

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

Peter Kämpfer,<sup>1</sup> Hans-Jürgen Busse,<sup>2</sup> John A. McInroy<sup>3</sup> and Stefanie P. Glaeser<sup>1</sup>

### Correspondence

Peter Kämpfer  
peter.kaempfer@umwelt.uni-giessen.de

<sup>1</sup>Institut für Angewandte Mikrobiologie, Universität Giessen, Germany

<sup>2</sup>Institut für Mikrobiologie, Veterinärmedizinische Universität, A-1210 Wien, Austria

<sup>3</sup>Department of Entomology and Plant Pathology, Auburn University, Alabama, USA

A slightly yellow-pigmented strain (IMT-174<sup>T</sup>) 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-C<sub>15</sub>:<sub>0</sub>, iso-C<sub>15</sub>:<sub>0</sub> 2-OH, iso-C<sub>17</sub>:<sub>1</sub>ω<sub>9c</sub> and iso-C<sub>17</sub>:<sub>0</sub> 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-174<sup>T</sup> represents a novel species, for which the name *Chryseobacterium sediminis* sp. nov. (type strain IMT-174<sup>T</sup>=LMG 28695<sup>T</sup>=CIP 110895<sup>T</sup>) 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 new tank (Kirk *et al.*, 2013), *Chryseobacterium aquaticum* from a water reservoir (Kim *et al.*, 2008), *Chryseobacterium hispalense* and *Chryseobacterium wanjuae* 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-174<sup>T</sup>, 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-174<sup>T</sup> stained Gram-negative and produced visible (diameter about 2 mm) yellowish colonies within 48 h

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strains IMT-174<sup>T</sup> is KR349467.

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, R2A agar and TSA, but not on MacConkey agar (Oxoid).

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 dihydrolyase, 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-534<sup>T</sup>, *Chryseobacterium lactis* LMG 12278<sup>T</sup>, *Chryseobacterium indologenes* CCUG 14556<sup>T</sup> and *Chryseobacterium viscerum* 687B-08<sup>T</sup>) and revealed a profile for IMT-174<sup>T</sup> typical of members of the genus *Chryseobacterium*, with the following most abundant fatty acids: iso-C<sub>15:0</sub>, iso-C<sub>17:0</sub> 3-OH, iso-C<sub>17:1</sub>ω9c and iso-C<sub>15:0</sub> 2-OH (which was detected as a summed feature: iso-C<sub>15:0</sub> 2-OH/C<sub>16:1</sub>ω7c, but as shown in several studies before could be clearly identified as iso-C<sub>15:0</sub> 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

**Table 1.** Comparison of characteristics of strain IMT-174<sup>T</sup> with those of closely related species of the genus *Chryseobacterium*

Strains: 1, *Chryseobacterium sediminis* sp. nov. IMT-174<sup>T</sup>; 2, *Chryseobacterium rhizoplanae* JM-534<sup>T</sup>; 3, *Chryseobacterium lactis* LMG 12278<sup>T</sup>; 4, *Chryseobacterium joostei* CIP 105533<sup>T</sup>; 5, *Chryseobacterium indologenes* CCUG 14556<sup>T</sup>; 6, *Chryseobacterium viscerum* 687B-08<sup>T</sup>. All data are from this study and that of Kämpfer *et al.* (2015). +, Positive; (+), weakly positive; –, negative. All strains produced acid from D-glucose and not from D-ribose or D-xylose. All strains were positive for digestion of casein and aesculin hydrolysis.

Characteristic	1	2	3	4	5	6
Acid production from:						
Sucrose	–	–	–*	–†	–	+
Arabinose	–	+	–*	–†	–	–
Salicin	–	+	–*	–†	–	–
Trehalose	–	+	+	+	+	+
Growth at 36–37 °C	+	+	+	–†	+	+
Growth on MacConkey agar	–	–	+	+	–	+

\*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*

Strains: 1, *Chryseobacterium sediminis* sp. nov. IMT-174<sup>T</sup>; 2, *C. rhizoplanae* JM-534<sup>T</sup>; 3, *C. lactis* LMG 12278<sup>T</sup>; 4, *C. joostei* CIP 105533<sup>T</sup>; 5, *C. indologenes* CCUG 14556<sup>T</sup>; 6, *C. viscerum* 687B-08<sup>T</sup>. All data are from this study.

Fatty acid	1	2	3	4	5	6
iso-C <sub>13:0</sub>	–	–	1.2	2.0	–	–
Unknown 13.565*	9.0	5.5	6.9	6.4	13.1	7.5
Unknown 14.949*	1.7	–	–	–	–	–
iso-C <sub>15:0</sub>	29.8	38.6	39.4	44.8	28.4	34.3
iso-C <sub>15:1</sub> F	–	–	1.8	1.2	3.4	2.1
iso-C <sub>15:0</sub> 3-OH	2.6	2.8	2.7	2.4	2.1	3.4
anteiso-C <sub>15:0</sub>	–	–	0.6	–	–	–
C <sub>16:0</sub>	2.5	1.4	1.6	1.7	1.2	1.0
C <sub>15:0</sub> 3-OH	2.4	–	–	–	–	–
C <sub>16:0</sub> 3-OH	–	1.3	1.2	1.0	0.8	1.8
iso-C <sub>16:0</sub> 3-OH	1.0	–	–	1.3	0.8	1.6
Unknown 16.582*	1.4	1.4	1.2	1.4	1.3	1.3
iso-C <sub>17:0</sub>	2.4	1.9	1.9	2.2	1.1	0.9
iso-C <sub>17:0</sub> 3-OH	16.0	20.4	15.6	14.2	14.3	20.2
iso-C <sub>17:1</sub> ω9c	23.1	17.9	19.2	15.6	23.4	14.5
C <sub>18:1</sub> ω5c	–	–	–	0.4	–	–
Summed feature 3†	9.3	8.7	6.5	5.4	9.8	11.2

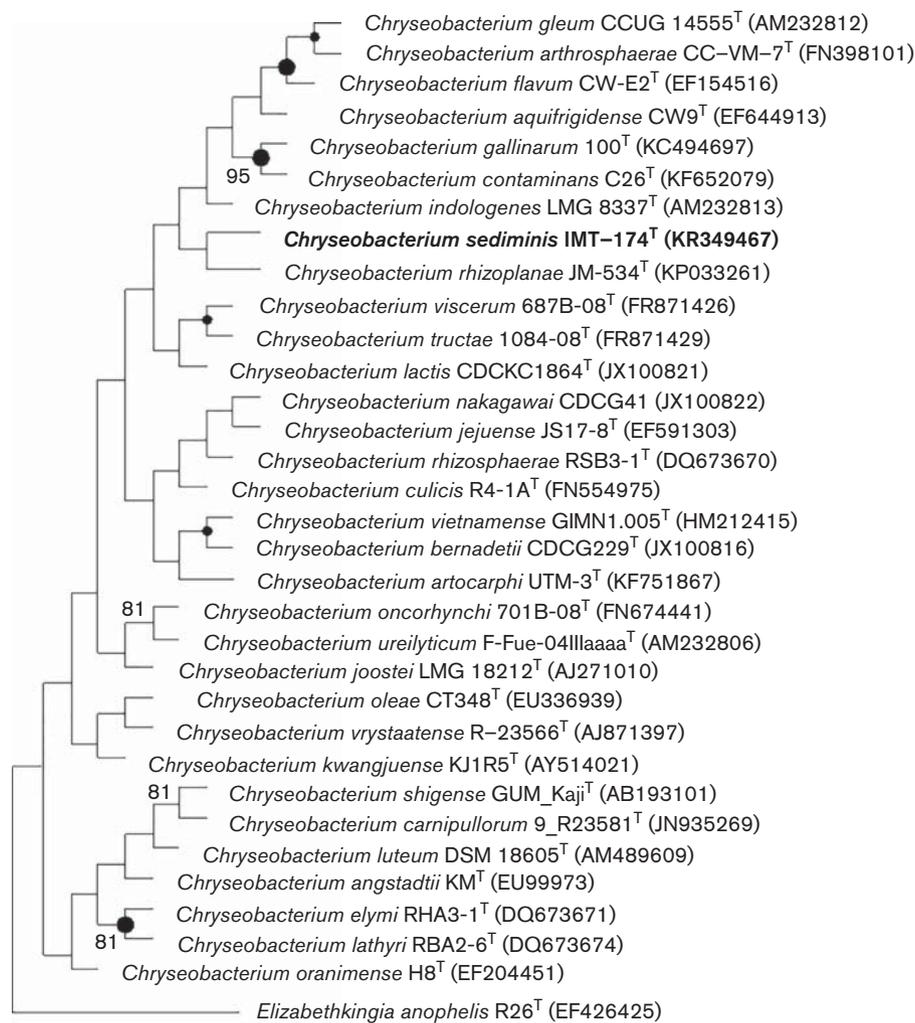
\*Unknown fatty acid; numbers indicate equivalent chain-length.

†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-C<sub>15:0</sub> 2-OH and/or C<sub>16:1</sub>ω7t. As shown in several studies, summed feature iso-C<sub>15:0</sub> 2-OH/C<sub>16:1</sub>ω7c could be clearly identified as iso-C<sub>15:0</sub> 2-OH (Vandamme *et al.*, 1994; Montero-Calasanz *et al.*, 2013).

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 *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 *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<sup>T</sup> obtained by sequence analysis using the dideoxy sequencing method was a continuous stretch of



**Fig. 1.** Maximum-parsimony tree based on nearly full-length 16S rRNA gene sequences showing the phylogenetic affiliation of strain IMT-174<sup>T</sup> to the most closely related type strains of the genus *Chryseobacterium*. The tree was calculated in ARB and based on 16S rRNA gene sequences between gene termini 96 to 1394 (according to *Escherichia coli* numbering; Brosius *et al.*, 1978). *Chryseobacterium* species which did not cluster directly with strain IMT-174<sup>T</sup> 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. *Elizabethkingia anophelis* R26<sup>T</sup> was used as an outgroup. Bar, 0.1 substitutions per nucleotide position.

1396 nt spanning gene positions 48 to 1470 (according to the *Escherichia coli* numbering published by Brosius *et al.*, 1978). Pairwise sequence similarities with the most closely related type strains were obtained using the EzTaxon type strain database (Kim *et al.*, 2012). Detailed phylogenetic 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 DNAPARS 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 anophelis* 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. rhizoplanae*

(99.0%), *C. viscerum* (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-174<sup>T</sup> clustered directly with the type strain of *C. rhizoplanae*, but not with the type strains of the other closely related species. In general, the clustering of strain IMT-174<sup>T</sup> was not supported by high bootstrap values independent of the treeing method applied.

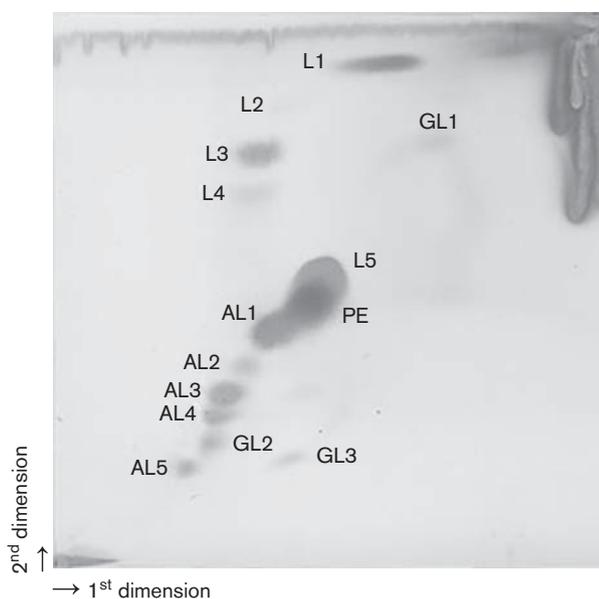
DNA–DNA hybridization experiments were performed with strain IMT-174<sup>T</sup> and the type strains of the most closely related species of the genus *Chryseobacterium*, *C. rhizoplanae* JM-534<sup>T</sup>, *C. lactis* LMG 12278<sup>T</sup>, *C. indologenes* CCUG 14556<sup>T</sup> and *C. viscerum* 687B-08<sup>T</sup> 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-174<sup>T</sup> showed low DNA–DNA similarities to *C. rhizoplanae* JM-534<sup>T</sup> (56%, reciprocal 41%), *C. lactis* LMG 12278<sup>T</sup> (45%, reciprocal 25%), *C. viscerum* 687B-08<sup>T</sup> (48%, reciprocal 28%) and *C. indologenes* CCUG 14556<sup>T</sup> (41%, reciprocal 19%).

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

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

*Chryseobacterium sediminis* (se.di'mi.nis. L. gen. n. *sediminis* 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 Oxoid) 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 aesculin, 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



**Fig. 2.** Polar lipids profile of strain IMT-174<sup>T</sup> after separation by two-dimensional TLC and detection using 5% ethanolic molybdato-phosphoric acid. PE, phosphatidylethanolamine; AL1–5, unidentified aminolipids; GL1–3, unidentified glycolipids; L1–5, unidentified polar lipids not detectable with any of the spray reagents specific for lipids containing a phosphate group, an amino group or a sugar moiety.

compounds are not utilized as a sole source of carbon: L-arabinose, acetate, propionate, *N*-acetylgalactosamine, *N*-acetylglucosamine, cellobiose, D-galactose, gluconate, salicin, D-fructose, glycerol, D-mannitol, maltitol,  $\alpha$ -melibiose, L-rhamnose, D-ribose, sucrose, D-xylose, adonitol, i-inositol, D-sorbitol, putrescine, *cis*-aconitate, *trans*-aconitate, 4-aminobutyrate, adipate, azelate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, 2-oxoglutarate, pyruvate, suberate, citrate, mesaconate, L-alanine,  $\beta$ -alanine, L-ornithine, L-phenylalanine, L-serine, L-aspartate, L-histidine, L-leucine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. The chromogenic substrates *p*-nitrophenyl  $\alpha$ -D-glucopyranoside, *p*-nitrophenyl  $\beta$ -D-glucopyranoside (weakly), *p*-nitrophenyl  $\beta$ -D-xylopyranoside, bis-*p*-nitrophenyl phosphate, bis-*p*-nitrophenyl phenylphosphonate, bis-*p*-nitrophenyl phosphorylcholine, 2-deoxythymidine-2'-*p*-nitrophenyl phosphate, L-alanine-*p*-nitroanilide,  $\gamma$ -L-glutamate-*p*-nitroanilide and L-proline-*p*-nitroanilide are hydrolysed. *p*-Nitrophenyl  $\beta$ -D-galactopyranoside and *p*-nitrophenyl  $\beta$ -D-glucuronide are not hydrolysed. The major cellular fatty acids are iso-C<sub>15:0</sub>, iso-C<sub>15:0</sub> 2-OH, iso-C<sub>17:1</sub>OH and iso-C<sub>17:0</sub> 3-OH. The polyamine pattern is characterized by the major compound *sym*-homospermidine 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<sup>T</sup> (=LMG 28695<sup>T</sup>=CIP 110895<sup>T</sup>), isolated from a river sediment in Guyana.

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