Chryseobacterium sediminis sp. nov., isolated from a river sediment

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A slightly yellow-pigmented strain (IMT-174T) isolated from a river sediment in Guyana was studied in detail for its taxonomic position. Cells of the isolate appeared rod-shaped and stained Gram-negative. Comparative 16S rRNA gene sequence analysis showed that the isolate had the highest sequence similarities to type strains of Chryseobacterium rhizoplanae (99.0%), Chryseobacterium viscerum (98.9%), Chryseobacterium lactis (98.8%) and Chryseobacterium indologenes (98.6%). The 16S rRNA gene sequence similarities to all other species of the genus Chryseobacterium were below 98.5%. Fatty acid analysis of whole-cell hydrolysates of the strain resulted in a pattern typical of members of the genus Chryseobacterium, with fatty acids iso-C₁₅ : ₀, iso-C₁₅ : ₂-OH, iso-C₁₇ : ₁ω₁₀c and iso-C₁₇ : ₀ 3-OH as major compounds. The polyamine pattern contained predominantly sym-homospermidine. The major quinone was menaquinone MK-6 and the only lipid identified in the polar lipid profile was phosphatidylethanolamine. In addition, 13 unidentified lipids were detected in moderate to major amounts. DNA–DNA hybridizations with type strains of C. rhizoplanae, C. viscerum, C. lactis and C. indologenes resulted in values below 70%. In addition to the genotypic differences, differentiating biochemical and chemotaxonomic properties confirmed that isolate IMT-174T represents a novel species, for which the name Chryseobacterium sediminis sp. nov. (type strain IMT-174T=LMG 28695T=CIP 110895T) is proposed.

The genus Chryseobacterium, described more than 20 years ago by Vandamme et al. (1994), is a still-growing genus with a large number of species isolated from a broad range of habitats. Several of the species of the genus Chryseobacterium were isolated from aquatic environments, among those Chryseobacterium angstadtii, which was isolated from water of a newt tank (Kirk et al., 2013), Chryseobacterium aquaticum from a water reservoir (Kim et al., 2008), Chryseobacterium hispalense and Chryseobacterium wanjense from rainwater ponds (Montero-Calasanz et al., 2013; Weon et al., 2006), Chryseobacterium hispanicum from a drinking water distribution system (Gallego et al., 2006), Chryseobacterium taihuense from an eutrophic lake (Wu et al., 2013), Chryseobacterium piperi from a freshwater creek (Strahan et al., 2011), Chryseobacterium daecheongense from a freshwater lake sediment (Kim et al., 2005) and several novel species from plant material (Kämpfer et al., 2014, 2015).

Here we describe strain IMT-174T, which was isolated from river sediment in Guyana. Initially, the strain was isolated and grown on tryptic soy agar (TSA; Oxoid) at 30°C and also further maintained and subcultivated on this agar at 30°C for 48 h. Analyses of the 16S rRNA gene sequence, and further biochemical, physiological and chemotaxonomic analyses, were carried out to characterize the strain.

All cultural and morphological characteristics were recorded from cultures after growth on TSA. Gram staining was performed according to the method of Gerhardt et al. (1994) and a motility test was done under a light microscope with cells grown for 3 days in tryptic soy broth (TSB; Oxoid) at 30°C. Temperature-dependent growth was tested at 4, 11, 30, 36, 40 and 45°C on nutrient agar. NaCl tolerance was investigated at different concentrations of NaCl (0.5 and 1.0–8.0 %, w/v, in 1 % increments) in TSB. pH-Dependent growth was tested in TSB adjusted with HCl and NaOH to pH values between pH 4.0 and 12.0 in 1.0 pH unit increments.

Strain IMT-174T stained Gram-negative and produced visible (diameter about 2 mm) yellowish colonies within 48 h...
at 30 °C. The isolate did not grow at temperatures below 4 °C or above 37 °C. The strain grew very slowly at 36 °C and at a NaCl concentration of 1–2 % (w/v), but not with 3 % (w/v) or more NaCl.

Colonies showed a translucent, glistening appearance with entire edges. A yellow pigment of the flexirubin type (KOH method according to Reichenbach, 1989) was produced on nutrient agar. Oxidase activity was positive with the Oxidase reagent (bioMérieux) used according to the instructions of the manufacturer. Cells of the strain were non-motile rods (approx. 1 µm wide and 2 µm long). No spores could be detected. The strain grew well on complex agar media, like nutrient agar, brain heart infusion agar, and at a NaCl concentration of 3 % (w/v) or more NaCl.

The strain was characterized physiologically/biochemically using the methods described previously by Kämpfer et al. (1991) in addition to some biochemical tests, among them the production of hydrogen sulphide using the lead acetate paper and triple-sugar-iron methods; indole reaction with Ehrlich’s and Kovacs’ reagents; activity of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, DNase (Oxoid), and triple-sugar-iron methods; indole reaction with Ehrlich’s and Kovacs’ reagents; activity of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, DNase (Oxoid CM321; supplemented with 0.01 % toluidine blue), β-galactosidase (ONPG) and urease on Christensen’s urea agar (Kämpfer, 1990); hydrolysis of casein, gelatin (plate method), starch and tyrosine (Smibert & Krieg, 1994). Very similar to many other members of the genus Chryseobacterium, the strain was not able to utilize many carbon sources, but was able to hydrolyse some chromogenic substrates. The biochemical/physiological data are given in Table 1 and in the species description.

The analysis of the cellular fatty acid profiles from whole-cell hydrolysates was performed as described previously (Kämpfer & Kroppenstedt, 1996) by using a HP gas chromatograph (HP 6890) with Sherlock MIDI software version 2.11 and TSBA peak-naming table version 4.1. Prior to fatty acid extraction, the strain was cultured on TSA at 37 °C for 48 h.

The results of fatty acid analysis are shown in Table 2 in comparison with those of the most closely related type strains (Chryseobacterium rhizoplanae JM-534T, Chryseobacterium lactis LMG 12278T, Chryseobacterium indologenes CCUG 14556T and Chryseobacterium viscerum 687B-08T) and revealed a profile for IMT-174T typical of members of the genus Chryseobacterium, with the following most abundant fatty acids: iso-C15:0, iso-C17:0 3-OH, iso-C17:1ω9c and iso-C15:0 2-OH (which was detected as a summed feature: iso-C15:0 2-OH/C16:1ω7c) but as shown in several studies before could be clearly identified as iso-C15:0 2-OH; Vandamme et al., 1994; Montero-Calasanz et al., 2013). Only minor differences were found in comparison with the profiles of the type strains of the most closely related species of the genus Chryseobacterium.

Polyamines, quinones and polar lipids were extracted from biomass grown in 3.3 × PYE broth [1 % (w/v) peptone from casein, 1 % (w/v) yeast extract, pH 7.2]. Polyamines

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Table 1. Comparison of characteristics of strain IMT-174T with those of closely related species of the genus Chryseobacterium

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succrose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Salicin</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Trehalose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 36–37 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth on MacConkey agar</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Results in agreement with those reported by Holmes et al. (2013).
†Results in agreement with those reported by Hugo et al. (2003).

Table 2. Long-chain fatty acid composition of species of the genus Chryseobacterium

<table>
<thead>
<tr>
<th>Fatty acid</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
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<tr>
<td>iso-C13:0</td>
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<td>–</td>
<td>1.2</td>
<td>2.0</td>
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<td>–</td>
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<tr>
<td>Unknown</td>
<td>13.565*</td>
<td>9.0</td>
<td>5.5</td>
<td>6.9</td>
<td>6.4</td>
<td>13.1</td>
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<tr>
<td>iso-C15:0</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C15:1 F</td>
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<td>–</td>
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<td>–</td>
</tr>
<tr>
<td>iso-C15:0 3-OH</td>
<td>2.6</td>
<td>2.8</td>
<td>2.7</td>
<td>2.4</td>
<td>2.1</td>
<td>3.4</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>–</td>
<td>–</td>
<td>0.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C16:0</td>
<td>2.5</td>
<td>1.4</td>
<td>1.6</td>
<td>1.7</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>C15:0 3-OH</td>
<td>2.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>iso-C16:0 3-OH</td>
<td>1.3</td>
<td>1.2</td>
<td>1.0</td>
<td>0.8</td>
<td>1.8</td>
<td></td>
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<tr>
<td>iso-C16:0 3-Ω</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>1.3</td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>1.4</td>
<td>1.4</td>
<td>1.2</td>
<td>1.4</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>2.4</td>
<td>1.9</td>
<td>1.9</td>
<td>2.2</td>
<td>1.1</td>
<td>0.9</td>
</tr>
<tr>
<td>iso-C17:0 3-OH</td>
<td>16.0</td>
<td>20.4</td>
<td>15.6</td>
<td>14.2</td>
<td>14.3</td>
<td>20.2</td>
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<tr>
<td>iso-C17:0 9c</td>
<td>23.1</td>
<td>17.9</td>
<td>19.2</td>
<td>15.6</td>
<td>23.4</td>
<td>14.5</td>
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<tr>
<td>C18:0 9c</td>
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<td>–</td>
<td>0.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Summed feature 3†</td>
<td>9.3</td>
<td>8.7</td>
<td>6.5</td>
<td>5.4</td>
<td>9.8</td>
<td>11.2</td>
</tr>
</tbody>
</table>

*Unknown fatty acid; numbers indicate equivalent chain-length.
†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 iso-C15:0 2-OH and/or C16:1ω7c. As shown in several studies, summed feature iso-C15:0 2-OH/C16:1ω7c could be clearly identified as iso-C15:0 2-OH; Vandamme et al., 1994; Montero-Calasanz et al., 2013.

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were extracted according to the method of Busse & Auling (1988) and analysed according to the method of Busse et al. (1997). Quinones and polar lipids were extracted and analysed as described by Tindall (1990a, b) and Altenburger et al. (1996). The HPLC apparatus used was reported by Stolz et al. (2007). The major polyamine was \textit{sym}-homospermidine and the quinone system consisted exclusively of menaquinone MK-6, which is in line with the genus description (Vandamme et al., 1994; Kämpfer et al., 2009). In the polar lipid profile (Fig. 1), phosphatidylethanolamine, one unidentified aminolipid (AL1) and three unidentified polar lipids detectable only after total lipid staining (L1, L3, L5) predominated. Moderate to minor amounts of two polar lipids (L2, L4), four unidentified aminolipids (AL2, AL3, AL4, AL5) and three unidentified glycolipids (GL1, GL2, GL3) were present as well. Similar polar lipid profiles have been reported for several species of the genus \textit{Chryseobacterium} (Kämpfer et al., 2003; Herzog et al., 2008; Nguyen et al., 2013).

Phylogenetic analysis was based on nearly full-length 16S rRNA gene sequences. The 16S rRNA gene fragment of strain IMT-174\textsuperscript{T} obtained by sequence analysis using the dideoxy sequencing method was a continuous stretch of

\begin{figure}
\centering
\includegraphics[width=\textwidth]{diagram.png}
\caption{Maximum-parsimony tree based on nearly full-length 16S rRNA gene sequences showing the phylogenetic affiliation of strain IMT-174\textsuperscript{T} to the most closely related type strains of the genus \textit{Chryseobacterium}. The tree was calculated in ARB and based on 16S rRNA gene sequences between gene termini 96 to 1394 (according to \textit{Escherichia coli} numbering; Brosius et al., 1978). Chryseobacterium species which did not cluster directly with strain IMT-174\textsuperscript{T} were removed from the tree without changing the overall tree topology. Numbers at nodes represent bootstrap values \(>70\%\) (100 replications). Nodes marked with filled circles were also present in the respective maximum-likelihood tree. Larger circles mark nodes with \(70\%\) bootstrap support in the maximum-likelihood tree. \textit{Elizabethkingia anophelis} R26\textsuperscript{T} was used as an outgroup. Bar, 0.1 substitutions per nucleotide position.}
\end{figure}

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analyses 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 LTPs119 (November 2014). Sequences missing in the database were aligned using the SILVA Incremental Aligner (SINA; Pruesse et al., 2012) version v1.2.11 and implemented into the database. The sequence alignment was checked manually. A maximum-likelihood tree was reconstructed using RAxML v7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis (100 resamplings) and a maximum-parsimony tree (Fig. 2) was reconstructed using DNA Pars version 3.6 (Felsenstein, 2005). Both trees were based on 100 resamplings (bootstrap analysis; Felsenstein, 1985) and gene sequence positions 96 to 1394 (Escherichia coli numbering; Brosius et al., 1978). The type strain of Elizabethkingia anopliis was used as an outgroup. Pairwise 16S rRNA gene sequence similarities were calculated using the ARB neighbour-joining tool without the application of an evolutionary model for similarity matrix generation.

Pairwise 16S rRNA gene sequence analysis indicated highest 16S rRNA gene sequence similarity to type strains of four species of the genus Chryseobacterium, C. rhizoplanæ (99.0 %), C. viscærum (98.9 %), C. lactis (98.8 %) and C. indologenes (98.6 %). Sequence similarities to all other type strains of species of the genus Chryseobacterium were below 98.5 % 16S rRNA gene sequence similarity. In the phylogenetic trees calculated, strain IMT-174T clustered directly with the type strain of C. rhizoplanæ, but not with the type strains of the other closely related species. In general, the clustering of strain IMT-174T was not supported by high bootstrap values independent of the treeing method applied.

DNA–DNA hybridization experiments were performed with strain IMT-174T and the type strains of the most closely related species of the genus Chryseobacterium, C. rhizoplanæ JM-534T, C. lactis LMG 12278T, C. indologenes CCUG 14556T and C. viscærum 687B-08T according to the method of Ziemke et al. (1998) (except that for nick translation, 2 μg of DNA was labelled during 3 h of incubation at 15 °C). Strain IMT-174T showed low DNA–DNA similarities to C. rhizoplanæ JM-534T (56 %, reciprocal 41 %), C. lactis LMG 12278T (45 %, reciprocal 25 %), C. viscærum 687B-08T (48 %, reciprocal 28 %) and C. indologenes CCUG 14556T (41 %, reciprocal 19 %).

On the basis of the results of this polyphasic study, it is obvious that strain IMT-174T represents a novel species, for which the name Chryseobacterium sediminæis sp. nov. is proposed.

Description of Chryseobacterium sediminæis sp. nov.

Chryseobacterium sediminæis (se.di’mi.nis. L. gen. n. sediminæis of a sediment).

Cells stain Gram-negative. They are non-motile, and appear as non-spore-forming rods, approximately 1 μm in width and 2 μm in length. Aerobic, oxidase-positive and catalase-positive. Good growth can be observed after 48 h of growth on nutrient agar, brain heart infusion agar, TSA and R2A agar (all Oxoïd) at 10–30 °C. No growth occurs on MacConkey agar (Oxoid) at 28 °C. Unable to grow at temperatures below 4 °C or above 40 °C. Cells grow in the presence of 1.0–2.0 % NaCl as an additional ingredient of nutrient agar. Colonies on nutrient agar produce a yellowish colour and appear circular, translucent and glistening with entire edges. The yellow pigment of the flexirubin type is non-diffusible and non-fluorescent. Acid is produced from d-glucose. No acid is produced from L-arabinose, maltose, trehalose, salicin, sucrose, adonitol, D-arabitol, dulcitol, erythritol, myo-inositol, lactose, D-mannitol, melibiose, methyl α-D-glucoside, raffinose, L-rhamnose, D-sorbitol or D-xylose. Urease activity, and hydrolysis of aesculins, casein, gelatin, starch, DNA and tyrosine are positive, while indole production, hydrogen sulphide production and activity of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and β-galactosidase are negative. The following compounds are utilized weakly as a sole source of carbon: d-glucose, maltose and d-mannose. The following
comounds are not utilized as a sole source of carbon:
L-arabinose, acetate, propionate, N-acetylglucosamine, N-acetylglucosaminidase, cellulose, D-galactose, gluconate, salicin, D-fructose, glycerol, D-mannitol, maltitol, Z-melibiose, L-rhamnose, D-ribose, sucrose, D-xylene, adonitol, i-inositol, D-sorbitol, putrescine, cis-aconitate, trans-aconitate, 4-aminobutyrate, adipate, azelate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, 2-oxoglutamate, pyruvate, suberate, citrate, mesaconate, L-alanine, β-alanine, L-ornithine, L-phenylalanine, L-serine, L-aspartate, L-histidine, L-leucine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. The chromogenic substrates p-nitrophenyl α-D-glucopyranoside, p-nitrophenyl β-D-glucopyranoside (weakly), p-nitrophenyl β-D-xylopyranoside, bis-p-nitrophenyl phosphate, bis-p-nitrophenyl phenylphosphonate, bis-p-nitrophenyl phosphorylcholine, 2-deoxymyoinosiose-2’-p-nitrophenyl phosphate, L-alanine-p-nitroanilide, γ-L-glutamate-p-nitroanilide and L-proline-p-nitroanilide are hydrolysed. p-Nitrophenyl β-D-galactopyranoside and p-nitrophenyl β-D-glucuronide are not hydrolysed. The major cellular fatty acids are iso-C₁₅:₀, iso-C₁₅:₀-2-OH, iso-C₁₇:₁ω9c and iso-C₁₇:₀-3-OH. The polyamine profile is characterized by the major compound sym-homoserpinime and the quinone system contains only menaquinone MK-6. The polar lipid profile contains the major lipids phosphatidylethanolamine, the unidentified aminolipid AL1 and the three unidentified polar lipids L1, L3 and L5 and moderate to minor amounts of two polar lipids (L2, L4), four unidentified aminolipids (AL2, AL3, AL4, AL5) and three unidentified glycolipids (GL1, GL2, GL3).

The type strain is IMT-174^T (=LMG 28695^T = CIP 110895^T), isolated from a river sediment in Guyana.

Acknowledgements

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References


