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Chryseobacterium paridis sp. nov., an endophytic bacterial species isolated from the root of *Paris polyphylla* Smith var. *yunnanensis*

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Abbreviations: GCM, the Global Catalogue of Microorganisms; MK, menaquinone; NJ, neighbor-joining; MP, maximum-parsimony; ML, maximum-likelihood; AAI, average amino acid identity; ANI, average nucleotide identity; dDDH, digital DNA-DNA hybridization; PE, phosphatidylethanolamine; AL, unidentified aminolipid; GL, unidentified glycolipid; L, unidentified lipid.

Abstract

A Gram-negative, yellow-pigmented, rod-shaped bacterial strain YIM B02567^T was isolated from the root of *Paris polyphylla* Smith var. *yunnanensis* in China. Strain YIM B02567^T grew optimally at 25–30°C and at pH 7.0 in the absence of NaCl on nutrient agar. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain YIM B02567^T belong to the genus *Chryseobacterium*, and closely related to *Chryseobacterium piperi* CTM^T and *Chryseobacterium soli* DSM 19298^T. Whole genome sequencing indicated that the genome size was 4,774,612 bp and had a DNA G+C content of 34.5 %. Values of the ANI and the dDDH between strain YIM B02567^T and its closely related *Chryseobacterium* species were below 81.72 % and 24.7 %. Strain YIM B02567^T contained menaquinone-6 as the sole isoprenoid quinone, anteiso-$C_{15:0,}$ iso-$C_{17:1}$ ω9*c* and iso-$C_{17:0}$ 3-OH as major fatty acids and phosphatidylethanolamine as major polar lipid. Based on the polyphasic analyses, strain YIM B02567^T could be differentiated genotypically and phenotypically from recognized species of the genus *Chryseobacterium*. The isolate therefore represents a novel species, for which the name *Chryseobacterium paridis* sp. nov. is proposed. The type strain is YIM B02567^T (=CGMCC 1.18657^T).

Keywords: *Chryseobacterium paridis* sp. nov.; Novel species; polyphasic taxonomy; the root of *Paris polyphylla* Smith var. *yunnanensis*

Introduction

The Genus *Chryseobacterium*, proposed by Vandamme et al. (1994) and was emended from the genus *Flavobacterium*, based on the genotypic, biochemical and phenotypic characteristics of the organisms (Bernardet et al. 1996). At the time of writing, more than one hundred valid published species of the genus *Chryseobacterium* have been reported (https://lpsn.dsmz.de/genus/chryseobacterium); many of these are abundant in diverse environments, including soil (Benmalek et al. 2010), water (Montero-Calasanz et al. 2013), plants (Du et al. 2015), rhizospheres (Park et al. 2006), raw milk (Hantsis-Zacharov et al 2007), chicken (Kämpfer et al. 2014) and fish (Ilardi et al. 2009). Interestingly, several species of the genus *Chryseobacterium*, isolated from plants or rhizospheres. In this genus, it has many rhizospheric microorganisms，for example，*Chryseobacterium cucumeris* isolated from cucumber (*Cucumis sativus* L.) root (Jeong et al. 2017). *Chryseobacterium ginsengisoli* isolated from the rhizosphere of ginseng (Nguyen et al. 2013), *Chryseobacterium ginsenosidimutans* isolated from soil of a *Rhus vernicifera*-cultivated field (Im et al. 2011). In this study, we described a new species of the genus *Chryseobacterium*, designated YIM B02567^T, isolated from the root of *Paris polyphylla* var. *yunnanensis*.

Materials and methods

Bacterial isolation and maintenance

Healthy root samples of *P. polyphylla* var. *yunnanensis* were collected from Shilin in Yunnan province, south-west PR China. Samples were sterilized and pulverized before distribution on nutrient agar (NA) medium as described by Yang et al. (2016). After incubation at 28 °C for 2 weeks, different clonies were randomly selected and their 16S rRNA genes were PCR-amplified. Strain YIM B02567^T was selected as a putative novel species of the genus *Chryseobacterium* for further taxonomic characterizations. The purified strain was preserved both on NA slants at 4 °C and in 20 % (v/v) glycerol at -80 °C for further use.

16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA of strain YIM B02567^T was extracted using a genomic DNA extraction kit (Tiangen, China). The 16S rRNA gene was amplified by PCR using forward primer 27F (5′-AGA GTT TGA

TCC TGG CT-3′) and reverse primer 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′). Amplified products were purified and cloned into vector pClone007 (TsingKe, China). The 16S rRNA gene sequence (1536 bp) of strain YIM B02567^T was checked manually and submitted to the GenBank database. The similarities of 16S rRNA gene sequences between strain YIM B02567^T and closely related type strains were calculated using the EZBioCloud server (https://www.ezbiocloud.net/) (Yoon et al. 2017). The 16S rRNA gene sequences were aligned by using Clustal Omega (Sievers et al. 2011) software and Kimura's two-parameter model (Kimura 1980). Phylogenetic trees were constructed with the Neighbour-joining (NJ) (Saitou and Nei 1987), Maximum-likelihood (ML) (Felsenstein 1981) methods using Mega X software (Kumar et al. 2018). Bootstrap analysis with 1000 replicates was conducted to assess confidence levels for the branches (Felsenstein 1985).

Whole genome sequencing and analysis

The whole-genome sequencing of strain YIM B02567^T was performed using BGISEQ platform by China General Microbiological Culture Collection Center (CGMCC) as part of the Global Catalogue of Microorganisms (GCM) 10K project (Shi et al. 2021). The sequence data were assembled using SOAPdenovo 2.04 (Li et al. 2015). The average nucleotide identity (ANI) values between strains YIM B02567^T and reference strains were calculated using FastANI (Jain et al. 2018). Average amino acid identity (AAI) values were calculated from protein sequences by using an online AAI calculator (http://enve-omics.ce.gatech.edu/aai/). The estimated genome-sequence based digital DNA-DNA hybridization (dDDH) values were calculated using formula 2 at the Genome-to-Genome Calculator (CGGC) website (https://ggdc.dsmz.de/ggdc.php) as described by Meier-Kolthof et al. (2013).
For further confirming the taxonomy status, phylogenomic analysis of strain YIM B02567^T and related species was performed. Genome sequences of the related species' type strains were collected from NCBI GenBank Database. And all of these genomes were annotated by using PROKKA (Seemann 2014). The orthologous gene inferring was using by OrthoFinder (Emms et al. 2015). The selected orthologs were aligned by using the Clustal Omega (Sievers et al. 2011) and concatenating all alignments. Gblocks (Castresana 2000) was used to select the conserved blocks from the concatenation. The reconstruction of a ML tree was using IQ-tree (Nguyen et al. 2015).

Morphology and physiology and biochemical analysis

Cell morphology was observed by scanning electron microscope after growth for 2 days in Reasoner's 2A (R2A) medium at 30 °C. The Gram reaction was performed using 3 % (w/v) KOH for cell lysis. The growth of the strain was assessed by incubating inoculated R2A plates in a bacteria culture box at 30 °C for 7 days. Growth was examined at different temperatures (low to 20 °C, up to 50 °C, at intervals of 5 °C) and NaCl concentrations (up to 5.0 %, at intervals of 0.5 %, w/v) for 7 days. The pH range for growth was tested between 4.0 and 10.0, at intervals of 1.0 pH unit in R2A broths prepared by using the buffer system described by Nie et al. (2012). Catalase activity was determined from the production of gas bubbles on the addition of a drop of 3 % (v/v) H_2O_2. Oxidase activity was detected using API oxidase reagent (bioMérieux) according to the manufacturer's instructions. Carbon source utilization was checked in Biolog GENIII microplates. Additional biochemical characteristics and enzymatic activities were further determined using the API 20NE and API ZYM kits (bioMérieux) according to the instructions provided by the manufacturers.

Chemotaxonomic characterization

The fatty acid profile, polar lipids and respiratory quinones of strain YIM B02567^T were analyzed in this study. To assess the fatty acids, strain YIM B02567^T were cultured on R2A agar plates at 30 ℃ for 2 days. After saponification and methylation, fatty acids were extracted using a standard protocol and the Sherlock Microbial Identification (Sherlock version 6.1; MIDI database: TSBA6) according to the manufacturer's instructions (Sasser 2001) and analysed on Agilent 7890A gas chromatography apparatus. Respiratory quinones and polar lipids were extracted from freeze-dried cells using the method described by Collins et al. (1977). Subsequently, quinones were analysed by a reversed-phase HPLC system (Agilent Technologies 1260 Infinity) with a C18 column (25 cm×4.6 mm, 5 μm). Extracted total lipids from strain YIM B02567^T were examined by a two-dimensional TLC procedure on silica gel G60 plates (Hasegawa et al 1983; Minnikin et al. 1984). For the presence of all lipids, TLC plates were sprayed with 5 % molybdophosphoric acid. Besides, 0.2 % ninhydrin was used to detect aminolipids, molybdenum blue spray reagent was used to detect phospholipids and *a*-naphthol reagent was used to detect glycolipids.

Results and discussion

Phylogenetic and whole-genome analysis

The cloned sequence of the 16S rRNA gene of YIM B02567^T has been deposited in NCBI GenBank under accession number (MW911623). Analysis of its 16S rRNA gene sequence revealed that strain YIM B02567^T belonged to the genus *Chryseobacterium* and had highest gene sequence similarities to *C. soli* DSM 19298^T (97.8 %), *Chryseobacterium ginsenosidimutans* THG 15^T (97.7 %), *Chryseobacterium soldanellicola* DSM 17072^T (97.5 %) and *C. piperi* CTM^T (97.4 %). The NJ phylogenetic tree based on 16S rRNA gene sequences showed that strain YIM B02567^T, *C. soli* and *C. piperi* formed a monophyletic clade (Fig 1), but did not clustered with C. *ginsenosidimutans* and *C. soldanellicola.* This topology relationship was supported by the ML tree. (Fig S3). Furthermore, a ML phylogenomic tree reconstructed using 1113 orthologous genes confirm that strain YIM B02567^T is most closely with *C. piperi* CTM^T (Fig 2).

The draft genome sequence of strain YIM B02567^T was submitted to GenBank and GCM Type strains Genome Database. The GenBank and the GCM accession numbers are JAENHK000000000 and GCM60020044 respectively. The draft genome of strain YIM B02567^T contained 12 scaffolds, with a total length of 4,774,612 bp and the N50 length of 2,588,358 bp. The DNA G+C content of strain YIM B02567^T was determined from the genome to be 34.5 %. The annotated result of YIM B02567^T genome contains 4236 genes, included 4153 protein-coding genes, 3 rRNA genes, 63 tRNA genes and 3 other RNA genes. The ANI values between strain YIM B02567^T and its closely related strains *C. piperi* CTM^T and *C. soli* DSM 19298^T were 81.72 % and 78.94 %, respectively. ANI values between YIM B02567^T and other species of *Chryseobacterium* are shown in Table S1. Strain YIM B02567^T has AAI values ranging from 78.77 %–86.67 % with the all reference genomes (Table S1). The ANI and AAI values were significantly lower than the widely accepted threshold for describing prokaryote species (95-96 %; Kim et al. 2014, Konstantinidis and Tiedje 2005). The dDDH values of strain YIM B02567^T to *C. piperi* CTM^T and *C. soli* DSM 19298^T were 24.7 % and 22.2 %, which were significantly lower than 70 % similarity of the species defined threshold (Chun et al. 2018). Therefore, according to the results of OGRIs (overall genome relatedness indices), strain YIM B02567^T can represent a novel species of the genus *Chryseobacterium.*

Morphology, physiology and biochemical analysis

Cells of strain YIM B02567^T were Gram-reaction-negative, aerobic, rod-shaped (Fig. S1). Colonies on R2A agar were deep orange and smooth after incubation at 30 °C for 2 days. Strains were able to grow

at temperatures ranging between 10 and 45 ℃ (optimum, 30 ℃), pH 5.0–8.0 (optimum, pH 7.0) and in the presence of up to 2.0 % (w/v) NaCl with optimum growth at non-additional NaCl on R2A.YIM B02567^T were catalase and oxidase positive. In the API ZYM tests, strain YIM B02567^T was positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, and weakly positive for cystine arylamidase activities. The substrate utilization of the strain contained dextrin, D-maltose, D-trehalose, D-fructose, L-fucose, D-mannitol, Glycerol, D-glucose-6-phosphate, D-fructose-6-phosphate, Tween 40, D-glucose-6-phosphate, D-fructose-6-phosphate, gelatin, glycyl-L-prolin, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-serine, glucuronamide, α-keto-glutaric acid, acetoacetic acid and propionic acid. Detail characteristics of the novel strain were summarized in the species description and compared to those of closely related strains in Table 1.

Chemotaxonomic characterization

The major cellular fatty acids of strain YIM B02567^T were iso-$C_{15:0}$ (41.8 %), iso-$C_{17:0}$ 3-OH (16.9 %), iso-$C_{17:1}$ ω9*c* (14.0 %), Summed Feature 3 ($C_{16:1}$ ω5*c* and/or $C_{16:1}$ ω6*c*, 13.9 %). Strain YIM B02567^T showed a similar major fatty acid composition to the related type strains of *Chryseobacterium* species. However, some qualitative and quantitative differences in the fatty acid compositions were observed between the novel strain and the other closely related *Chryseobacterium* species (Table 2). The major polar lipids of strain YIM B02567^T was phosphatidylethanolamine (PE).Three unidentified aminolipids (AL), five unidentified glycolipids (GL) and three unidentified lipids (L) were also detected (Fig. S2). The predominant sole respiratory ubiquinone was found to be MK-6, which is the typical ubiquinone of the genus *Chryseobacterium*.

Taxonomic conclusion

Based on morphological, physiological, and chemotaxonomic properties, and phylogenetic analysis, strain YIM B02567^T could be considered a representative of a novel species belonging in the genus *Chryseobacterium*, for which the name *Chryseobacterium paridis* sp. nov. is proposed.

Description of *Chryseobacterium paridis* sp. nov.

Chryseobacterium paridis (pa'ri.dis. L. gen. n. *paridis of Paris*, a plant genus, from which the type strain was isolated).

Cells are Gram-reaction-negative, short rods. Colonies are deep orange, slimy and smooth on R2A after 2 days of incubation at 30°C. Growth occurs at 10–45°C (optimum, 30 °C), at pH 5.0–8.0 (optimum, pH 7.0) and in the presence of 0–2 % NaCl (optimum, 0 %). Catalase and oxidase activities are positive. Positive for hydrolysis of esculin and gelatin, but negative for reduction of nitrate to nitrite, indole production, fermentation of glucose, arginine dihydrolase, hydrolysis of urea and 4-nitrophenyl-*β*D-galactopyranoside. In Biolog GENIII microplates, positive for utilization of dextrin, D-maltose, D-trehalose, L-fucose, D-fructose, D-mannitol, glycerol, Tween 40, D-glucose-6-phosphate, D-fructose-6-phosphate, gelatin, glycyl-L-prolin, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-serine, glucuronamide, *α*-keto-glutaric acid, acetoacetic acid and propionic acid; negative for the remaining utilization tests. In the API ZYM system, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase; weakly positive for cystine arylamidase; but negative for lipase (C14), trypsin, *α*-chymotrypsin, naphthol-AS-BI-phosphohydrolase, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-glucosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase and *α*-fucosidase. Only MK-6 is detected as the isoprenoid quinone. Major cellular fatty acids are anteiso-$C_{15:0,}$ iso-$C_{17:1}$ *ω*9*c* and iso-$C_{17:0}$ 3-OH. Phosphatidylethanolamine is detected as major polar lipid.

The type strain is YIM B02567^T, isolated from a root of *P. polyphylla* var. *yunnanensis* collected from Shilin, Yunnan Province, southwest PR China. The DNA G+C content of the type strain is 34.5 % (genome). The GenBank accession number for the 16S rRNA gene of strain YIM B02567^T is MW911623. The whole genome sequences have been deposited at GenBank and GCM under accession JAENHK000000000 and GCM60020044, respectively.

Declarations

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Conflict of interest The authors declare that there are no conflicts of interest.

Availability of data and material The GenBank accession number for the 16S rRNA gene sequence of strain YIM B02567^T is MW911623. The draft genome sequence has been deposited in GenBank and GCM under accession numbers JAENHK000000000 and GCM60020044, respectively.

Code availability Not applicable

Authors' contributions Zhen Zhang, Cong-Jian Li and Xing-Wang Jiang performed the experiments; Zhen Zhang and Ling-Ling Yang analyzed the data and wrote the manuscript; Xiao-Yang Zhi guided the experiments and revised the manuscript.

Compliance with ethical standards

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication The manuscript is submitted with the consent of all authors.

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Table 1. Diferential characteristics of YIM B02567^T and related species of the genus *Chryseobacterium.*

Characteristics	**1**	**2**	**3**	**4**	**5**
Optimum temperature (°C)	28−30	20−30	28-30	25−30	25−30
Range of temperature (°C)	10−40	5−37	5-30	5−42	10−37
Optimum pH	7.0	6.5−8.0	6.0−7.0	5.0	7.0
Range of pH	6.0−9.0	6.0−10.0	5.0−9.0	5.0−7.0	5.5−10.0
NaCl tolerance (%)	2	2	3	4	1
Indole production	–	+	–	–	–
Arginine dihydrolase	–	–	–	–	+
Hydrolysis of urea	–	+	+	–	+
4-Nitrophenyl *β*D-galactopyranoside	–	+	–	–	–
Enzyme activity of:					
Esterase (C4)	+	w	+	+	–
Cystine arylamidase	w	w	+	–	–
Trypsin	–	–	+	–	–
Naphthol-AS-BI-phosphohydrolase	–	+	+	+	+
α-Glucosidase	–	+	+	+	+
β-Glucosidase	–	–	–	–	+
N-acetyl-*β*-Glucosaminidase	–	+	+	–	+
Utilization of:					
D-Glucose	–	w	+	w	+
L-Arabinose	–	–	–	w	+
D-Mannose	–	w	+	w	+
D-Mannitol	+	w	–	–	–
DNA G+C content (%)*	34.5	35.2	36.4	35.4	35.7

Taxa: 1, YIM B02567^T; 2, *C. piperi* CTM^T (Strahan et al. 2011); 3, *C. soli* DSM 19298^T (Weon et al. 2008); 4, *C. soldanellicola* PSD1-4^T (Park et al. 2006); 5, *C. ginsenosidimutans* THG 15^T (Im et al. 2011). *, The DNA G+C contents were calculated based on their genome sequences in this study, except *C. ginsenosidimutans* THG 15^T. All strains were Gram-negative rods, catalase and oxidase positive. In API 20 NE and API ZYM kits, all strains were positive for the following characteristics: hydrolysis of esculin and gelatin; alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase. All strains were negative for the following characteristics: reduction of nitrate to nitrite, fermentation of glucose, lipase (C14), *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-mannosidase and *α*-fucosidase. Symbols: +, positive; −, negative; w, weakly positive.

Table 2. Cellular fatty acid composition of strains YIM B02567^T and the type strains of related *Chryseobacterium* species.

Fatty Acid	**1**	**2**	**3**	**4**	**5**
Saturated					
$C_{16:0}$	2.0	1.1	1.6	1.4	−
$C_{16:0}$ 3-OH	1.7	1.8	1.3	1.6	−
Branched-chain:					
iso-$C_{13:0}$	tr	1.1	1.0	1.8	−
iso-$C_{15:0}$	41.8	36.6	36.5	40.4	50.3
anteiso-$C_{15:0}$	tr	tr	2.6	2.7	3.8
iso-$C_{17:1}$ ω9*c*	14.0	22.0	16.9	12.2	9.3
iso-$C_{17:0}$	tr	tr	1.3	tr	−
Hydroxy:					
iso-$C_{15:0}$ 3-OH	3.5	4.2	2.8	3.6	5.2
iso-$C_{16:0}$ 3-OH	tr	1.8	1.3	1.6	−
iso-$C_{17:0}$ 3-OH	16.9	17.9	20.7	19.8	21.9
$C_{17:0}$ 2-OH	tr	tr	1.1	tr	−
Summed Feature 3	13.9	12.6	11.9	11.8	9.5

Taxa: 1, YIM B02567^T; 2, *C. piperi* CTM^T; 3, *C. soli* JS6-6^T; 4, *C. soldanellicola* PSD1-4^T;.5, *C. ginsenosidimutans* THG 15^T (Im et al. 2011). Data for columns 2−4 were taken from Strahan et al. (2011). Summed features represent groups of two or three fatty acids that cannot be separated using MIDI system. Summed feature 3, $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*. Symbols: tr, trace amount (<1.0 %); −, not detected.

Figure Legends

Fig. 1. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain YIM B02567^T and members of the genus *Chryseobacterium*. Bootstrap values (expressed as percentages of 1000 replications) of above 50% are shown at branch points. Bar, 0.006 substitutions per nucleotide position.

Fig. 2. Maximum-likelihood phylogenomic tree showing the position of strain YIM B02567^T and related taxa. Bootstrap values of 100 % are shown. The scale bar denotes the number of accepted substitutions per site.

Figures

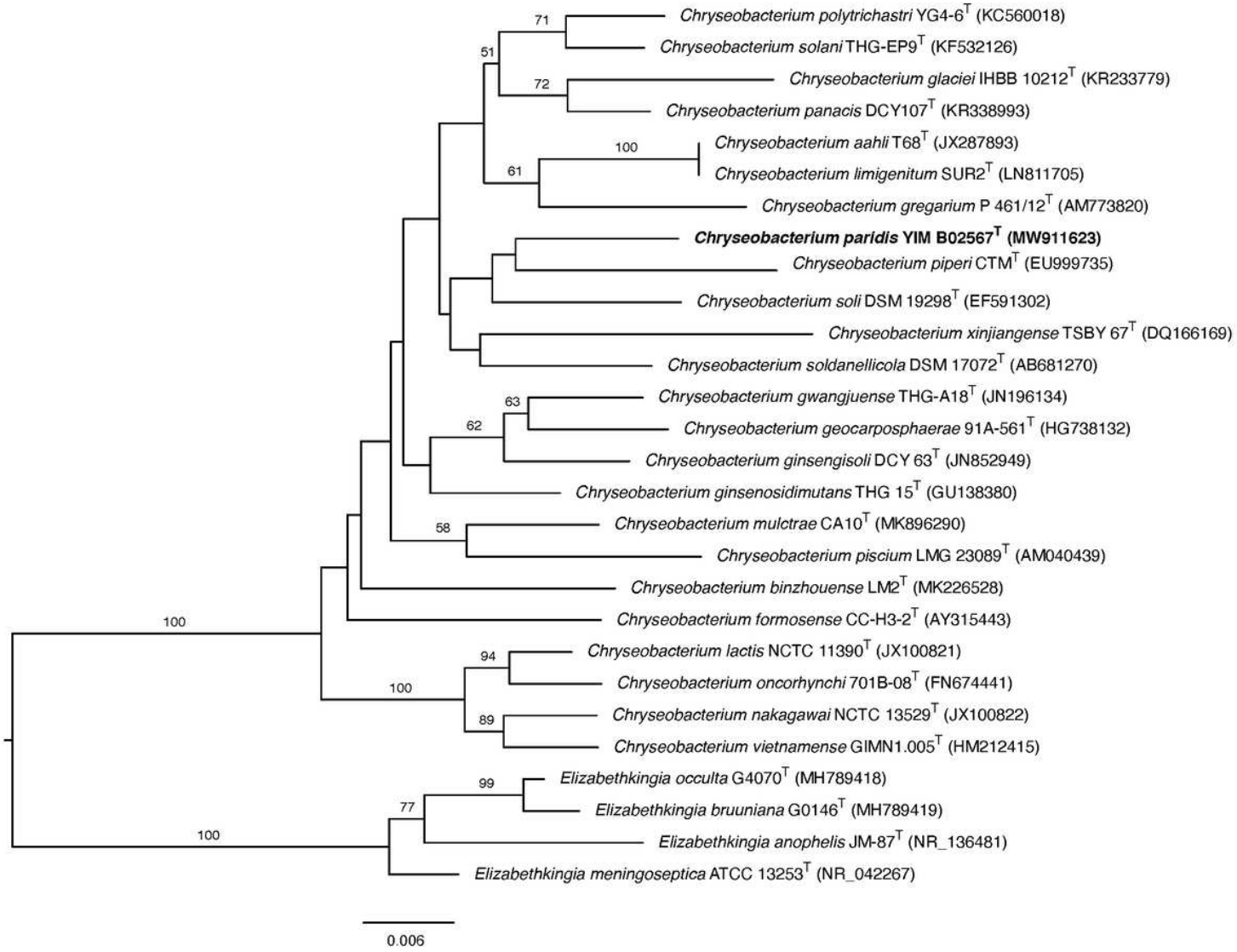

Figure 1

A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain YIM B02567T and members of the genus Chryseobacterium. Bootstrap values (expressed as percentages of 1000 replications) of above 50% are shown at branch points. Bar, 0.006 substitutions per nucleotide position.

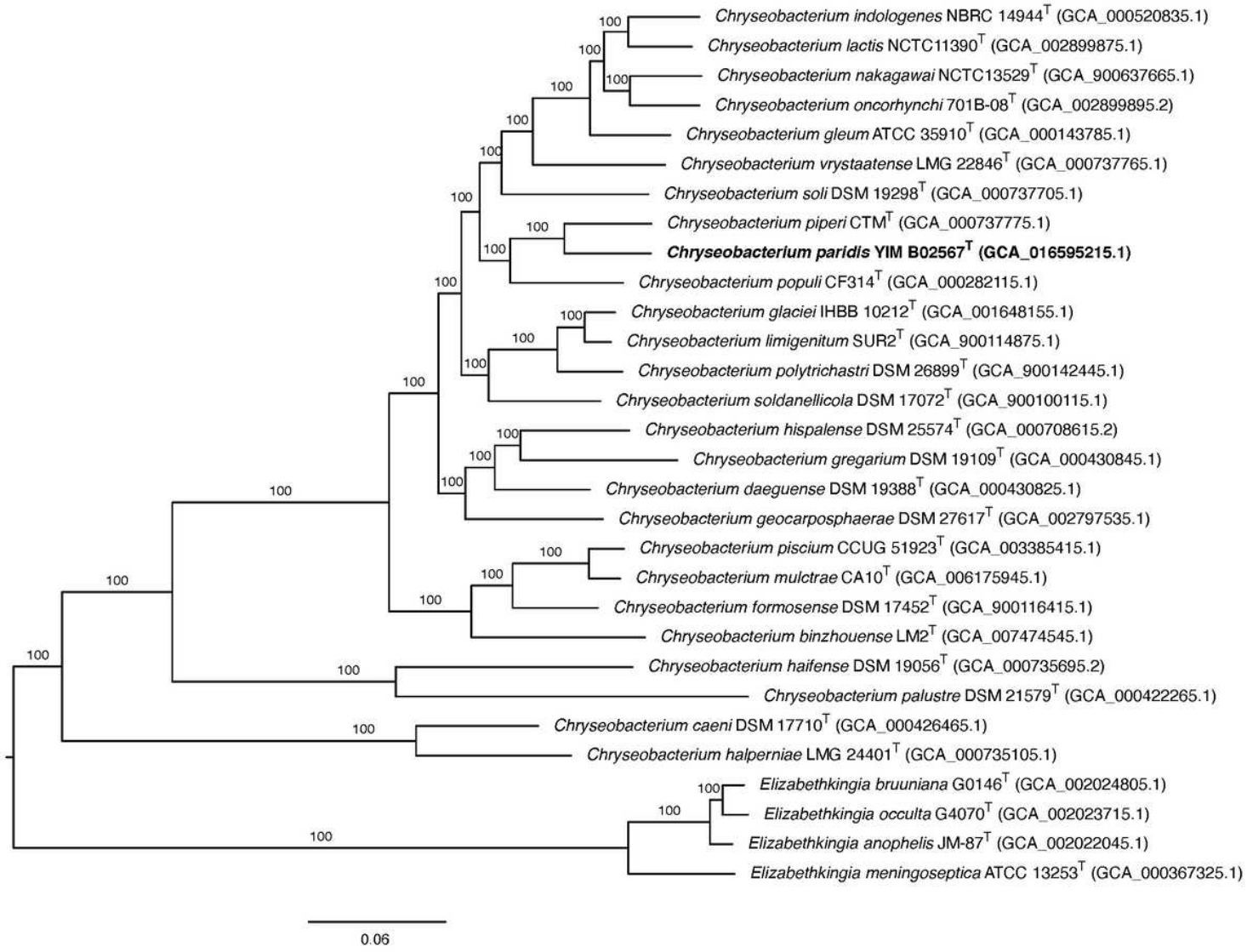

Figure 2

Maximum-likelihood phylogenomic tree showing the position of strain YIM B02567T and related taxa. Bootstrap values of 100 % are shown. The scale bar denotes the number of accepted substitutions per site.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials3clean.pdf