**Ornithinimicrobium flavum** sp. nov., isolated from the leaf of *Paris polyphylla*

Xiao-Mei Fang,‡ Dong Yan,‡ Jing-Lin Bai,† Jing Su,† Hong-Yu Liu,† Bai-Ping Ma,‡ Yu-Qin Zhang† and Li-Yan Yu†,*

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**Abstract**

A Gram-positive bacterium originating from the surface-sterilized leaf of *Paris polyphylla* var. *yunnanensis* (Franch.) was characterized by using a polyphasic approach. The isolate formed yellow, smooth, circular colonies on nutrient agar with 0.2 % starch (NSA). Cells were non-motile, non-sporulating, irregular rods or cocci. Strain CPCC 203535\(^T\) had the highest 16S rRNA gene sequence similarity to the type strain of *Ornithinimicrobium kibberense* (96.9 %) and formed the deepest branch in the genus *Ornithinimicrobium* in the neighbour-joining (NJ) phylogenetic tree based on 16S rRNA gene sequences. The major menaquinones of strain CPCC 203535\(^T\) were MK-8(H2), MK-8(H4) and MK-8. The peptidoglycan contained ornithine as the diagnostic diamino acid. The polar lipid profile consisted of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylinositol (PI) and unknown lipid (UL). The major fatty acids iso-C\(_{14:0}\), iso-C\(_{15:0}\), iso-C\(_{16:0}\) and anteiso-C\(_{15:0}\) were consistent with the fatty acid patterns reported for members of the genus *Ornithinimicrobium*. The DNA G+C composition is 71.4 mol%. The results of physiological and biochemical tests allowed phenotypic differentiation of strain CPCC 203535\(^T\) from its closest phylogenetic species in the genus *Ornithinimicrobium*. Strain CPCC 203535\(^T\) represents a novel species of the genus *Ornithinimicrobium*, for which the name *Ornithinimicrobium flavum* sp. nov. is proposed, with CPCC 203535\(^T\) (=NBRC 109452\(^T\)=KCTC 29164\(^T\)) as the type strain.

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The genus *Ornithinimicrobium*, a member of the family *Intrasporangiaceae*, currently accommodates 6 species ([http://www.bacterio.net/](http://www.bacterio.net/)) and each species is represented by a single strain. The type strain of *Ornithinimicrobium humiphilum* was isolated from garden soil [1], followed by *Ornithinimicrobium kibberense* isolated from a cold desert of the Indian Himalayas [2], *Ornithinimicrobium pekingense* isolated from activated sludge of a wastewater treatment bioreactor [3], *Ornithinimicrobium murale* isolated from an indoor wall [4], *Ornithinimicrobium tianjinense* isolated from a water sample of a recirculating aquaculture system [5] and *Ornithinimicrobium algicola* isolated from green alga [6]. Cells of this genus are Gram-stain-positive, not acid-fast and nonsporeforming. Oxidase-negative, catalase-positive. The major menaquinone is MK-8(H2), MK-8(H4) and MK-8 may occur in minor amounts. The cellular fatty acid profile is distinctly dominated by the occurrence of iso-branched-chain acids [7]. In the present study, we report polyphasic characterisation of strain CPCC 203535\(^T\) and propose a novel species. It is the first endophytic strain of *Ornithinimicrobium* isolated from a terrestrial plant.

Strain CPCC 203535\(^T\) was isolated from the surface-sterilized leaf of *Paris polyphylla* var. *yunnanensis* (Franch.), the roots of which were used widely as an antihelminthic, antimicrobial, and anti-tumor agent in China [8]. This medicinal plant was collected from Xishuangbanna in Yunnan province (China). The isolation of bacteria from the plant was conducted as described by Fang *et al.* [9]. Strain CPCC 203535\(^T\) was isolated on isolation medium plate containing starch 2 g, K\(_2\)HPO\(_4\)0.5 g, MgSO\(_4\)0.5 g, KNO\(_3\)1 g, NaCl 0.4 g, FeSO\(_4\)·7H\(_2\)O 0.01 g and agar 15 g per litre (pH 7.2). The purified isolate was cultured on nutrient agar with 0.2 % starch (NSA) at 28 °C for 7 days, and the stock cultures were maintained at −80 °C by adding 20 % (v/v) glycerol.

All the experiments were performed in parallel on strain CPCC 203535\(^T\) and the type strains *Ornithinimicrobium kibberense* DSM 17687\(^T\) (96.9 % 16S rRNA gene sequence similarity).

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**Keywords:** *Ornithinimicrobium flavum*; new taxa-actinobacteria; *Paris polyphylla*; CPCC 203535.

**Abbreviations:** DPG, diphosphatidylglycerol; ISP2, International Streptomycetes Project media 2; NA, nutrient agar; NJ, neighbour-joining; NSA, nutrient agar with 0.2 % starch; PG, phosphatidylglycerol; PI, phosphatidylinositol; UL, unknown lipid.

‡These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of strain CPCC 203535\(^T\) (=NBRC 109452\(^T\)=KCTC 29164\(^T\)) is KC550155.

One supplementary figure and one supplementary table are available with the online Supplementary Material.
The colonial properties of the test strains were determined on NSA, nutrient agar (NA) and International Streptomyces Project media 2 (ISP 2) (Difco) plates incubated at 28 °C. Smears from these plates were prepared, Gram-stained according to Hucker’s modification, as described by Isik et al. [10]. Acid-fastness tests were after a modification of the Ziehl–Neelsen method [10]. Cell morphology was observed microscopically (JEM-1400, JEOL; transmission electron microscopy mode) after incubation on NSA medium at 28 °C for 5 days. Motility of cells was examined on NSA swarming agar (0.4 %, w/v). Growth temperatures were determined by incubating the organism at 0, 4, 10, 28 to 37 °C (at intervals of 1.0 °C), 40 and 45 °C using Nutrient Broth with 0.2 % starch (NSB) adjusted to pH 7.0. The pH range for growth was tested at pH 4.0–11.0 (at intervals of 0.5 pH units) at 28 °C, using the buffer system described by Xu et al. [11]. Tolerance to NaCl was examined on NSA with different NaCl concentrations (0–10 %, w/v, at intervals of 0.5 %) at 28 °C. Carbon source utilization tests and qualitative enzyme tests were determined with Biolog GEN III (MicroPlate) and API ZYM test kits (bioMérieux) according to the manufacturer’s instructions. The abilities of the strain to hydrolyze L-tyrosine, starch, to produce H2S, indole, and the methyl-red and Voges–Proskauer reactions were tested as described by Williams et al. [12]. Catalase and oxidase activities were determined following the procedure described by Zhang et al. [13]. Other physiological tests (e.g. gelatin hydrolysis, milk coagulation and peptonization) were determined according to previously described procedures by Yuan et al. [14].

Strain CPCC 203535T grew well, and formed smooth, circular, opaque, yellow, convex colonies with entire margins on NSA plates, while it grew poorly on NA plates and the colonies were thin, small and cream to yellow in colour after incubation for 7 days at 28 °C. Cells did not grow on ISP2. Cells were irregular short rods (0.8–1.7×0.6–1.0 µm) or cocci (0.6–1.0 µm) that occurred singly, in pairs, short chains or clusters (Fig. 1). Gram-stain-positive, not acid-fast, nonsporeforming and nonmotile. Growth was observed at 10–37 °C, pH 6.5–10.0 and NaCl tolerance range was 0–2 % (w/v). The optimum growth was at 28–30 °C, pH 6.5–7.0 and 0–1 % (w/v) NaCl. Strain CPCC 203535T and the members in the genus Ornithinimicrobium had in common the following physiological characteristics: they were oxidase-negative, catalase-positive; positive for hydrolysis of starch, but negative for Voges–Proskauer reaction. The different physiological and biochemical characteristics were able to differentiate strain CPCC 203535T from its closest type strain Ornithinimicrobium tianjinense and type strain O. kibberense (Table 1).

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed with methods described by Li et al. [15]. Purified PCR products were sequenced with an ABI PRISM automatic sequencer (model 3730XL). The obtained sequences were compared with available 16S rRNA gene sequences from GenBank using the BLAST program and a web-based tool in http://www.ezbiocloud.net as described by Kim et al. [16] to determine the approximate phylogenetic affiliation. The 16S rRNA gene sequences were aligned with available nucleotide sequences of closely related members of the genus Ornithinimicrobium and Serinicoccus, which were retrieved from the DDBJ/GenBank/EMBL databases, by using the CLUSTAL X 1.8 program [17]. The phylogenetic tree was inferred using the Neighbour-Joining method [18]. The evolutionary distances were computed using the Maximum Composite Likelihood method [19] and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7 [20]. The G+C content of the genomic DNA was determined using the thermal denaturation (Tm) method [21], with Escherichia coli K-12 as a control.

An almost-complete 16S rRNA gene sequence of strain CPCC 203535T (1515nt) was determined. BLAST search results indicated that strain CPCC 203535T showed low levels of similarity with all type strains with validly published names. Strain CPCC 203535T had the highest 16S rRNA gene sequence similarity to the type strain of Ornithinimicrobium kibberense DSM 17687T (96.9 %) and formed the deepest branch in the genus Ornithinimicrobium in the neighbour-joining (NJ) phylogenetic tree (Fig. 2) based on 16S rRNA gene sequences. The bootstrap support for the clustering of all members of the genus Ornithinimicrobium in the NJ tree was high (93 %). Based on the low 16S rRNA gene sequence similarity values to all Ornithinimicrobium type strains, strain CPCC 203535 belonged to a unique genomic species.

Biomass for chemical and molecular studies was obtained by cultivation in liquid medium at 28 °C for 5 days on a rotary shaker (about 180 r.p.m.). Whole-cell sugars and diamino acids patterns were determined by TLC as described by Staneck and Roberts [22]. Polysaccharides were extracted and identified by two-dimensional TLC by using the method of Embley et al. [23]. Menaquinones were extracted, purified by the method of Collins et al. [24] and analyzed by reversed-phase HPLC [25]. Fatty acids were purified, identified and

![Image](https://www.microbiologyresearch.org/images/fig1.jpg)
Table 1. Differential characteristics of the novel isolate and its phylogenetic neighbours in the genus *Ornithinimicrobium*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum growth medium</td>
<td>NSA</td>
<td>NSA, NA, ISP2</td>
<td>NSA, NA, ISP2</td>
</tr>
<tr>
<td>Growth tests:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature range for growth</td>
<td>10–37 °C (optimum 28–30 °C)</td>
<td>10–40 °C (optimum 28–37 °C)</td>
<td>10–40 °C (optimum 28–30 °C)</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>6.5–10.0 (optimum 6.5–7.0)</td>
<td>5.0–10.0 (optimum 7.0–8.0)</td>
<td>5.5–9.5 (optimum 6.5–7.0)</td>
</tr>
<tr>
<td>NaCl range (w/v)</td>
<td>0–2 % (optimum 0–1 %)</td>
<td>0–7 % (optimum 0–1 %)</td>
<td>0–4 % (optimum 0–1 %)</td>
</tr>
<tr>
<td>Hydrolysis of Tween 40</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of L-tyrosine</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Utilisation as sole carbon:

- Maltose: –
- Trehalose: –
- Cellobirose: –
- Gentibiose: –
- Sucrose: –
- Turanose: –
- Stachyose: –
- Raffinose: –
- Lactose: –
- d-Salicylic acid: –
- N-Acetyl neuraminic acid: –
- α-D-Glucose: –
- d-Fructose: –
- Inosine: –
- L + Sodium lactate: –
- Glycerol: –
- d-Glucose-6-PO4: –
- L-Histidine: –
- L-Pyroglutamic acid: –
- L-Serine: –
- L-Lactic acid: –
- d-Malic acid: –
- β-Hydroxy-d,L-butyric acid: –

Enzyme activity:

- Alkaline phosphatase: +
- Lipase(C14): +
- Valine arylamidase: +
- α-Chymotrypsin: +
- β-Glucuronidase: +
- β-Glucosidase: +

| Major menaquinone | MK-8(H4), MK-8(H2), MK-8 | MK-8(H4) | MK-8(H4) |
| DNA G+C content (mol%) | 71.4 | 71.0 | 68.3 |
| Isolate habitat | Medicinal plant | Cold desert | Water sample |

Strains: 1, Strain CPCC 203535T; 2, *O. kibberense* DSM 17687T; 3, *O. tianjinense* CGMCC 1.12160T. Results were obtained from this study performed under the same conditions. NSA, nutrient agar with 0.2 % starch; NA, nutrient agar; ISP 2, International Streptomyces Project media 2. +, Positive; w, weakly positive; −, negative.

quantified by GC using the Sherlock Microbial Identification System (MIDI). MIDI Sherlock version 6.0 and the ACTIN1 database were employed for this analysis [26].

Strain CPCC 203535T possessed chemotaxonomic characteristics that were consistent with memberships of the genus *Ornithinimicrobium*: the peptidoglycan contained ornithine as the diagnostic diamino acid, glucose and arabinose as whole-cell sugars, DPG, PG and PI as the polar lipids (Fig. S1, available with the online Supplementary Material), MK-8(H4) (73.5 %), MK-8(H2) (16.8 %), MK-8(9.7 %) as the major menaquinones and iso-C_{14:0} 3, iso-C_{15:0} 3, iso-C_{16:0} and anteiso-C_{15:0} (Detail in Table S1) as the major fatty acids. These characters supported the strain’s assignment to
the genus *Ornithinimicrobium*, although it had the highest G+C content at 71.4 mol% (the range for the other type strains in the genus was 68.3–71 mol%).

The phenotypic characteristics of strain CPCC 203535\(^T\) were compared to the type strains of *Ornithinimicrobium kibberense* and *O. tianjinense* (Tables 1 and S1). This analysis showed that strain CPCC 203535\(^T\) was phenotypically distinct from *O. kibberense* and *O. tianjinense*.

Thus, based on the evidence presented, it is proposed that strain CPCC 203535\(^T\) represents a novel species of the genus *Ornithinimicrobium*, with the name *Ornithinimicrobium flavum* sp. nov.

**DESCRIPTION OF ORNITHINIMICROBIUM FLAVUM SP. NOV.**

*Ornithinimicrobium flavum* (fla’ve’üm. L. neut. adj. flavum, yellow, referring to the colony colour).

Colonies are yellow, smooth, circular, opaque, and convex with entire margins on NSA medium. Aerobic cells are irregular short rods (0.8–1.7×0.6–1.0 µm) or cocci (0.6–1.0 µm). Gram-stain-positive, not acid-fast, nonsporeforming and nonmotile. Good growth on NSA medium between 28–30 °C, pH 6.0–7.0 and 0–5 % (w/v) and NaCl (optimum 0–1 %). Catalase-positive and oxidase-negative. Nitrate is reduced to nitrite. Milk is coagulated and peptonized. Hydrolyzes starch but not gelatin, urea and L-tyrosine. Methyl-red and Voges–Proskauer reaction and H\(_2\)S and indole production negative. Positive for α-glucosidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase, esterase lipase, leucine arylamidase, lipase, naphthol-AS-BI-phosphohydrolase and valine arylamidase. Utilizes acetic acid, acetoclastic acid, β-Hydroxyl-D-1,4 butyric acid, dextrin, D-fructose-6-PO\(_4\), D-glucuronic acid, formic acid, glucuronamide, pectin, tween 40 as sole carbon sources, but not lactose, α-hydroxy-butyric acid, α-keto-glutaric acid, β-methyl-D-glucoside, bromo-succinic acid, D-arabitol, D-aspartic acid, cellobiose, D-fucose, D-malic acid, maltose, D-mannose, D-mannitol, melibiose, raffinose, D-saccharic acid, D-salicin, D-serine, D-sorbitol, trehalose, turanose, gentiobiose, glycerol, glycolyl-L-proline, inosine, L-rhamnose, L-arginine, 1-histidine, 1-pyrroglutamic acid, L-serine, methyl pyruvate, myo-inositol, N-acetyl-D-galactosamine, N-acetyl-D-gluconosamine, N-acetyl-β-D-mannosamine, N-acetyl neuraminic acid, quinic acid, stachyose or sucrose. Whole-cell hydrolysates contain ornithine as the diagnostic diamino acid and whole cell sugars are glucose and arabinose. The polar lipid profile consists of DPG, PG, PI, UL and MK-8(H\(_4\)), MK-8(H\(_2\)), MK-8 are major menaquinones. The fatty acid profile consists of mainly iso-C\(_{15:0}\), iso-C\(_{17:0}\) 3-OH and anteiso-C\(_{15:0}\). The DNA G+C content of the type strain is 71.4 mol%.

The type strain CPCC 203535\(^T\) (=NBRC 109452\(^T\)=KCTC 29164\(^T\)) was isolated from the leaf of a medicinal plant, *P. polyphylla* var. *yunanensis* (Franch.), which was collected from Xishuangbanna in Yunnan province of China.

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### Conflicts of interest

The authors declare no conflicts of interest. This article does not contain any studies with human participants or animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

### Ethical statement

The authors certify that this manuscript is original and has not been published and will not be submitted elsewhere for publication. No data have been fabricated or manipulated to support our conclusions. The submission has been received explicitly from all co-authors. And authors whose names appear on the submission have contributed
sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

References

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