Actinocorallia lasiicapitis sp. nov., an actinomycete isolated from the head of an ant (Lasius fuliginosus L.)

Chongxi Liu,† Yao Li,† Lan Ye,† Junwei Zhao,†,2 Chenyu Piao,† Zhilei Li,† Jiansong Li,† Wensheng Xiang†,2 and Xiangjing Wang†

1Key Laboratory of Agriculture Biological Functional Gene of Heilongjiang Provincial Education Committee, Northeast Agricultural University, No. 59 Mucai Street, Xiangfang District, Harbin 150030, China
2State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

A novel actinomycete, designated strain 3H-GS17T, was isolated from the head of an ant (Lasius fuliginosus L.) and characterized using a polyphasic approach. 16S rRNA gene sequence similarity studies showed that strain 3H-GS17T belongs to the genus Actinocorallia with high sequence similarity to Actinocorallia glomerata JCM 9376T (98.13 %) and Actinocorallia longicatena JCM 9377T (97.64 %). The chemotaxonomic properties of strain 3H-GS17T were also consistent with those of members of the genus Actinocorallia. The cell wall contained meso-diaminopimelic acid and whole-cell sugars were ribose, mannose, glucose, galactose and madurose. The predominant menaquinones were MK-9(H4), MK-9(H6) and MK-9(H8). The phospholipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylylino- sol Mannoside. The major fatty acids were C16:0 and C18:1ω7c. A combination of DNA–DNA hybridization experiments and phenotypic tests were carried out between strain 3H-GS17T and its closely related strains, which further clarified their relatedness and demonstrated that 3H-GS17T could be distinguished from these strains. Therefore, strain 3H-GS17T is concluded to represent a novel species of the genus Actinocorallia, for which the name Actinocorallia lasiicapitis sp. nov. is proposed. The type strain is 3H-GS17T (=DSM 100595T=CGMCC 4.7282T).

The genus Actinocorallia was first proposed by Iinuma et al. (1994) as a member of the family Thermomonosporaceae and its description was emended by Zhang et al. (2001). Members of the genus form spore chains on the aerial mycelium. The genus is characterized chemotaxonomically by the presence of meso-diaminopimelic acid in the cell wall, diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol as the major phospholipids, MK-9 (H4), MK-9(H6) and MK-9(H8) as the predominant menaquinones, and hexadecanoic, 14-methylpentadecanoic, octadecanoic and 19-methylododecanoic acids as the predominant fatty acids (Trujillo & Goodfellow, 2012). At the time of writing, the genus comprises seven species with validly published names (http://www.bacterio.net/actinocorallia.html). During a study of the diversity of symbiotic actinomycetes in ants, strain 3H-GS17T was isolated from the head of Lasius fuliginosus L. In this study, we performed a polyphasic taxonomic analysis on this strain, and propose that it is a representative of a novel species of the genus Actinocorallia.

Strain 3H-GS17T was isolated from the head of L. fuliginosus L. collected from Northeast Agriculture University (Harbin, Heilongjiang, China). L. fuliginosus individuals were caught under a willow tree adjacent to their formicary. Ten individuals were surface-disinfected in 70 % ethanol for 60 s and then washed three times in sterile distilled water. Surface-disinfected individual ants were divided into head, mesosoma and gaster, and each body part was separately put in 500 µl of sterile water with shaking on a rotary shaker at 180 r.p.m at 28 °C for 30 min. Subsequently, a 200 µl sample of the suspension of the heads was spread on a plate of Gause’s synthetic agar no. 1 (Atlas, 1993) supplemented with the online Supplementary Material.

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 20 3H-GS17T is KR261652.

Three supplementary figures and one supplementary table are available with the online Supplementary Material.
with cycloheximide (50 mg l\(^{-1}\)) and nalidixic acid (20 mg l\(^{-1}\)). After 21 days of aerobic incubation at 28 °C, colonies were transferred and purified on oatmeal agar (ISP medium 3) (Shirling & Gottlieb, 1966) and maintained as glycerol suspensions (20 %, v/v) at −80 °C. The type strains of *Actinocorallia glomerata* and *Actinocorallia longicatena* were purchased from the Japan Collection of Microorganisms (JCM) and cultured under the same conditions for comparative analysis.

Morphological characteristics were observed by light microscopy (Nikon ECLIPSE E200) and electron microscopy (Hitachi S-3400N) using cultures grown on ISP 3 at 28 °C for 3–12 weeks. Spore motility was assessed by light microscopy (Nikon ECLIPSE E200) observation of cells suspended in phosphate buffer (pH 7.0, 1 mM). Cultural characteristics were determined after 2 weeks at 28 °C using ISP 1–7, potato-glucose agar (PDA), modified Bennett’s agar, humic acid–vitamin agar (HV) and Czapek’s agar (Jones, 1949; Waksman, 1961, 1967; Shirling & Gottlieb, 1966; Hayakawa & Nonomura, 1987). ISC-NSB colour charts were used to determine the designations of colony colours (Kelly, 1964). The utilization of sole carbon and nitrogen sources, decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, peptonization and coagulation of milk, liquefaction of gelatin and production of H\(_2\)S were examined as described previously (Gordon et al., 1974; Yokota et al., 1993). Production of esterase and urease were tested as described by Smibert & Krieg (1994). Growth at different temperatures (0, 4, 10, 15, 20, 28, 32, 35, 37 and 40 °C) was determined on ISP 3 agar after incubation for 2 weeks. Tolerance of pH (pH 4–11, at intervals of 1 pH units), using the buffer system described by Xie et al. (2012), and NaCl tolerance (0–7 %, with an interval of 1 %, w/v) for growth were determined after 2 weeks of growth in ISP 2 broth in shake flasks (250 r.p.m.) at 28 °C.

Biomass for chemical studies were grown by strain 3H-GS17\(^{T}\) in modified GY (Jia et al., 2013) medium in shake flasks at 28 °C for 10 days. Cells were harvested by centrifugation, washed with distilled water and freeze-dried. The isomers of diaminopimelic acid (DAP) in the cell wall were derivatized according to McKerrow et al. (2000) and analysed by HPLC using an Agilent TC-C18 column (250×4.6 mm, i.d. 5 µm). Whole-cell sugars were analysed according to the procedures developed by Lechevalier & Lechevalier (1980). The N-acyl group of muramic acid in the peptidoglycan was determined according to the method of Uchida et al. (1999). Phospholipids in cells were extracted and identified using the methods of Minnikin et al. (1984). Menaquinones were extracted from freeze-dried biomass and purified according to Collins (1985), then analysed by HPLC-UV as described previously (Wu et al., 1989). Mycolic acids were checked by the acid methanolysis method as described by Minnikin et al. (1980). To determine cellular fatty acid compositions, strain 3H-GS17\(^{T}\) was cultivated in GY medium for 7 days in shake flasks at 28 °C. Fatty acid methyl esters were extracted from the biomass as described by Gao et al. (2014) and analysed by GC-MS using the method of Xiang et al. (2011).

Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene sequence was carried out using a standard procedure (Kim et al., 2000). The PCR product was purified and cloned into the vector pMD19-T (Takara) and sequenced using an Applied Biosystems DNA sequencer (model 3730XL). The almost full-length 16S rRNA gene of strain 3H-GS17\(^{T}\) (1508 nt) was obtained and aligned with multiple sequences obtained from the GenBank/EMBL/DDBJ databases using Clustal X 1.83 software. Phylogenetic trees were generated with the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms using MEGA software version 6.06 (Tamura et al., 2013). The stability of the topology of the phylogenetic tree was assessed using the bootstrap method with 1000 repetitions (Felsenstein, 1985). A distance matrix was generated using Kimura’s two-parameter model (Kim, 1980). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). 16S rRNA gene sequence similarities between strains were calculated on the basis of pairwise alignment using the EzTaxon-e server (Kim et al., 2012). The G+C contents of the genomic DNA were determined using the thermal denaturation (T\(_{m}\)) method (Mandel & Marmur, 1968) with *Escherichia coli* JM109 DNA used as the control. DNA–DNA relatedness tests between strain 3H-GS17\(^{T}\), *A. glomerata* JCM 9376\(^{T}\) and *A. longicatena* JCM 9377\(^{T}\) were carried out as described by De Ley et al. (1970) with the modifications described by Huss et al. (1983), using a model Cary 100 bio UV/VIS spectrophotometer equipped with a Peltier-thermostatted 6×6 multicell changer and a temperature controller with *in situ* temperature probe (Varian). The concentration and purity of DNA samples were determined by measuring the optical density at 260, 280 and 230 nm. The DNA samples used for hybridization were

![Fig. 1. Scanning electron micrograph of strain 3H-GS17\(^{T}\) grown on ISP 3 agar for 12 weeks at 28 °C; bar, 1 µm.](http://ijs.microbiologyresearch.org)
The DNA renaturation rates were determined in a sonic cell disruptor (ultrasonic time 3 s, interval time 4 s, sodium citrate buffer), then sheared using a JY92-II ultrasonic processor. Strain 3H-GS17 produced well-developed and branched substrate mycelium and the spore surface was smooth (Fig. 1). Non-motile and oval spores (0.4–0.8 µm) were borne singly on the substrate mycelium, but lacked aerial mycelium. Non-motile and oval spores (0.4–0.8 µm) were borne singly on the substrate mycelium, but lacked aerial mycelium. Non-motile and oval spores (0.4–0.8 µm) were borne singly on the substrate mycelium, but lacked aerial mycelium. Non-motile and oval spores (0.4–0.8 µm) were borne singly on the substrate mycelium, but lacked aerial mycelium.

Chemotaxonomic analyses revealed that strain 3H-GS17 exhibited characteristics that are typical of members of the genus Actinocorallia. It contained meso-diaminopimelic acid as the cell-wall diamino acid and whole-cell sugars included ribose, mannose, glucose, galactose and madurose. The N-acyl type of muramic acid was determined to be acetyl. The phospholipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside (phospholipid type II; Lechevalier & Lechevalier, 1970) (Fig. S2). The menaquinones detected were MK-9(H4) (43.7%), MK-9(H6) (29.3%) and MK-9(H8) (27.0%). The major cellular fatty acids were C16:0 (32.5%) and C18:1ω7c (13.8%); minor amounts of C17:1ω7c (8.9%), C17:0 (8.4%), C18:0 (7.4%), C19:0 10-methyl (6.9%), C16:1ω7c (5.1%), C15:0 (4.3%), C17:0 10-methyl (4.2%), C19:1ω9c (2.4%), C17:0 cyclo (1.3%), anteiso-C17:0 (1.2%), C14:0 (1.0%), iso-C16:1ω8c (0.8%), iso-C15:0 (0.7%), iso-C15:0 3-OH (0.7%) and anteiso-C15:0 (0.4%) were also present. Mycolic acids were not detected.

Based on analysis against the EzTaxon-e server, strain 3H-GS17 was affiliated to the genus Actinocorallia, and was related most closely to A. glomerata JCM 9376T (98.13 % 16S rRNA gene sequence similarity) and A. longicatena JCM 9377T (97.64 %). Phylogenetic analysis based on the 16S rRNA gene sequences indicated that the isolate clustered in the maximum-likelihood tree. Bar, 0.005 nucleotide substitutions per site.
with other members of the genus *Actinocorallia* and formed a monophyletic clade in the neighbour-joining tree (Fig. 2). This clade was also recovered by the maximum-likelihood algorithm (Fig. S3). DNA–DNA hybridization was employed to further clarify relatedness between 3H-GS17 and *A. glomerata* JCM 9376 and *A. longicatena* JCM 9377; the levels of DNA–DNA relatedness between them were 51.0±1.0 and 42.1±0.8 %, respectively, which were below the threshold value of 70 % for prokaryotic species delineation as recommended by Wayne *et al.* (1987). The G+C content of the genomic DNA of strain 3H-GS17 was 70.7±0.7 mol %.

Besides the genotypic evidence presented above, strain 3H-GS17 could also be distinguished from its closely related strains and the type strains of other species of the genus *Actinocorallia* by several phenotypic characteristics (Table 1, and Fig. S1). Strain 3H-GS17 formed single spores on the substrate mycelium, which distinguished it from other members of the genus *Actinocorallia*. The isolate was able to grow at 4 °C, in contrast to *A. glomerata* JCM 9376 and *A. longicatena* JCM 9377, which could not. The novel strain could not hydrolyse starch or utilize L-arabinose, D-fructose, D-galactose, L-rhamnose and glycine, while the closely related species could. The isolate could coagulate milk and utilize raffinose and L-alanine, whereas the closely related species could not. In addition, a comparative study between strain 3H-GS17 and other type strains of the genus *Actinocorallia* revealed that it differed from them in several phenotypic characteristics.

In conclusion, it is evident from the genotypic and phenotypic data that strain 3H-GS17 represents a novel species of the genus *Actinocorallia*, for which the name *Actinocorallia lasiicapitis* sp. nov. is proposed.

**Description of Actinocorallia lasiicapitis sp. nov.**

*Actinocorallia lasiicapitis* (la.si.i.ca.pi.tis. N.L. masc. n. *Lasius* an ant genus; L. neut. n. *caput*, *capitis* head; N.L. gen. n. *lasicapitis* of the head of a *Lasius* ant).

Aerobic, Gram-stain-positive, non-motile actinomycete which forms branched non-fragmenting vegetative hyphae.

---

**Table 1. Differential characteristics of strain 3H-GS17 and other members of the genus *Actinocorallia***

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4*</th>
<th>5†</th>
<th>6‡</th>
<th>7*,†</th>
<th>8§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spore-chain arrangement</td>
<td>Single</td>
<td>Pseudosporangium</td>
<td>Straight or flexuous</td>
<td>Straight or flexuous</td>
<td>Smooth</td>
<td>Synnemata</td>
<td>Hooks, spirals</td>
<td>Hooks</td>
</tr>
<tr>
<td>Spore-surface ornamentation</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Warty</td>
<td>Folded, warty</td>
</tr>
<tr>
<td>Hydrolysis of starch</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Coagulation of milk</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>Reduction of nitrate</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Utilization as sole carbon source:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>D-Raffinose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Utilization as sole nitrogen source:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Alanine</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glycine</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Growth at 4 °C</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Growth pH range</td>
<td>5–10</td>
<td>6–8</td>
<td>6–8</td>
<td>ND</td>
<td>5.1–10.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Data from Tamura *et al.* (2007).
†Data from Lee (2006).
‡Data from Iinuma *et al.* (1994).
§Data from Meyer (1979).
No aerial mycelium is produced on any of the tested media. Spores are non-motile with a smooth surface and borne singly on the substrate mycelium. Positive for peptoneization and coagulation of milk, production of urease and hydrolysis of asucin, but negative for hydrolysis of starch, production of esterase and H₂S, decomposition of cellulose, liquefaction of gelatin and reduction of nitrate. D-Glucose, lactose, maltose, raffinose, D-ribose, D-sucrose and D-xylse are utilized as sole carbon sources but l-arabinose, D-Fructose, D-galactose, inositol, D-mannitol, l-rhamnose and D-sorbitol are not. L-Alanine, l-arginine, l-aspartic acid, l-asparagine, creatine, l-glutamic acid, l-glutamine, l-threonine and L-tyrosine are utilized as sole nitrogen sources but glycine and l-serine are not. The diagnostic amino acid of the peptidoglycan is meso-diaminopimelic acid. Whole-cells contain ribose, mannose, glucose, galactose and maladose. The menaquinones are MK-9(H₈), MK-9(H₆) and MK-9(H₄). The phospholipid profile consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylino- sositol and phosphatidylinositol mannoside. The major fatty acids (>10 %) are C₁₀:0 and C₁₈:0ω7c.

The type strain is 3H-GS17T (=DSM 100595T=CGMCC 4.7282T), isolated from the head of L. fuliginosus collected from Northeast Agricultural University (Harbin, Heilongjiang, China). The G+C content of the DNA of the type strain is 70.7 mol %.

Acknowledgements

This work was supported in part by grants from the National Outstanding Youth Foundation (No. 31225024), the National Natural Science Foundation of China (Nos. 31471832, 31171913, 31500010, 31572070 and 31372006), the National Key Technology R&D Program (No. 2012BAD19B06), Chang Jiang Scholar Candidates Program for Provincial Universities in Heilongjiang (CSCP), the Youth Science Foundation of Heilongjiang Province (No. QC2014C013), the 'Young Talents' Project of Northeast Agricultural University (14QC02), the Science and Technology Research Project of Heilongjiang Provincial Educational Commission (No. 12541001), the China Postdoctoral Science Foundation (2014MS61319) and the Heilongjiang Postdoctoral Fund (LBH-Z14027).

References


