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Chryseobacterium endalhagicum sp. nov., isolated from seed of leguminous plant

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Abstract

A Gram-stain-negative, yellow-pigmented bacterium, designated as L7^T, was isolated from seeds of *Alhagi sparsifolia* Shap., a leguminous plant that grows in northwest PR China. Strain L7^T was found to be non-flagellated, non-spore forming rods which can grow at 10–37 °C, pH 6.0–8.5 and in 0–3% (v/w) NaCl concentration. The 16S rRNA gene sequence analysis showed that strain L7^T belongs to the genus *Chryseobacterium* with sequence similarities to *Chryseobacterium vietnamense* GIMN1.005^T (98.1%), *C. bernardetii* NCCTC13530^T (98.0%), *C. vrystaatense* LMG 22846^T (97.9%), *C. nakagawai* NCTC13529^T (97.7%), *C. shigense* DSM 17126^T (97.6%) and *C. rhizosphaerae* RSB3-1^T (97.5%). The average nucleotide identity of strain L7^T to 31 reference strains were 78.6–85.6%, lower than the species delineation threshold of 95%. MK-6 was the only respiratory quinone of L7^T and major fatty acids were iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$, iso$C_{17:1}\omega9c$ and/or $C_{16:0}$ 10-methyl. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol, three unidentified aminophospholipids, two unidentified aminolipids, three unidentified glycolipids and two unidentified lipids. The G+C content of the genome was 38.58 mol%. On the basis of polyphasic taxonomy analyses in this study, strain L7^T is considered to represent a novel species in the genus *Chryseobacterium*, for which the name *Chryseobacterium endalhagicum* sp. nov. is proposed. The type strain is L7^T (=MCCC 1K05687^T=JCM 34506^T)

INTRODUCTION

The genus *Chryseobacterium* belongs to the family *Weeksellaceae*, and was first described by Vandamme in 1994 [1]. There are 120 species of the genus that have been reported until now (https://lpsn.dsmz.de/genus/chryseobacterium), and most species could form yellow colonies on solid medium. Species of *Chryseobacterium* were isolated from varied environments, such as terrestrial and aquatic environments [2–5], even including organisms and food [6–8].

In this study, strain L7^T was isolated from seeds of *Alhagi sparsifolia* Shap., a leguminous plant grown in northwest China. Comparing physiological, biochemical and genetic characteristics with reference strains *C. vietnamense* GIMN1.005^T, *C. bernardetii* NCTC13530^T, *C. vrystaatense* LMG 22846^T, *C. nakagawai* NCTC 13529^T, *C. shigense* DSM 17126^T, *C. rhizosphaerae* RSB3-1^T and type strain *C. gleum* ATCC35910^T, strain L7^T represents a novel species in the genus *Chryseobacterium*.

METHODS

Isolation and ecology

Strain L7^T was isolated from seeds of *Alhagi sparsifolia* Shap. collected from Turpan basin of Xinjiang (N 45°16′, E 85°2′). We took about 1.0 g of the seeds and surface sterilized them according to the method of Yuan [9]: firstly, we rinsed the seeds with distilled water three times, and then soaked them with 0.01% (v/w) Tween-20 for 1 min; followed by soaking with 5% (v/w) sodium hypochlorite solution, then rinsed with sterile water three times and dried with sterile paper. We then ground the sterile seeds into an homogenate, took 0.1 ml the slurry and spread it on Tryptic Soy Agar (TSA, Qingdao Rishui Bio-techologies Co., Ltd), incubated at 30 °C for 72 h, then selected single colonies and purified the colonies to obtain a pure culture. We isolated a bacterium which formed yellow colonies on the plate, named it L7, and preserved it in Tryptic Soy Broth (TSB, Qingdao Rishui Bio-techologies Co., Ltd) with 15% (v/v) glycerol at −80 °C. *C. vietnamense* GIMN1.005^T

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Keywords: microbial taxonomy; novel species; Chryseobacterium.
Abbreviations: ANI, average nucleotide identity; DDH, DNA-DNA hybridization; PGAP, prokaryotic genome annotation pipeline.
Two supplementary tables and two supplementary figures are available with the online version of this article.

Table 1. 16S rRNA similarity and genomic differences between strain L7^T and reference type strains

Strain	16S rRNA similarity (%)	ANI (%)	DDH (%)
C. vietnamense GIMN1.005^T	98.1	79.5	23.6
C. bernardetii NCTC 13530^T	98.0	79.3	23.3
C. vrystaatense LMG 22846^T	97.9	83.4	27.3
C. nakagawai NCTC 13529^T	97.7	79.0	23.1
C. shigense DSM 17126^T	97.6	83.7	27.6
C. rhizosphaerae RSB3-1^T	97.5	79.5	23.5
C. angstadtii KM^T	97.5	83.8	27.6
C. carnipullorum DSM 25581^T	97.5	83.8	27.4
C. aurantiacum F30^T	97.5	79.0	23.1
C. oleae DSM 25575^T	97.4	84.8	29.3
C. candidae JC507^T	97.4	79.8	23.6
C. culicis DSM 23031^T	97.4	79.2	23.2
C. jejuense DSM 19299^T	97.4	79.1	23.1
C. pennipullorum 7_F195^T	97.3	78.8	22.9
C. kwangjuense KJ1R5^T	97.2	85.6	30.6
C. luteum DSM 18605^T	97.2	84.1	28.5
C. indologenes NBRC 14944^T	97.2	79.0	22.9
C. lactis NCTC 11390^T	97.0	79.2	23.1
C. arthrosphaerae CC-VM-7^T	96.9	79.3	23.5
C. ureilyticum DSM 18017^T	96.9	79.3	23.1
C. viscerum 687B-08^T	96.8	79.4	23.3
C. oranimense DSM 19055^T	96.8	84.1	28.1
C. gleum ATCC 35910^T	96.8	79.5	23.4
C. cucumeris GSE06^T	96.7	79.5	23.3
C. aureum 17S1E7^T	96.7	79.3	23.5
C. artocarpi UTM-3^T	96.7	79.2	22.9
C. oncorhynchi 701B-08^T	96.6	79.2	23.1
C. sediminis IMT-174^T	96.6	79.6	23.4
C. gallinarum DSM 27622^T	96.5	79.2	23.0
C. contaminans DSM 27621^T	96.2	79.2	23.0
C. daecheongense DSM 15235^T	96.2	78.6	22.1

(GDMCC1.2012), *C. rhizosphaerae* RSB3-1^T (KCTC22548), and *C. gleum* ATCC35910^T (GDMCC1.870) were obtained from the Korean Collection for Type Cultures (KCTC) and Gongdong Microbial Culture Collection Centre (GDMCC) as reference strains for phenotypic comparison. To characterize the features of strain L7^T, the isolate and reference strains were routinely cultured on TSA at 28 °C for 24 h unless otherwise noted.

16S rRNA gene phylogeny

The genomic DNA of strain L7^T was extracted using TIANamp Bacteria DNA Kit (TIANGEN Biotech Co., Ltd.) according to the protocol. Using the genomic DNA of L7^T as a template the 16S rDNA was amplified with Taq PCR Master Mix (Sangon Biotech Co., Ltd.) and universal bacterial primers 27F/1492R [10]. The PCR product was purified using TIANgel Mini Purification Kit (TIANGEN Biotech Co., Ltd.), and sequencing was done by Sangon Sequencing (Sangon Biotech Co., Ltd.). The 16S rRNA gene sequence analysis of strain L7^T and closely related strains from the EZ BioCloud (https://www.ezbiocloud.net/) [11] were aligned using CLUSTAL W programme [12]. Phylogenetic trees were reconstructed according to the neighbour-joining and maximum-likelihood methods [13, 14] using MEGA X software [15], bootstrap analyses were performed using 1000 replications.

Genome features

The whole-genome of strain L7^T was sequenced and assembled by Majorbio Whole Genome Analysis Service (Majorbio Co., Ltd.) using the Illumina Hiseq ×10 platform and SOAPdenovo2 [16], respectively. The assembled genome was annotated using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [17]. To confirm the taxonomic status of strain L7^T, average nucleotide identity (ANI) and digital DDH (dDDH) values were analysed using the ANI online tool of Chunlab (http://www.ezbiocloud.net/ezgenome/ani) [18] and Genome-to-Genome Distance Calculator version 2.1 (http://ggdc.dsmz.de/ggdc.php#) [19], respectively. The genome sequences of reference strains were downloaded from GenBank database.

Morphological and physiological analysis

The morphology of strain L7 ^{T} was observed by transmission electron microscopy (JEM-1230; JEOL), and the production of flexirubin-type pigments was tested using 20% (w/v) KOH [20]. Gram-staining was performed using a Gram-staining kit (Solarbio). The range of growth temperatures was tested in TSB over the range of 5–50 °C (4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 45 and 50). The pH range for growth was tested from pH 5.0 to pH 10.0 (in 0.5 pH unit intervals) at 28 °C. The pH was adjusted using MES (pH 4.0–6.0), PIPES (pH 7.0–8.0), HEPES (pH 8.0–9.0), and Gly-NaOH (pH 9.0–10.0) [21]. The catalase and oxidase activities were tested using 3% (w/v) H_2O_2 and 1% (w/v) dimethylaniline [22], respectively. The biochemical properties of strain L7^T were determined using the API 20NE kit (bioMérieux), GNIII microplate (Biolog) and API ZYM kit (bioMérieux) according to protocols.

Chemotaxonomic analysis

Cellular fatty acids were analysed using biomass of strain L7^T and reference strains which were obtained by culturing in nutrient broth at 28 °C. The fatty acid methyl esters were prepared, separated and identified according to the instructions of the Microbial Identification System (MIDI) [23].

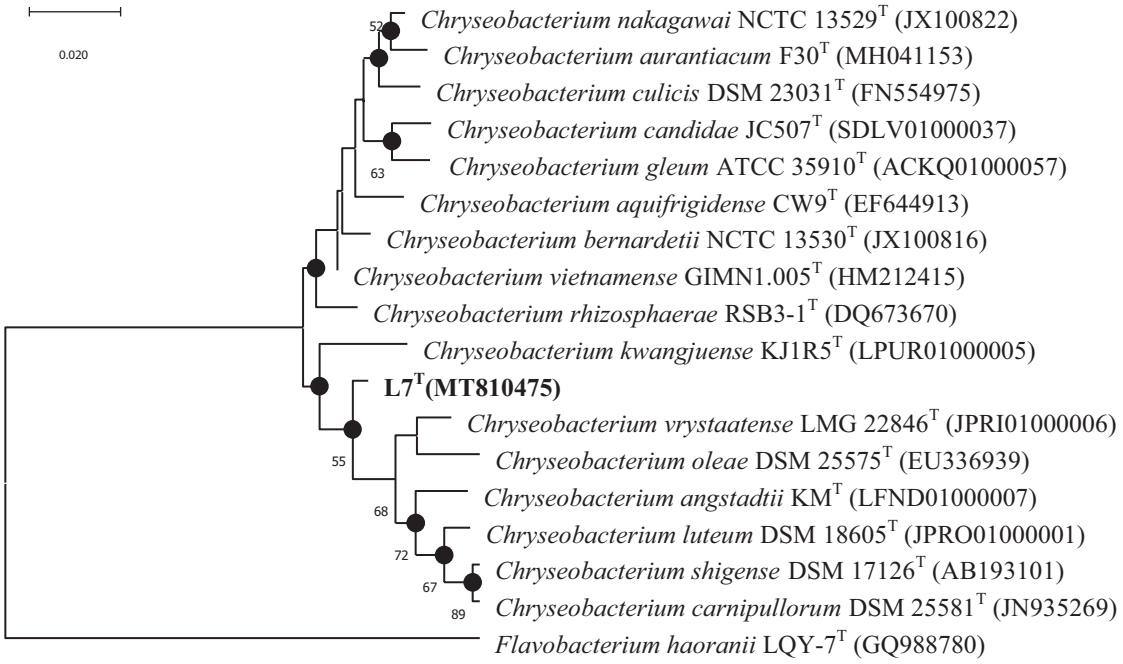

Fig. 1. Maximum-likelihood tree based on 16S rRNA gene sequences available in the GenBank database (accession numbers in parentheses), showing the positions of strain L7^T and related type strains belonging to the genus *Chryseobacterium*. Bootstrap values (expressed as the percentage of 1000 replications) are shown at the branch points; only values greater than 50% are shown. Filled circles indicate nodes that were also recovered in the tree with neighbour-joining algorithm. *Flavobacterium haoranii* LQY-7^T (GQ988780) was used as outgroup. Bar, 0.02 nucleotide substitutions per nucleotide position.

The respiratory quinone content of strain L7^T was analysed by following the method of Lee [5]. Polar lipids were extracted and analysed by two-dimensional TLC (silica gel plates, layer thickness 0.2 mm; Merck) according to the method modified by Indu [24]. Relevant data of strains *C. bernardetii* NCTC13530^T, *C. vrystaatense* LMG 22846^T, *C. nakagawai* NCTC 13529^T, and *C. shigense* DSM 17126 ^{T} were quoted from database and literature.

RESULTS AND DISCUSSION

Phylogenetic characteristics

Analysis of the 16S rRNA gene sequence of strain L7^T (accession no. MT810475) revealed that L7^T belonged to the genus *Chryseobacterium*, the most closely related strain was *Chryseobacterium vietnamense* GIMN1.005^T, with 98.1% 16S rRNA gene sequence similarity, followed by *C. bernardetii* NCCTC13530^T (98.0%), *C. vrystaatense* LMG 22846^T (97.9%), *C. nakagawai* NCTC13529^T (97.7%), *C. shigense* DSM 17126^T (97.6%), and *C. rhizosphaerae* RSB3-1^T (97.5%) (Table 1).

Phylogenetic trees of representative members in the genus *Chryseobacterium* were reconstructed. In Fig. 1, strain L7^T was separated from the most similar sequences, forming a distinct lineage within the genus *Chryseobacterium* and the neighbour-joining phylogenetic tree shows a similar structure (Fig. S1) considering the 16S rRNA gene sequence similarities below the established thresholds [25], it was concluded strain L7^T as a potential novel species of the genus *Chryseobacterium*.

Genomic features

The assembled draft genome sequence of strain L7^T was 4931506 bp, and was composed of ten contigs. The NCBI PGAP revealed three copies of the 5S, 16S, 23S rRNA gene, respectively, and 83 RNA genes (nine rRNA, 71 tRNA and three ncRNA). Among a total of 4371 predicted genes, 4288 genes were identified as protein-coding sequences. The genomic information of strain L7^T and 31 reference species are shown in Table S1. The ANI values between strain L7^T and the 31 type strains were 78.6–85.6%. DNA–DNA hybridization between strain L7^T and the 31 type strains were <31% (Table 1). The low ANI and DDH values indicated that strain L7^T represents a novel species in the genus *Chryseobacterium*.

Morphological and physiological analysis

Strain L7^T was shown to be rod-shaped (2–3 µm×0.5–0. 6 µm) and non-flagellated, Gram-stain-negative, non-spore forming, produced flexirubin-type pigments (Fig. S2). The temperature range for growth was 10–37 °C (optimum 28 °C). The pH range for growth was 6.0–8.5 (optimum 7.0). The NaCl range for growth was 0–3% (optimum 1 %). Activities of catalase and oxidase were positive. Carbon substrate utilization was positive for: dextrin, trehalose, gentiobiose, α-D-glucose, D-fructose, D-mannitol, glycerol, D-glucose-6-phosphate,

Table 2. Differential characteristics of the novel strain L7^T with its closest phylogenetic relatives. Strains: 1, L7^T; 2, *C. vietnamense* GIMN1.005^T; 3, *C. bernardetii* NCTC13530^T; 4, *C. vrystaatense* LMG 22846^T; 5, *C. nakagawai* NCTC 13529^T; 6, *C. shigense* DSM 17126^T; 7, *C. rhizosphaerae* RSB3-1^T; 8, *C. gleum* ATCC35910^T. +, Positive; -, negative; w, weakly positive; ND, no data

Characteristics	1	2	3*	4*	5*	6*	7	8
Source	leguminous plant	forest soil	human sputum	raw chicken	human kidney	bovine milk	rhizosphere	human vagina
Growth temperature (°C)	10–37	5–37†	18–37‡	4-32§	ND	5-30†††	10-37¶	18–40**
NaCl tolerance (%)	3	2†	ND	3§	3††	ND	4¶	7**
pH range for growth	6.0–8.5	6.0-9.0†	ND	ND	5.0–10.0††	5.0-8.0†††	5.0-10.0¶	6.0-10.0**
Indole production	–	–	ND	+§	-††	+†††	–	+
UREase	–	+	+‡	+§	ND	-†††	+	+
D-Glucose	W	W	ND	+§	+††	+†††	W	+
L-Arabinose	–	–	ND	ND	-††	-†††	W	+
D-Mannose	–	W	ND	+§	ND	ND	W	+
Maltose	–	W	ND	ND	ND	-†††	W	+
D-Mannitol	+	W	ND	+§	W††	-†††	+	–
D-Galacturonic acid	W	+	ND	ND	ND	ND	–	–
D-Glucuronic acid	+	+	ND	-/+‡‡	ND	-‡‡	W	–
Polar lipids§§	PE, 3 APL, 2AL, PL, 3 GL, 2L	ND	PE, 5AL, 4L¶¶	7L, 4AL‡‡	ND	6L, 4AL, PE‡‡	ND	PE, 7AL, 4 L**
DNA G+C content (mol%)***	38.58	36.07	36.32	37.06	35.40	37.46	36.28	36.81

*Data from literature.
†Data from Li *et al*. [26].
‡Data from Holmes *et al*. [27].
§Data from Beer *et al*. [28].
¶Data from Cho *et al*. [29].
**Data from Holmes *et al*. [30].
††Data from Lee *et al*. [31].
‡‡Data from Carmen Montero-Calasanz *et al*. [32].
§§PE, Phosphatidylethanolamine; AL, unidentified aminolipid; APL, unidentified aminophospholipid; GL, unidentified glycolipid; L, unidentified lipid.
¶¶Data from Kim *et al*. [33].
***The DNA G+C contents of the strains were calculated in this study using the genome sequence.
†††Data from Shimomura *et al*. [34].

D-fructose-6-phosphate, gelatin, glycyl-L-proline, L-arginine, L-aspartic acid, L-glutamic acid, L-serine, D-glucuronic acid, D-saccharic acid, methyl pyruvate, D-lactic acid methyl ester, L-lactic acid, citric acid, α-keto-glutaric acid, Tween 40, acetoacetic acid, acetic acid. Enzyme activity (API ZYM) was positive for: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase (Table S2). The main differential phenotypic characteristics of strain L7^T and the reference strains are given in Table 2.

Chemotaxonomic analysis

Major fatty acids (>10 %) of strain L7^T were iso-$C_{15:0}$ (23.56 %), iso-$C_{17:0}$ 3-OH (22.9 %), $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c* (13.7 %), iso$C_{17:1}$ *ω9c* and/or 10-methyl $C_{16:0}$ (11.9 %). The fatty acid composition of strain L7^T was significantly different from those of *C. vietnamense* GIMN1.005^T, *C. bernardetii* NCTC13530 ^{T}, *C. vrystaatense* LMG22846^T, *C. nakagawai* NCTC13529^T, *C. shigense* DSM17126 ^{T}, *C. rhizosphaerae* RSB3-1^T, and *C. gleum* ATCC35910^T (Table 3). Menaquinone-6 (MK-6) was found as the only respiratory quinone of strain L7^T. The major polar lipids of strain L7^T were phosphatidylethanolamine, phosphatidylglycerol, three unidentified aminophospholipids, two unidentified aminolipids,

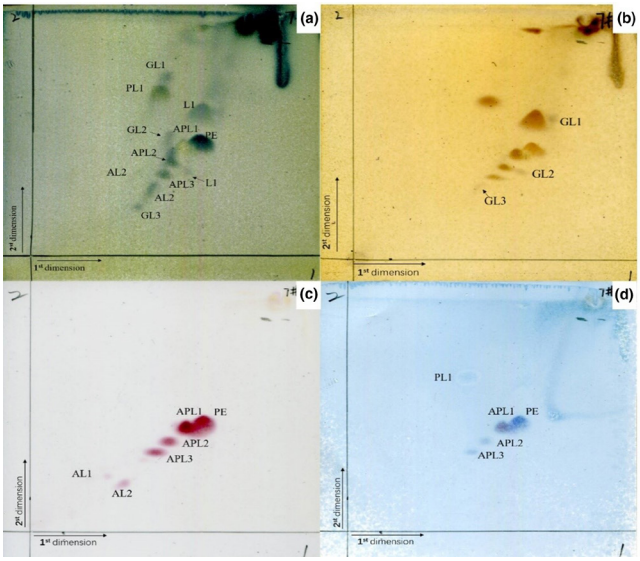

Fig. 2. Two-dimensional TLC of polar lipids photographs from strain L7^T. A, total lipids of strain L7^T; B, glycolipids of strain L7^T; C, aminophospholipids and phosphatidylethanolamine of strain L7^T; D, phospholipids of strain L7^T. AL, unidentified aminolipid; APL, unidentified aminophospholipid; GL, unidentified glycolipid; L, unidentified lipid; PL, phospholipid; PE, phosphatidylethanolamine.

three unidentified glycolipids and two unidentified lipids (Fig. 2, Table 2).

The phenotypic and chemotaxonomic characteristics of the isolate, summarized in Tables 1–3, in additional to the 16S rRNA gene phylogenetic analyses, suggest that strain L7^T represents a novel species of genus *Chryseobacterium*, for which the name *Chryseobacterium endalhagicum* sp. nov. is proposed.

DESCRIPTION OF *CHRYSEOBACTERIUM ENDALHAGICUM* SP. NOV.

Chryseobacterium endalhagicum (end. al. ha' gi. cum. Gr. pref. *endo-* within; N.L. fem. n. *Alhagi*, a botanical genus name; N.L. neut. adj. *endalhagicum*, living inside *Alhagi*).

Cells are Gram-stain-negative, rod-shaped, 0.5–0.6 µm wide, 2.0–3.0 µm long, non-motile. Colonies on TSA are slimy, round, have a smooth margin, and bright yellow in colour, produce flexirubin-type pigments. Growth occurs at 10–37 °C, with optimum growth at 28 °C. pH range for growth is 6.0–8.5, with optimum growth at pH 7.0. Tolerates up to 3% NaCl (w/v), optimum growth occurs at 1%. Catalase and oxidase activities are positive. Carbon substrate utilized were dextrin, trehalose, gentiobiose, α-D-glucose, D-fructose, D-mannitol, glycerol, D-glucose-6-phosphate, D-fructose-6-phosphate, gelatin, glycyl-L-proline, L-arginine, L-aspartic acid, L-glutamic acid, L-serine, D-glucuronic acid, D-saccharic acid, methyl pyruvate, D-lactic acid methyl ester, L-lactic acid, citric acid, α-keto-glutaric acid, Tween 40, acetoacetic acid, acetic acid. Enzyme activity (API ZYM) positive for: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphtol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase. MK-6 is the

Table 3. Cellular fatty acid contents of strain L7^T and type strains of phylogenetically related species

Strains: 1, L7^T; 2, *C. vietnamense* GIMN1.005^T; 3, *C. bernardetii* NCTC13530^T; 4, *C. vrystaatense* LMG 22846^T; 5, *C. nakagawai* NCTC 13529^T; 6, *C. shigense* DSM 17126^T; 7, *C. rhizosphaerae* RSB3-1^T; 8, *C. gleum* ATCC35910^T. Values are percentage of total fatty acids; fatty acids amounting to <1% of the total fatty acids in all strains listed are omitted. -, Not detected; TR, trace amount (<1%)

Fatty acid	1	2	3*	4†	5‡	6§	7	8
iso-$C_{15:0}$	23.6	28.2	29.7	46.8	35.3	41.7	29.7	24.0
iso-$C_{15:0}$3-OH	2.9	2.8	2.9	3.2	3.0	2.4	3.2	3.2
iso-$C_{16:0}$	4.0	3.0	–	TR	–	TR	3.0	3.1
$C_{16:0}$	2.6	4.1	4.0	1.3	2.0	1.0	–	2.3
iso-$C_{16:0}$3-OH	3.7	1.5	1.1	TR	–	ND	2.4	2.4
iso-$C_{17:0}$ 3-OH	22.9	26.3	17.8	12.9	–	16.3	24.4	26.0
Summed feature 3¶	13.7	10.1	14.1	9.4	12.9	9.9	9.6	14.8
Summed feature 8¶	–	–	1.4	–	–	ND	–	–
Summed feature 9¶	11.9	9.8	16.8	14.4	21.3	ND	12.1	10.9

*Data from Kim *et al.* [33].
†Data from https://www.ccug.se/strain?id=50970
‡Data from https://www.ccug.se/strain?id=60563
§Data from Montero-Calasanz *et al.* [32].
¶Summed features are fatty acids that can not be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 3 contains $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 8 contained $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$; summed feature 9 contains iso$C_{17:1}\omega9c$ and/or $C_{16:0}$ 10-methyl.

only quinone. Phosphatidylethanolamine, an unidentified phospholipid, two unidentified aminolipids, three unidentified aminophospholipids, three unidentified glycolipids and two unidentified lipids are the polar lipids. Major fatty acids (>10%) are iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$, iso$C_{17:1}$ $\omega 9$c and/or 10-methyl $C_{16:0}$. The genomic DNA G+C content is 38.58 mol%.

The type strain is L7^T (=MCCC 1K05687^T=JCM 34506^T), which was isolated from seeds of *Alhagi sparsifolia* Shap., a leguminous plant. The GenBank accession number for the 16S rRNA gene sequence and the draft genome sequence accession numbers of *Chryseobacterium endalhagicum* L7^T were MT810475 and JAELVM000000000, respectively.

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Conflicts of interest
The authors declare no conflicts of interest.

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