Allobranchiibius huperziae gen. nov., sp. nov., a member of Dermacoccaceae isolated from the root of a medicinal plant Huperzia serrata (Thunb.)

Meng-Jie Ai,† Ye Sun,† Hong-Min Sun, Hong-Yu Liu, Li-Yan Yu and Yu-Qin Zhang*

Abstract
A Gram-stain-positive, non-spore-forming actinobacterial strain, designated CPCC 204077\textsuperscript{T}, was isolated from the surface-sterilized root of a medicinal plant Huperzia serrata (Thunb.) collected from Sichuan Province, south-west China. The peptidoglycan type of strain CPCC 204077\textsuperscript{T} was detected as A4\textsubscript{w} with an L-Lys-L-Ser-D-Asp interpeptide bridge. Galactose, glucose, rhamnose and ribose were the sugar compositions in the whole-cell hydrolysates. The major fatty acid was iso-C\textsubscript{16:0}. The genomic DNA G+C content was 71.0 mol\%. The phylogenetic tree based on 16S rRNA gene sequences showed that strain CPCC 204077\textsuperscript{T} stood for a distinct lineage within the family Dermacoccaceae alongside the genera Branchiibius, Demetria and Barrientosisimonas, with the highest 16S rRNA gene sequence similarities to Branchiibius edensensis Mer 29717\textsuperscript{T} (95.0 \%), Calidofontibacter indicus PC IW02\textsuperscript{T} (94.9 \%) and Demetria terragena HKI 0089\textsuperscript{T} (94.7 \%), and less than 94.7 \% sequence similarities to all other species. Signature nucleotides in the 16S rRNA sequence showed that the strain contained the Dermacoccaceae family-specific 16S rRNA signature nucleotides and a genus-specific diagnostic nucleotide signature pattern. Combining the genotypic and phenotypic analyses, we propose that strain CPCC 204077\textsuperscript{T} represents a novel species of a new genus in the family Dermacoccaceae with the name Allobranchiibius huperziae gen. nov., sp. nov. Strain CPCC 204077\textsuperscript{T} (=NBRC 110719\textsuperscript{T}=DSM 29531\textsuperscript{T}) is the type strain of the type species.

The family Dermacoccaceae was first dissected by Stackebrandt and Schumann to embrace the type genus Dermacoc- cus as well as the genera Kytococcus and Demetria [1–3]. Recently, seven more genera, namely Luteipulveratus, Yimella, Branchiibius, Calidofontibacter, Flexivirga, Barrientosisimonas and Rudaeicoccus have been described [4–10]. Members of the family have been isolated from multiple environments, such as terrestrial habitats (Antarctic soil, soil near wastewater treatment, plant tissue), aquatic habi- tats (fresh water, hot springs, sea water, deep-sea sediment, branchia of Japanese codling), human blood, indoor air sample, and agar plates in the laboratory. All of them share a common taxon-specific 16S rRNA signature nucleotide pattern at positions 140–223 (C–G), 144–178 (C–G), 248–276 (C–G), 258–268 (G–C), 379–384 (C–G), 407–435 (A– U, G–C), 502–543 (A–U), 586–755 (C–G), 602–636 (C–G), 610 (R), 612–628 (Y–G), 615–625 (G–C), 616–624 (G–Y), 630 (Y), 668–738 (A–U), 839–847 (U–A), 863 (U) and 1133–1141 (A–U) [1]. In this paper, we propose that strain CPCC 204077\textsuperscript{T} represents a novel species of a new genus in the family Dermacoccaceae.

The endophytic actinobacterium, designated CPCC 204077\textsuperscript{T}, was isolated from the root of a medicinal plant Huperzia serrata (Thunb.) collected from low brush (29°41′ 24.98″ N, 103°10′ 16.23″ E, 1187 m H) located in Hongya County of Sichuan Province, south-west China. The plants were divided into an aerial part and an underground part which were then sterilized separately. We transferred the sterilized plant tissues into aseptic dishes on a superclean bench to dry the disinfectant attached to the surface, then cut off the ends and ground up the middle part of the plant sample in a sterilized container. The disinfectants used in this test included sodium hypochlorite (75 \%, v/v) and alcohol (50 \%, v/v). The filamentous plant fragments were used for isolation of endophytic strains. Tap water-yeast extract (TWYE) agar containing (l L-Lys-L-Ser-D-Asp interpeptide bridge. Galactose, glucose, rhamnose and ribose were the sugar compositions in the whole-cell hydrolysates. The major fatty acid was iso-C\textsubscript{16:0}. The genomic DNA G+C content was 71.0 mol\%. The phylogenetic tree based on 16S rRNA gene sequences showed that strain CPCC 204077\textsuperscript{T} stood for a distinct lineage within the family Dermacoccaceae alongside the genera Branchiibius, Demetria and Barrientosisimonas, with the highest 16S rRNA gene sequence similarities to Branchiibius edensensis Mer 29717\textsuperscript{T} (95.0 \%), Calidofontibacter indicus PC IW02\textsuperscript{T} (94.9 \%) and Demetria terragena HKI 0089\textsuperscript{T} (94.7 \%), and less than 94.7 \% sequence similarities to all other species. Signature nucleotides in the 16S rRNA sequence showed that the strain contained the Dermacoccaceae family-specific 16S rRNA signature nucleotides and a genus-specific diagnostic nucleotide signature pattern. Combining the genotypic and phenotypic analyses, we propose that strain CPCC 204077\textsuperscript{T} represents a novel species of a new genus in the family Dermacoccaceae.

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0.25 g, K₂HPO₄ 0.5 g, agar 15.0 g, tap water 1000 ml, pH 7.5) was employed as the isolation medium. Aztreonam (25 mg l⁻¹), acididine (25 mg l⁻¹) and potassium dichromate (30 mg l⁻¹) were added to the medium to prevent the growth of Gram-stain-negative bacteria and fungi that might be present. After 3 weeks of incubation at 28 °C, the visible colonies were picked and streaked onto PYG medium (l⁻¹) (peptone 3.0 g, yeast extract 5.0 g, glycerol 10.0 ml, betaine hydrochloride 1.25 g, sodium pyruvate 1.25 g, agar 15 g) and incubating at 28 °C to obtain the separated colonies. The purified culture was maintained on PYG agar at 4 °C and as glycerol suspensions (20 %, v/v) at −80 °C.

Growth of the strain CPCC 204077T was tested at 28 °C for 4–6 weeks on PYG medium, YM medium, Marine Agar (Difco), TSA medium, R2A Agar (Difco) and nutrient agar [11]. Growth conditions of strain CPCC 204077T were tested using PYG medium, incubating the cultures for up to 3 weeks. Different temperatures for growth (4, 10, 15, 20, 28, 30, 32, 35, 37, 40 and 45 °C) were tested on PYG agar [pH 7.0, NaCl 0 % (w/v)]. NaCl tolerance was tested with different NaCl concentrations [0, 1, 2, 3, 5, 7 and 10 % (w/v)] on PYG agar (28 °C, pH 7.0). The pH range for growth (4.0–11.0, at intervals of 1.0 pH unit) was observed in PYG broth (28 °C, 14–28 days) using the buffer system described by Xu et al. [12]. The oxidase activity was detected using API oxidase reagent (bioMerieux) according to the manufacturer’s instructions. Catalase activity was detected by observing the production of oxygen bubbles with addition of a drop of hydrogen peroxide solution (3 %, v/v). Metabolic characters were examined using Biolog GEN III (MicroPlate), API 50CH, API 20NE and API ZYM test kits (bioMerieux) according to the manufacturer’s instructions. Results were evaluated after incubation at 28 °C for 48–96 h. Other physiological tests of the strain were examined according to previously described procedures [13]. The Gram reaction was tested by the standard Gram-staining method and observed using light microscopy (Axio Scope. A1 Vario; Zeiss). The morphology of cells was observed by light microscopy and confirmed using scanning electron microscopy (Quanta 200; FEI) with gold-coated dehydrated specimens of 7-day culture from PYG agar.

Cells of strain CPCC 204077T were aerobic, Gram-stain-positive. Scanning electron microscopic observations revealed irregular cocoid to short rod-shaped cells that varied in size (0.4–0.6 × 1.1–1.2 μm) on PYG agar (Fig. 1). Strain CPCC 204077T grew well on PYG medium, while moderate growth occurred on YM medium, TSA medium, nutrient agar, and no growth was observed on Marine Agar (Difco) and R2A Agar (Difco). Colonies on PYG medium after a week at 28 °C were circular, convex and smooth with ivory white to pale yellow colour and approximately 1.0 mm in diameter. Growth of strain CPCC 204077T was observed at 10–32 °C, pH 5.0–8.0, in the presence of 0–1 % (w/v) NaCl. The optimum growth occurred at 28–30 °C, pH 7.0, with the absence of NaCl. The strain hydrolysed gelatin and urea but not CM-cellulose and starch. Peptonization of milk, nitrate reduction and H₂S production tests were negative. Detailed physiological and biochemical characteristics of strain CPCC 204077T are given in the species description. Molecular systematic and chemotaxonomic studies of the strain CPCC 204077T were conducted with cells after cultivation in shake flasks on a rotary shaker (150 r.p.m.) using PYG broth at 28 °C to its logarithmic phase, except that the mass used for cellular fatty acid analysis was harvested from TSB. The diagnostic sugars in the whole-cell hydrolysates of strain CPCC 204077T were examined using TLC as described by Lechevalier and Lechevalier [14, 15]. A peptidoglycan sample was prepared following the procedure described by Schleifer and Kandler [16]. Amino acids and peptides in cell-wall hydrolysates were analysed according to the method described by Schumann [17]. The polar lipids were examined by two-dimensional TLC and identified using previously described procedures [18]. Menaquinones were extracted according to Collins et al. [19], and analysed by HPLC [3]. Cellular fatty acids were extracted, methylated and analysed using the Sherlock Microbial Identification System (MIDI) according to the manufacturer’s instructions [20, 21]. MIDI Sherlock Version 6.0 and the ACTIN1 database were employed in this analysis.

The peptidoglycan amino acid composition of strain CPCC 204077T was found to contain Lys (0.8), Ala (1.9), Ser (0.9), Glu (1.0), Asp (1.0) and Mur (1.3) (molar ratios in parentheses), and the enantiometric analysis revealed the ratio of amino acids as L-Lys (1.4), D-Ala (0.4), L-Ala (1.3), L-Ser (0.8), D-Asp (1.0) and D-Glu (1.0) (approximate molar ratios in parentheses). The peptides of mild hydrolysat detected were L-Ala-D-Glu, L-Lys-L-Ser, L-Lys-D-Ala and D-Ala-L-Lys-L-Ser. Based on these results, it is concluded that the peptidoglycan of strain CPCC 204077T was A4α (L-Lys-L-Ser-D-Asp). Galactose, glucose, rhamnose and ribose
were detected in the whole-cell hydrolysates. The polar lipids consisted of a large amount of diphasphatidylglycerol (DPG) and phosphatidylethanolamine (PE), and a small amount of phosphatidylglycerol (PG), phosphatidylinositol (PI), one phospholipid (PL) and one glycolipid (GL) (Fig. S1, available in the online Supplementary Material). The only menaquinone was MK-8(H4). The predominant cellular fatty acid (>10 %) was iso-C16:0 (58.7 %) (Table S1).

For analysis of the 16S rRNA gene sequence of strain CPCC 204077T, genomic DNA extraction, amplification and sequencing of purified products were carried out as described by Li et al. [22]. The 16S rRNA gene sequence of the isolate was compared with available 16S rRNA gene sequences from GenBank using the BLAST program and the EzTaxon-e server (http://www.ezbiocloud.net) [23] to determine an approximate phylogenetic affiliation. Multiple alignments with sequences of the most closely related taxa and calculations of levels of sequence similarity were carried out using MEGA version 5.0 [24]. Phylogenetic trees were inferred using the neighbour-joining method [25] with K$_{\text{sub}}$ values [26, 27] and complete deletion gaps, and the maximum-parsimony [28] and maximum-likelihood [29] methods. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein [30] with 1000 replicates. The G+C content of the genomic DNA was determined using the thermal denaturation method [31] using a UV-1700 spectrophotometer (SHIMADZU) equipped with a DCW-2008 thermo bath, with Calidifontibacter indicus JCM 16038T as a reference.

The G+C content in the genomic DNA of strain CPCC 204077T was detected as 71.0 mol%. The almost full-length 16S rRNA gene sequence (1513 bp) of strain CPCC 204077T was obtained. BLAST search results showed that the novel isolate exhibited the highest similarities with members of the family Dermacoccaceae, such as Branchiibius hedensis Mer 29717T (95.0 %) and Calidifontibacter indicus PC IW02T (95.0 %), followed by Barrientosiimonas humi 39T (94.9 %) and Demetria terragena HKI 0089T (94.7 %). In the phylogenetic tree based on the 16S rRNA gene sequences of all genera within the family Dermacoccaceae, strain CPCC 204077T formed a distinct lineage alongside the genus Branchiibius (Fig. 2), standing for a genus position, which verified that strain CPCC 204077T should be assigned to a novel taxon in the family Dermacoccaceae. The analysis of 16S rRNA signature nucleotides demonstrated that strain CPCC 204077T shared the family-specific signature nucleotide pattern at positions 140–223 (C–G), 144–178 (C–G), 248–276 (C–G), 258–268 (G–C), 379–384 (C–G), 407–435 (A–U, G–C), 502–543 (A–U), 586–755 (C–G), 602–636 (C–G), 610 (R), 612–628 (Y–G), 615–625 (G–C), 616–624 (G–Y), 630 (Y), 668–738 (A–U), 839–847 (U–A), 863 (U) and 1133–1141 (A–U), which was defined for the family Dermacoccaceae.

![Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the relationship of strain CPCC 204077T to representatives of the family Dermacoccaceae. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony methods. Bootstrap values (those above 50 %) are shown as percentages of 1000 replicates. Bar, 1 nt substitution per 100 nt.](https://www.microbiologyresearch.org/article/download/4210-4215)
Table 1. Differentiating characteristics of strain CPCC 204077^T and phylogenetically related genera of the family Dermacoccaceae

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
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<th>8</th>
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<tr>
<td>Interpeptide bridge (Aia)</td>
<td>1-Lys-ND</td>
<td>Lys-ND</td>
<td>Lys-ND</td>
<td>Lys-ND</td>
<td>Lys-ND</td>
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<tr>
<td>Amino acid composition (ratio) of peptidoglycan</td>
<td>d-Ala(0.4), l-Ala(1.3), l-Ser(0.8), d-Glu(1.0), d-Asp(1.0)</td>
<td>d-Ala(1.3), l-Ser(1.0), Gly(0.4), d-Glu(2.0), d-Asp(0.9)</td>
<td>d-Ala(1.3), l-Ser(0.8), Gly(0.4), d-Glu(2.0), d-Asp(0.9)</td>
<td>d-Ala(1.3), l-Ser(0.8), Gly(0.4), d-Glu(2.0), d-Asp(0.9)</td>
<td>d-Ala(1.3), l-Ser(0.8), Gly(0.4), d-Glu(2.0), d-Asp(0.9)</td>
<td>d-Ala(1.3), l-Ser(0.8), Gly(0.4), d-Glu(2.0), d-Asp(0.9)</td>
<td>d-Ala(1.3), l-Ser(0.8), Gly(0.4), d-Glu(2.0), d-Asp(0.9)</td>
<td>d-Ala(1.3), l-Ser(0.8), Gly(0.4), d-Glu(2.0), d-Asp(0.9)</td>
<td>d-Ala(1.3), l-Ser(0.8), Gly(0.4), d-Glu(2.0), d-Asp(0.9)</td>
<td>d-Ala(1.3), l-Ser(0.8), Gly(0.4), d-Glu(2.0), d-Asp(0.9)</td>
<td>d-Ala(1.3), l-Ser(0.8), Gly(0.4), d-Glu(2.0), d-Asp(0.9)</td>
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<tr>
<td>Whole-cell sugars</td>
<td>Gal, Glc, Rha, Rib</td>
<td>Gal, Glc, Rha, Rib</td>
<td>Gal, Man, Rha, Rib, Glc, MK-8(H_4)</td>
<td>Gal, Man, Rha, Rib, Glc, MK-8(H_4)</td>
<td>Gal, Man, Rha, Rib, Glc, MK-8(H_4)</td>
<td>Gal, Man, Rha, Rib, Glc, MK-8(H_4)</td>
<td>Gal, Man, Rha, Rib, Glc, MK-8(H_4)</td>
<td>Gal, Man, Rha, Rib, Glc, MK-8(H_4)</td>
<td>Gal, Man, Rha, Rib, Glc, MK-8(H_4)</td>
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<tr>
<td>Major menaquinone(s)</td>
<td>MK-8(H_4)</td>
<td>MK-8(H_4)</td>
<td>MK-8(H_4)</td>
<td>MK-8(H_4)</td>
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<td>MK-8(H_4)</td>
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<tr>
<td>Major fatty acids</td>
<td>i-C_{16:0}, ai-C_{17:0}, ai-C_{17:0}, ai-C_{18:1}, i-C_{20:0}</td>
<td>i-C_{16:0}, ai-C_{17:0}, ai-C_{18:1}, i-C_{20:0}</td>
<td>i-C_{16:0}, ai-C_{17:0}, ai-C_{18:1}, i-C_{20:0}</td>
<td>i-C_{16:0}, ai-C_{17:0}, ai-C_{18:1}, i-C_{20:0}</td>
<td>i-C_{16:0}, ai-C_{17:0}, ai-C_{18:1}, i-C_{20:0}</td>
<td>i-C_{16:0}, ai-C_{17:0}, ai-C_{18:1}, i-C_{20:0}</td>
<td>i-C_{16:0}, ai-C_{17:0}, ai-C_{18:1}, i-C_{20:0}</td>
<td>i-C_{16:0}, ai-C_{17:0}, ai-C_{18:1}, i-C_{20:0}</td>
<td>i-C_{16:0}, ai-C_{17:0}, ai-C_{18:1}, i-C_{20:0}</td>
<td>i-C_{16:0}, ai-C_{17:0}, ai-C_{18:1}, i-C_{20:0}</td>
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<tr>
<td>DNA G+C content (mol %)</td>
<td>71.0</td>
<td>68.4</td>
<td>68.4</td>
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</table>
Chemotaxonomically, it is quite clear that strain CPCC 204077 differs from other members of the family Dermacoccaceae even though they all share a number of common characteristics. In the cellular fatty acid profile, strain CPCC 204077 shared the common major fatty acid of iso-C₁₆:0 with its close phylogenetic neighbours. MK-8(H₄) was detected in the menaquinone system, without MK-8(H₂). One glycolipid was found in the polar lipid extracts, while mannose was not detected in its cellular sugar composition (Table 1). These data support the differentiation of strain CPCC 204077 from other genera in the family Dermacoccaceae.

Therefore, on the basis of the phenotypic and phylogenetic distinctiveness presented above, strain CPCC 204077 is proposed to represent a novel species of a new genus in the family Dermacoccaceae, for which the name Allobranchiibius huperziae gen. nov., sp. nov., is proposed.

**DESCRIPTION OF ALLOBRANCHIIBIUS GEN. NOV.**

Allobranchiibius (Al.lo.bran.chi.i’bi.us. Gr. adj. allos another, the other, different; N.L. masc. n. Branchiibius a bacterial genus name; N.L. masc. n. Allobranchiibius the other Branchiibius, referring to the fact that it is phylogenetically related to Branchiibius).

Gram-stain-positive, irregular coccoïd to short rod-shaped cells that vary in size (0.4–0.6 × 1.1–1.2 μm), are non-spore-forming and non-motile. The peptidoglycan type is A4γα with an L-Lys–L-Ser–D-Asp interpeptide bridge. Galactose, glucose, rhamnose and ribose are detected in whole-cell sugars. The polar lipids are composed of diphasphatidyglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, one unidentified phospholipid and one unidentified glycolipid. The menaquinone was MK-8 (H₄). The major cellular fatty acid was iso-C₁₆:0. The DNA G+C content of the type strain is 71.0 mol%. The signature nucleotide pattern is 127:234 (A–A), 185:192 (C–C), 187 (C), 199:218 (U–U), 200:217 (U–U), 203:214 (G–C), 1012:1017 (A–A), 1014 (C), 1015 (A), 1025:1036 (U–U), 1026:1035 (U–G), 1027:1034 (U–G), 1090 (A), 1245:1292 (A–U), 1267 (C) and 1450 (A). Phylogenetically, the genus is a member of the family Dermacoccaceae. The type species is *Allobranchiibius huperziae*.

**DESCRIPTION OF ALLOBRANCHIIBIUS HUPERZIAE SP. NOV.**

*Allobranchiibius huperziae* (hu.per’zi.ae. N.L. gen. n. Huperziae of Huperzia, referring to the isolation of the type strain from a plant of *Huperzia serrata*).

Besides the characteristics given in the genus description, the properties of the species are detailed as follows. The pH, NaCl concentration and temperature range for growth are pH 5.0–8.0, 0–1 % NaCl (w/v) and 10–32 °C, with optimum growth at pH 7.0, 0 % NaCl (w/v) and 28–30 °C, respectively. Colonies on PYG medium after a week at 28 °C are circular, convex and smooth with ivory white to pale yellow colour. Positive for catalase and hydrolysis of gelatin, Tween 40 and urea, while negative for hydrolysis of starch and CM-cellulose, oxidase, peptonization of milk, H₂S production and nitrate reduction. According to API ZYM enzyme assays, positive for acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase lipase (C8), esterase (C4), leucine arylamidase, lipase (C14), N-acetyl-β-glucosaminidase, naphthol-AS-B1-phosphohydrolase, trypsin, valine arylamidase, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-galactosidase, but not α-fucosidase and α-mannosidase. Produces acid from arbutin, D-arabitol, D-fructose, D-glucose, D-ribose, D-sucrose (sucrose), trehalose, D-xyllose, erythritol, ascorbic ferric citrate, glycerol, potassium 2-ketogluconate, potassium 5-ketogluconate, potassium gluconate and xylitol. Can utilize azetromin, D-galactose, glycerol and d-ribose as sole carbon sources. The G+C content of the type strain is 71.0 mol%.

The type strain is CPCC 204077 (=NBRC 110719T=DSM 29531T), and was isolated from a medicinal plant *Huperzia serrata* (Thunb.) collected from Sichuan Province, China.

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**Conflicts of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Ethical statement**

This research did not contain any studies with animals performed by any of the authors.

**References**


