</td>
</tr>
<tr>
<td>Production of flexirubin pigments</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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

\(^t\)Data from Yi et al. (2005).

## Description of Chryseobacterium treverense sp. nov.

Chryseobacterium treverense [tre.ve.ren'se. L. neut. adj. treverense pertaining to Augusta Trevirorum, the Latin 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-reactive-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, glycerol, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, L-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, histidine, hyponcholine, testosterone and xanthine are not hydrolysed. Activity is detected for acid phosphatase, alkaline phosphatase, ester lipase C8, leucine aminopeptidase, valine aminopeptidase, naphthol-AS-BI-phosphatase, alkaline phosphatase, ester lipase C8, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphatase, alkaline phosphatase, ester lipase C8, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphatase, alkaline phosphatase, ester lipase C8, leucine arylamidase, valine arylamidase.

The only identified phospho-arginine dihydrolase, phospho-esterase C4, phospho-glucuronidase, phospho-a-galactosidase, phospho-b-galactosidase, phospho-a-fucosidase, phospho-N-acetyl-b-glucosaminidase, lipase C14, phospho-mannnosidase, trypsin or urease. Nitrate is not reduced. Tests for indole, acetoin, H2S, lecithinase and xanthine are not hydrolysed. Activity is detected for acid phosphatase, alkaline phosphatase, ester lipase C8, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphatase, alkaline phosphatase, ester lipase C8, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphatase, alkaline phosphatase, ester lipase C8, leucine arylamidase, valine arylamidase.

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

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References


