Streptomyces amphotericinicus sp. nov., an amphotericin-producing actinomycete isolated from the head of an ant (Camponotus japonicus Mayr)

Tingting Cao,1 Shan Mu,1 Chang Lu,1 Shanshan Zhao,1 Dongmei Li,1 Kai Yan,1 Wensheng Xiang1,2,* and Chongxi Liu1,*

Abstract
A novel actinomycete, designated strain 1H-SSA8T, was isolated from the head of an ant (Camponotus japonicus Mayr) and was found to produce amphotericin. A polyphasic approach was employed to determine the status of strain 1H-SSA8T. Morphological and chemotaxonomic characteristics were consistent with those of members of the genus Streptomyces. The menaquinones detected were MK-9(H6), MK-9(H8) and MK-9(H4). The phospholipid profile consisted of diphasatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine and phosphatidylinositol mannoside. The major fatty acids were identified as iso-C16:0, C16:0, C15:0 and anteiso-C15:0. Analysis of the 16S rRNA gene sequence showed that strain 1H-SSA8T belongs to the genus Streptomyces with high sequence similarity to Streptomyces ramulosus NRRL B-2714T (99.2%). Two tree-making algorithms based on 16S rRNA gene sequences showed that the isolate formed a phylectic line with Streptomyces himastatinicus ATCC 53653T (98.7%). The MLSA utilizing partial sequences of the housekeeping genes (atpD, gyrB, recA, rpoB and trpB) also supported the position. However, evolutionary distances were higher than the 0.007 MLSA evolutionary distance threshold proposed for species-level relatedness. Moreover, the low level of DNA–DNA relatedness and phenotypic differences allowed the novel isolate to be differentiated from its most closely related strain S. ramulosus NRRL B-2714T and strain S. himastatinicus ATCC 53653T. It is concluded that the organism can be classified as representing a novel species of the genus Streptomyces, for which the name Streptomyces amphotericinicus sp. nov. is proposed. The type strain is 1H-SSA8T (=CGMCC 4.7350T=DSM 103128T).

Streptomyces is the largest genus of the phylum Actinobacteria [1, 2]. The ability of Streptomyces to produce antibiotics and other industrially significant secondary metabolites remains unsurpassed [3–7]. However, traditional microbial sources such as soil actinobacteria often lead to the costly rediscovery of known compounds and the discovery of new biologically active compounds is rather inefficient [8]. Thus, screening programs are often oriented to expand the range of isolation sources [9, 10]. Insects are the most abundant and diverse animal class on earth and they are associated with an amazing variety of microorganisms. The ability of insects to live in unique niche habitats is often facilitated by their associated microbe [11]. For the past few years, the relationship between actinomycetes and ant has been intensely studied [12–15]. Actinomycetes, especially the genera Streptomyces and Pseudonocardia isolated from ants likely offer protection against microfungal weeds that infect the attine fungal gardens by their strong antifungal activities [16]. Therefore, we carried out a programme to discover actinomycetes producing natural products from Camponotus japonicus Mayr, a species of ant. Strain 1H-SSA8T with antifungal activity was isolated. In this study, we performed a polyphasic taxonomic analysis on this strain and proposed that this strain represented one new species of the genus Streptomyces. By the bioactivity-guided isolation methods, we found that the strain could produce amphotericin, which is a potential antifungal agent.

Strain 1H-SSA8T was isolated from the head of a Camponotus japonicus Mayr collected from Northeast Agriculture University (Harbin, Heilongjiang, China). Camponotus japonicus Mayr individuals were caught under a pine tree
adjacent to their formicary. Surface-disinfected individual ants were divided into head, mesosoma and gaster, and each body part was separately put in 500 µl sterile water with shaking on a rotary shaker at 180 r.p.m. at 28 °C for 30 min. Subsequently, a 200 µl aliquot of the suspension of the heads was spread on a plate of sodium succinate-asparagine agar (asparagine 0.2 g l⁻¹; sodium succinate 1 g l⁻¹; CaCl₂·2H₂O 0.2 g l⁻¹; FeSO₄·7H₂O 0.001 g l⁻¹; KCl 0.3 g l⁻¹; K₂HPO₄ 0.9 g l⁻¹; KH₂PO₄·3H₂O 0.6 g l⁻¹; agar 20 g l⁻¹, pH 7.2) and incubated for 21 days at 28 °C supplemented with cycloheximide (50 mg l⁻¹) and nalidixic acid (20 mg l⁻¹). Morphologically distinguishable colonies were purified and transferred onto oatmeal agar [International Streptomyces Project (ISP) 3 medium] [17] for repeated different inoculations under equivalent conditions and maintained as a glycerol suspension (20 %, v/v) at −80 °C. The type strain of Streptomyces ramulosus was purchased from the Japan Collection of Microorganisms (JCM) and Streptomyces himastatinicus was received from Chinese Academy of Sciences (CAS) Key Laboratory of Tropical Marine Bioresources and Ecology, South China Sea Institute of Oceanology. Reference strains were cultured under the same conditions for comparative testing.

Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene sequence were carried out according to the procedure developed by Kim et al. in 2000 [18]. The PCR product was purified and cloned into the vector pMD19-T (Takara) and sequenced using an Applied Biosystems 3730XL. The almost full-length 16S rRNA gene of strain 1H-SSA8 (1519 bp) was obtained and aligned with multiple sequences obtained from the GenBank/EMBL/DDJB databases using CLUSTAL X 1.83 software [19]. Phylogenetic trees were generated with the maximum-likelihood [20] and neighbours-joining [21] algorithms using molecular evolutionary genetics analysis (MEGA) software version 6.06 [22]. The stability of the topology of the phylogenetic tree was assessed using the bootstrap method with 1000 repetitions [23]. Phylogenetic distances were calculated with the Kimura two-parameter model for neighbour-joining method and General Time Reversible model with Invariant sites (GTR+G+I) for maximum-likelihood method [24, 25]. In addition, a heuristic search was performed using nearest neighbour interchange branch swapping in maximum-likelihood inference. 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 EzBioCloud server [26]. The gyrB gene was amplified with primers PF-1 and PR-2 [27] using the same PCR programme as for 16S rRNA gene. PCR amplification of atpD, recA, rpoB and trpB genes were performed as described by Guo et al. [28]. The sequence data were exported as single gene alignments or a concatenated five-gene alignment for subsequent analysis as described above. The sequences were trimmed manually on the same position of the Streptomyces MLST website database (http://pubmlst.org/streptomyces) before being used for further analysis. Trimmed sequences of the five housekeeping genes were concatenated head-to-tail in-frame in the order atpD (496 bp)-gyrB (417 bp)-recA (504 bp)-rpoB (549 bp)-trpB (573 bp). Phylogenetic analyses were performed as described above. Strain 1H-SSA8ᵀ, S. ramulosus NRRL B-2714ᵀ and S. himastatinicus ATCC 53653ᵀ were included in the phylogenetic trees. The G+C content of the genomic DNA was determined using the thermal denaturation (Tₘ) method [29] with Escherichia coli JM109 DNA used as the control. DNA–DNA relatedness tests were carried out as described by de Ley et al. [30] with the modifications described by Huss et al. [31], using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-Thermostatted 6×6 multiscell changer and a temperature controller with in situ temperature probe (Varias). The DNA samples used for hybridization were diluted with 0.1×SSC (saline sodium citrate buffer) and the concentration was adjusted spectrophotometrically at 260 nm to around 1, then sheared using a JY92-II ultrasonic cell disruptor (ultrasonic time 3 s, interval time 4 s, 90 times). The DNA renaturation rates were determined in 2×SSC at 70 °C. The experiments were performed with three replications and the DNA–DNA relatedness value was expressed as a mean value.

Morphological characteristics were observed by light microscopy (Nikon ECLIPSE E200) and scanning electron microscopy (Hitachi S-3400N) using cultures grown on ISP 3 medium at 28 °C for 4 weeks. Samples for scanning electron microscopy were prepared as described by Guan et al. [32]. Spore motility was assessed by light microscopic (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 International Streptomyces Project (ISP) media 1–7 [33], nutrient agar (NA) [34], Bennett’s agar (BA) [35] and Czapek’s agar (CA) [36]. The ISCC-NBS colour charts were used to determine the designations of colony and soluble pigment colours [37]. Growth at different temperatures (4, 10, 13, 14, 15, 18, 20, 28, 37, 38, 39 and 40 °C) was determined on ISP 3 agar after incubation for 2 weeks. The pH range for growth was investigated between pH 2.0–12.0 (in increments of 1.0 pH unit) in GY broth [38] using the buffer system described by Xu et al. [39], and the effect of NaCl concentration on growth was determined in GY broth supplemented with 1–20 % NaCl (with an interval of 1 %, w/v) at 28 °C for 14 days on a rotary shaker. The utilization of sole carbon and nitrogen sources (0.5 %, w/v), decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, peptonization and coagulation of milk, liquefaction of gelatin and production of H₂S were examined as described previously [40, 41]. Production of esterase and urease were tested as described by Smibert and Krieg [42]. Each set of experiments was repeated twice at least.

Biomass for chemotaxonomic studies was prepared by growing the organism in ISP 2 broth in shake flasks (250 r.p.m.) at 28 °C for 1 week. Cells were harvested by
centrifugation, washed twice with distilled water, recenter-
fuged and freeze-dried. The isomers of dianimipimelic acid
(DAP) in the cell wall were derivatized according to McKer-
row et al. [43] and analyzed by a HPLC method using an
Agilent TC-C18 Column (250 × 4.6 mm i.d. 5 µm). The
whole-cell sugars were analyzed according to the procedures
developed by Lechevalier and Lechevalier [44]. Phospholi-
pids in cells were examined by two-dimensional TLC and
identified using the method of Minnikin et al. [45]. Mena-
quiones were extracted from freeze-dried biomass and
purified according to Collins [46] and analyzed by a HPLC-
UV method as described previously [47]. To determine cel-

ular fatty acid compositions, strain 1H-SSA8T was cul-
vated in ISP 2 broth for 5 days in shake flasks at 28C. Fatty
acid methyl esters were extracted from the biomass as
described by Gao et al. [48] and analyzed by GC-MS using
the method of Xiang et al. [49].

Antifungal activity bioassay in vitro followed the procedures
described by Bai et al. [50]. The test strains 1H-SSA8T and
fungi were placed on the same potato or carrot dextrose agar
plate. The plates without the test strain were used as the neg-
ative control. The fungal strain Sclerotinia sclerotiorum was
kindly provided by Soybean Research Institute of Northeast
Agricultural University (Harbin, China). Colletotrichum
orbi culare, Corynespora cassiicola, Setosphaeria turci-
caf, Curvularia lunata, Phytophthora capsici, Helminthospo-
raydis, Alternaria solani, Fusarium oxysporum, Phytophthora infestans, Thanatephorus cucumeris and Spha-
celotheca reiliana were kindly provided by the Institute of
Vegetables and Flowers, Chinese Academy of Agricultural
Sciences (Beijing, China). Phytophthora capsici and Phytoph-
thora infestans were incubated on carrot medium (carrot
200 g, dextrose 20 g, agar 20 g, distilled water 1 l, pH 6.4) at
20C, the others were incubated on PDA (potato 200 g,
dextrose 20 g, agar 20 g, distilled water 1 l, pH 6.4) agar at
28C except Sclerotinia sclerotiorum at 20C.

Strain 1H-SSA8T was inoculated into a 250 ml flask contain-
ing 25 ml seed medium (yeast extract 0.4 %, malt extract
powder 0.1 %, glucose 2 %, CaCO3 0.2 %, pH 7.0) and incu-
bated at 28C for 24 h, shaken at 250 r.p.m. Then, 4.0 ml
culture was transferred into 1 litre Erlenmeyer flask contain-
ing 100 ml of the seed medium under the same culture con-
dition as described above. Finally, 750 ml culture was trans-
ferred into 30 l fermentation medium (adding 1 % sol-
uble starch to the seed medium). Fermentation was carried
out at 28C for 6 days on a rotary shaker at 100 r.p.m. The
final 30 l fermentation broth was extracted with ethanol and
the extract was purified by silica gel column chromatogra-
phy, then the isolated secondary metabolites were identified
by NMR and MS analysis [51].

The almost complete 16S RNA gene sequence of strain 1H-
SSA8T (1519 bp) has been determined and deposited as
KX777593 in the GenBank/EMBL/DDBJ databases. EzBio-
cloud analysis of the 16S RNA gene sequence revealed that
strain 1H-SSA8T should be classified into the genus Strepto-
myces with the highest sequence similarity to S. ramulosus
NRRL B-2714T (99.2 %). The phylogenetic tree (Fig. 1)
based on 16S rRNA gene sequences showed that strain 1H-
SSA8T formed a distinct branch with S. himastatinicus ATCC 53653T (98.7 %) in the neighbour-joining tree and
the topology structure was also supported by the maxi-

mum-likelihood algorithm (Fig. S1, available in the online
version of this article). To further clarify the affiliation
of strain 1H-SSA8T to S. himastatinicus ATCC 53653T and S.
rulosus NRRL B-2714T, partial sequences of housekeep-
ing genes including atpD, gyrB, recA, rpoB and trpB were
obtained. GenBank accession numbers of the sequences, the
length of each gene and similarities of related species with

\[ \begin{array}{c}
\text{Streptomyces platensis JCM 4662}^T \ (\text{AB045882}) \\
\text{Streptomyces hygroscopicus subsp. gleobus NBRC 13786}^T \ (\text{AB184479}) \\
\text{Streptomyces lividans subsp. rufus LMG 20087}^T \ (\text{AJ781351}) \\
\text{Streptomyces caniferus NBRC 15389}^T \ (\text{AB184640}) \\
\text{Streptomyces decoucious NRRL B-2666}^T \ (\text{LGU01000106}) \\
\text{Streptomyces catenulae NRRL B-2342}^T \ (\text{JODY10000075}) \\
\text{Streptomyces nigrescens NRRC 12894}^T \ (\text{AB184225}) \\
\text{Streptomyces lividans subsp. lividans NRRC 13452}^T \ (\text{AB184414}) \\
\text{Streptomyces amphotericinus 1H-SSA8T (KX777593) } \\
\end{array} \]

\[ \begin{array}{c}
\text{Streptomyces himastatinicus ATCC 53653}^T \ (\text{EF408736}) \\
\text{Streptomyces yogyakartensis NBRC 100779}^T \ (\text{AB249942}) \\
\text{Streptomyces javensis NBRC 100777}^T \ (\text{AB249940}) \\
\text{Streptomyces violaceusvar. violaceus NBRC 13459}^T \ (\text{AB184420}) \\
\text{Streptomyces rimosus subsp. rimosus ATCC 10970}^T \ (\text{ANSJ00000404}) \\
\text{Streptomyces sclerotialus NRRL ISP-5269}^T \ (\text{JOBC01000056}) \\
\text{Streptomyces albus NRRC 13014}^T \ (\text{NR_112341}) \\
\text{Streptomyces varsoviensis NRRL ISP-5348}^T \ (\text{JOBF01000056}) \\
\text{Kitasatospora setae JCM 9304}^T \ (\text{NR_097063}) \\
\end{array} \]

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing relationship between strain 1H-SSA8T and related taxa. Only bootstrap values above 50 % (percentages of 1000 replications) are indicated. Asterisks indicate branches also recovered in the maximum-likelihood tree; Bar, 0.005 nucleotide substitutions per site.
strain 1H-SSA8T are displayed in Table 1 (all sequences of S. himastatinicus ATCC 53653T were retrieved from complete genomes submitted in the Genomes OnLine Database and GenBank accession number is ACEX01). The phylogenetic trees based on individual housekeeping genes for this set of strains (Fig. S2a–e) or the neighbour-joining tree constructed from the concatenated sequence alignment (2539 bp) of five housekeeping genes (Fig. 2) supported the conclusion obtained from 16S rRNA, but bootstrap support for these groupings was significantly higher. Although the strain S. himastatinicus ATCC 53653T was quite similar to strain 1H-SSA8T by BLAST analyses of atpD, gyrB and rpoB genes (99.4, 99.0 and 99.3 %, respectively), the pairwise distances calculated for them were well above 0.007, considered to be the threshold for species determination [52]. In addition, DNA–DNA hybridization experiments revealed levels of relatedness of 29.7±0.8 and 45.5±2.3 % between strain 1H-SSA8T and S. ramulosus NRRL B-2714T and S. himastatinicus ATCC 53653T, respectively, which was well below 70 % cut-off point recommended for assigning bacterial strains to the same genomic species [53].

Morphological observation of 4 week cultures of strain 1H-SSA8T grown on ISP 3 medium revealed that it has the typical characteristics of the members of the genus Streptomyces [54]. Strain 1H-SSA8T formed well-developed substrate mycelium and aerial hyphae that differentiate into spiral spore chains consisting of 18 to 28 cylindrical spores (0.6–1.3×0.4–0.8 µm) which were smooth and non-motile (Fig. S3). Cultural characteristics of strain 1H-SSA8T are shown in Table 2. Strain 1H-SSA8T exhibited good growth on ISP 3 and ISP 2 media, moderate growth on BA and ISP 5 media, and poor growth on ISP 1, ISP 4, ISP 6, ISP 7, NA and CA media. Diffusible pigments or melanin were not formed on any of the tested media, which could unambiguously distinguish the isolate from S. ramulosus NRRL B-2714T and S. himastatinicus ATCC 53653T on ISP 3, ISP 4 and MBA media (Fig. S4). The strain could utilize L-arabinose, D-galactose, D-glucose, meso-inositol, lactose, D-maltose, D-sorbitol and D-xyllose but not mannitol, D-mannose, D-raffinose, sucrose, D-fructose, D-ribose or D-ribose as sole carbon sources. L-alanine, L-asparagine, L-aspartic acid, creatine, L-glutamic acid, L-glutamine, L-proline, L-threonine and L-tyrosine are utilized as sole nitrogen sources, but not D-arginine, L-serine or glycine. A comparison of phenotypic characteristics between the isolate and its closest relative was performed to differentiate them (Table 3). Strain 1H-SSA8T could be distinguished distinctly from S. ramulosus NRRL B-2714T and S. himastatinicus ATCC 53653T by the range of temperature, pH and tolerance range of NaCl. Moreover, differences including utilization of L-arabinose, D-sorbitol, creatine and L-alanine, hydrolyzing tween 40 and decomposition of cellulose could also make a contribution to differentiate the isolate from its two related species.

Strain 1H-SSA8T was found to exhibit a range of chemotaxonomic properties that are consistent with the description of the genus Streptomyces. It contained L-
diaminopimelic acid as cell wall diamino acid, indicating that the strain is of cell wall chemotype I [55, 56]. The whole-cell sugars included glucose and ribose. The phospholipid profile consisted of diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine and phosphatidylinositol mannoside (phospholipid type II sensu) (Fig. S5). The menaquinones detected were identified as MK-9(H6) (45.7 %), MK-9(H8) (27.3 %) and MK-9 (H4) (27.0 %). The cellular fatty acid profile was composed of iso-C16:0, C16:1ω7c, anteiso-C15:0, C16:0, C17:1ω7c, iso-C14:0, anteiso-C17:0, iso-C17:0, C18:0, C15:0, and C14:0.

Strain 1H-SSA8T showed broad-spectrum antifungal activity against all of the tested fungi. It possessed strong antifungal activity against C. orbiculare and A. solani with the inhibition rates over 50 %, and moderately inhibited the growth of P. capsici, S. sclerotiorum, C. lunata, F. oxysporum, C. cassicola, H. maydis and T. cucumeris with the inhibition rates over 20 % (Table S1). On the basis of spectroscopic analysis, the strain was found to produce amphotericin (Fig. S6).

Differences in various characteristics showed that strain 1H-SSA8T is phenotypically distinct from its phylogenetic relative, S. ramulosus NRRL B-2714T and S. himastaticinus ATCC 53653T. Therefore, it is evident that strain 1H-SSA8T represents a novel species of the genus Streptomyces, for which the name Streptomyces amphotericinus sp. nov. is proposed.

**DESCRIPTION OF STREPTOMYCES AMPHOTERICINUS SP. NOV.**

*Streptomyces amphotericinus* (am.pho.te.rí.ci’ni.cus. N.L. neut. n. *amphotericinum* amphotericin, an antibiotic; L. masc. suffix -icus adjetival suffix used with various meanings; N.L. masc. adj. *amphotericinicus* referring to the ability of the organism to produce amphotericin).

**Table 2. Cultural characteristics of strain 1H-SSA8T after 2 weeks incubation at 28 °C.**

<table>
<thead>
<tr>
<th>Agar medium</th>
<th>Growth</th>
<th>Aerial mycelium</th>
<th>Substrate mycelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISP 1</td>
<td>Poor</td>
<td>None</td>
<td>Transparent</td>
</tr>
<tr>
<td>ISP 2</td>
<td>Good</td>
<td>Yellowish gray</td>
<td>Dark grayish yellow</td>
</tr>
<tr>
<td>ISP 3</td>
<td>Good</td>
<td>Pure white</td>
<td>Dark yellow</td>
</tr>
<tr>
<td>ISP 4</td>
<td>Poor</td>
<td>Pure white</td>
<td>Grayish yellow</td>
</tr>
<tr>
<td>ISP 5</td>
<td>Moderate</td>
<td>Deep yellow</td>
<td>Dark grayish yellow</td>
</tr>
<tr>
<td>ISP 6</td>
<td>Poor</td>
<td>None</td>
<td>Transparent</td>
</tr>
<tr>
<td>ISP 7</td>
<td>Poor</td>
<td>Deep yellowish brown</td>
<td>Grayish brown</td>
</tr>
<tr>
<td>CA</td>
<td>Poor</td>
<td>Strong yellowish brown</td>
<td>Moderate brown</td>
</tr>
<tr>
<td>NA</td>
<td>Poor</td>
<td>None</td>
<td>Transparent</td>
</tr>
<tr>
<td>BA</td>
<td>Moderate</td>
<td>Deep yellow</td>
<td>Dark Yellow</td>
</tr>
</tbody>
</table>

**Table 3. Differential phenotypic properties between strain 1H-SSA8T and the type strains of the most closely related species of the genus Streptomyces**

Strains: 1, 1H-SSA8T; 2, S. ramulosus NRRL B-2714T; 3, S. himastaticinus ATCC 53653T. +, positive; −, negative. The data presented are from this study unless indicated otherwise.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth at/with:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>14–39</td>
<td>18–39</td>
<td>10–37</td>
</tr>
<tr>
<td>pH range</td>
<td>4–9</td>
<td>5–10</td>
<td>5–10</td>
</tr>
<tr>
<td>NaCl% (w/v) tolerance</td>
<td>up to 6 %</td>
<td>up to 12 %</td>
<td>up to 8 %</td>
</tr>
<tr>
<td>Carbon utilization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-sorbitol</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>maltose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>raffinose</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-ribose</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-rhamnose</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-arabinose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>meso-inositol</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Nitrogen utilization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-arginine</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-alanine</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>L-threonine</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Creatine</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Degradation of:</td>
<td></td>
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</tr>
<tr>
<td>Tween 40</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cellulose</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Gram-stain-positive and aerobic. Spore chains appear as spiral and spores (0.5–1.3 × 0.4–0.8 μm) are smooth, cylindrical and non-motile. Growth occurs at 14–39 °C and pH values of 4.0–9.0. Growth is observed in the presence of up to 6 % (w/v) NaCl. Optimal temperature and pH for growth are 28 °C and pH 7.0, respectively. Gives a positive result in tests for hydrolysis of starch, aesculin and tween 80, of gelatin, peptonization and coagulation of milk and decomposition of cellulose, and urease. Negative for hydrolysis of tween 20, tween 40, production of H2S and reduction of nitrate. The menaquinones are MK-9(H6), MK-9(H8) and MK-9 (H4). The phospholipid profile consists of diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine and phosphatidylinositol mannoside. The major fatty acids areiso-C16:0, C16:0, iso-C15:0 and anteiso-C15:0.

The type strain 1H-SSA8T (=CGMCC 4.7350T=DSM 103128T) was isolated from the head of an ant (Camponotus japonicus Mayr), collected from Northeast Agriculture University (Harbin, Heilongjiang, China). The DNA G+C content of the type strain is 68.8 mol%.

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

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