**Streptomyces solisilvae** sp. nov., isolated from tropical forest soil

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Abstract

A novel streptomyctete (strain HNM0141) was isolated from tropical forest soil collected from Bawangling mountain of Hainan island, PR China and its taxonomic position was established in a polyphasic study. The organism had chemical and morphological properties consistent with its classification as a member of the *Streptomyces violaceusniger* clade. On the basis of the results of 16S rRNA gene sequence analysis, HNM0141 showed highest similarity to *Streptomyces malaysiensis* CGMCC 4.1900 (99.4 %), *Streptomyces samsunensis* DSM 42010 (98.9 %), *Streptomyces yatensis* NBRC 101000 (98.3 %), *Streptomyces rhizophaericus* NBRC 100778 (98.0 %) and *Streptomyces sporoclavus* NBRC 100767 (97.9 %). The strain formed a well-delineated subclade with *S. malaysiensis* CGMCC 4.1900 and *S. samsunensis* DSM 42010. The levels of DNA–DNA relatedness between HNM0141 and *S. malaysiensis* CGMCC 4.1900 and *S. samsunensis* DSM 42010 were 62 and 44 %, respectively. On the basis of phenotypic and genotypic characteristics, HNM0141 represents a novel species in the *S. violaceusniger* clade for which the name *Streptomyces solisilvae* sp. nov. is proposed. The type strain is HNM0141 (=CCTCC AA 2016045 =KCTC 39905).
were determined according to ISCC-NBS colour charts [35]. Spore chain morphology and spore-surface ornamentation were observed using scanning electron microscopy (TM-3000; Hitachi). The isolate was also probed using the *S. violaceusniger* clade-specific oligonucleotide primers according to the method of Kumar *et al.* [4]. HNM0141T was also examined for its grown characteristics by using standard ISP media [34] at 28 °C for 14 days. Phenotypic characteristics of HNM00141T and its closest phylogenetic neighbors were examined together according to several standard methods. Growth at different temperatures (4, 15, 20, 25, 28, 37, 40, 45 and 50 °C) and at pH 4.0–12.0 (at intervals of 1.0 pH unit) were determined on ISP2 agar medium. Tolerance to NaCl concentrations ranging from 0 to 10% (at intervals of 1% NaCl) was tested in ISP2 broth after incubation for 14 days at 28 °C. The other phenotypic features, such as utilization of carbon and nitrogen sources and degradation activity, were examined as described by Williams *et al.* [36]. Susceptibility to antibiotics was tested according to the method of Zhang *et al.* [37]. The antibiotics were chloramphenicol (30 µg ml⁻¹), gentamicin (10 µg ml⁻¹), kanamycin (30 µg ml⁻¹), novobiocin (5 µg ml⁻¹), penicillin G (20 international units ml⁻¹), rifampin (5 µg ml⁻¹), streptomycin (50 µg ml⁻¹), sulfamethoxazole (23.75 µg ml⁻¹), tetracycline (30 µg ml⁻¹) and tobramycin (10 µg ml⁻¹).

Biomass used for cell-wall amino acids and sugars, phospholipids and menaquinones analyses was obtained by cultivating HNM0141T in ISP2 broth for 4 days at 28 °C and cells were harvested by centrifugation, washed twice in distilled water, recentrifuged and freeze-dried. Amino acids and sugars in whole-cell hydrolysates were analyzed by the methods of Lechevalier and Lechevalier [38]. Phospholipids were analyzed and menaquinones were extracted and purified by methods described previously [39]. Menaquinones were analyzed by reversed-phase HPLC on a YMC ODS-A (150×4.6 mm) column. Extraction of cellular fatty acids was carried out from wet biomass grown in ISP2 tubes held at 28 °C for 4 days. Extraction and analysis of fatty acids were carried out according to standard procedures of the MIDI system (http://www.midi-inc.com).

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were carried out as described by Huang *et al.* [40]. The sequences of closely related species with validly published names were retrieved from the EzTaxon-e server [41]. The 16S rRNA gene sequence of HNM0141T was multiply aligned with those of closely related species selected using the clustal x 1.8 software [42] and evolutionary distances were calculated using Kimura’s two-parameter model [43]. Phylogenetic trees were inferred using neighbour-joining [44], maximum-parsimony [45] and maximum-likelihood [46] methods with the MEGA 6.0 program [47]. Bootstrap analysis was conducted using 1000 replicates to estimate the confidence levels of the tree topologies [48]. DNA–DNA hybridization of HNM0141T with its closest neighbours, *S. malaysiensis* CGMCC4.1900T and *S. samsunensis* DSM42010T was carried out using the optical renaturation method [49, 50]. The DNA G+C content of HNM0141T was determined by the HPIC method [51].

HNM0141T produced a yellow–green substrate mycelium and a white–grey aerial spore mass which turned black with age on ISP3 agar medium. The organism also formed aerial mycelia which differentiated into spiral spore chains with rugose spore ornamentation (Fig. 1) and gave a PCR amplification product characteristic of members of the *S. violaceusniger* clade. All of these properties indicated that HNM0141T represented an authentic member of the *S. violaceusniger* clade [2, 4]. HNM0141T grew well on most of the media tested, but poorly on ISP1 agar. Melanoid pigments were not formed on ISP6 agar and no diffusible pigment was detected on the remaining media tested (Table S1, available in the online Supplementary Material).

HNM0141T grew at 20–40 °C (optimum 28 °C), with 0–5% (w/v) NaCl tolerance (optimum 0%) and at pH 5.0–10.0 (optimum pH 7.0). Detailed results for the physiological and biochemical properties are summarized in Table 1 and in the species description. It can be seen from Table 1 that there are several phenotypic characteristics that clearly differentiate HNM0141T from *S. malaysiensis* CGMCC4.1900T and *S. samsunensis* DSM42010T, which were isolated from Malaysian soil [8] and the rhizosphere of *Robinia pseudoacacia* [10] respectively.

The cell-wall peptidoglycan of HNM0141T contained LL-diaminopimelic acid as the diagnostic diamino acid [52]. The whole-cell hydrolysates consisted of glucose and galactose. The predominant menaquinones of HNM0141T were MK-9.
The spore mass colour is initially white and later becomes brown. Growth at pH 4.0 and in NaCl (5 %, w/v) is positive. Degradation tests (% w/v) showed positive results for starch, dextrin, sodium propionate, trehalose, sucrose, raffinose and xylose. The G+C content of the DNA was 70.9 mol%. It was evident that chemotaxonomic features of HNM0141 were consistent with its assignment to the genus Streptomyces [53].

Comparison of the almost complete 16S rRNA gene sequence obtained for HNM0141 (1480 nt) with corresponding sequences of type strains of species classified as members of the S. violaceusniger clade revealed that the isolate formed a well-delineated subclade with S. malaysiensis CGMCC4.1900T and S. samsunensis DSM42010T with a high bootstrap value (98 and 93 %, respectively) in the neighbour-joining tree (Fig. 2). The taxonomic status of the subclade was also supported by the other tree-making algorithms (Figs S2 and S3). HNM0141T shared 99.4 and 98.9 % sequence similarities with S. malaysiensis CGMCC4.1900T and S. samsunensis DSM42010T, respectively. HNM0141T was also closely related to Streptomyces yatensis NBRC 101000T (98.3 % similarity), Streptomyces rhizophaerus NBRC 100778T (98.0 % similarity) and Streptomyces sporiclatus NBRC 100767T (97.9 % similarity). All of these similarity values were lower than those found between the type strains of several species classified as members of the S. violaceusniger clade [2, 3, 10]. Therefore, HNM0141T is considered to represent a novel species.

The percentages of DNA–DNA relatedness (mean±SD of triplicate determinations) between HNM0141T and S. malaysiensis CGMCC4.1900T and between HNM0141T and S. samsunensis DSM42010T were 62.0±1.1 and 44.0±3.2 %, respectively, which were less than the threshold of 70 % used to define a strain as a member of a new species [54]. On the basis of the data presented, we suggest that strain HNM0141T represents a novel species of the S. violaceusniger clade and propose the name Streptomyces solisilvae sp. nov.

**DESCRIPTION OF STREPTOMYCES SOLISILVAE SP. NOV.**

Streptomyces solisilvae (so.li.sil’vae. L. n. solum, soil; L. n. silva, forest; N.L. gen. n. solisilvae, of/from forest soil).

An aerobic, Gram-stain-positive actinomycete which forms branching substrate and aerial mycelia that differentiate into spiral spore chains with rugose spore ornamentation. Grows well on most ISP media, but poorly on ISP1 agar. The spore mass colour is initially white–grey, then black at maturity on ISP3 agar where the substrate mycelium is yellow–green and diffusible pigments are not formed. Mela-noid pigments are not formed on ISP6 or ISP7 agar. Growth occurs at 20–40 °C, at pH 5–10 and in the presence of 0–5 % NaCl. Degrades casein, chitin, gelatin, starch, Tween 20 and 80 and xylan, but not adenine, ascin, arbutin, elastin, guanine, hypoxanthine or xanthine. D-Galactose, D-glucose, myo-inositol, sucrose, raffinose and xylose are utilized as sole source of carbon but adonitol, cellobiose, D-arabinose, dextrin, D-mannitol, D-sorbitol, inulin, L-sorbose, maltose, melezitose, melibiose, salicin, sodium propionate, trehalose or xylitol are not. L-Alanine, L-arginine, L-asparagine, L-leucine, L-phenylalanine, L-valine and L-lysine are utilized as sole sources of nitrogen but L-cysteine, L-glutamine, L-histidine, L-hydroxyproline, L-isoleucine, L-methionine, L-proline, L-serine or L-threonine are not. Sensitive to gentamicin, kanamycin, streptomycin and tobramycin but not to chloramphenicol, novobiocin, penicillin G, rifampin, sulfamethoxazole or tetracycline. The cell wall contains LL-DAP and is type I. The phospholipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and four unidentified phospholipids. The major cellular fatty acids are iso-C16:0, anteiso-C15:0, iso-C15:0, C16:0 and iso-C14:0. The predominant menaquinones are MK-9 (H4), MK-9 (H4) and MK-9 (H8).

The type strain, HNM0141 (=CCTCC AA 2016045 =KCTC 39905T), was isolated from tropical forest soil collected from Bawangling mountain of Hainan island, PR China. The DNA G+C content of the type strain is 70.9 mol%.

**Table 1.** Differentiation characteristics of HNM0141T, S. malaysiensis CGMCC4.1900T and S. samsunensis DSM42010T

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Degradation tests (% w/v)</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Aesculin hydrolysis (0.1)</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Arbutin hydrolysis (0.1)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin (0.4)</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Chitin (0.4)</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Growth on sole carbon sources (% w/v)</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Adonitol (1.0)</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Cellobiose (1.0)</td>
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<td>-</td>
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<tr>
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<td>+</td>
<td>-</td>
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<tr>
<td>myo-Inositol (1.0)</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Xylose (1.0)</td>
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<td>Sucrose (1.0)</td>
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<tr>
<td>Raffinose (1.0)</td>
<td>+</td>
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<tr>
<td>Sodium propionate (0.1)</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Growth on sole nitrogen sources (% w/v)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>+</td>
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<tr>
<td>L-Phenylalanine</td>
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<td>-</td>
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<td>L-Valine</td>
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<td>+</td>
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<tr>
<td>L-Histidine</td>
<td>+</td>
<td>-</td>
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<tr>
<td>L-Lysine</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Growth at pH 4.0</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Growth in NaCl (5 %, w/v)</td>
<td>-</td>
<td>+</td>
<td>-</td>
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</tbody>
</table>
Fig. 2. Neighbour-joining phylogenetic tree for HNM0141\(^T\) based on 16S rRNA gene sequences (1428 nt). Asterisks indicate that the conserved branches were also recovered using maximum-parsimony [45] and maximum-likelihood [46] tree-making algorithms. Bootstrap values based on 1000 replications are listed as percentages; only values over 50% are given. Bar, 0.005 substitutions per nucleotide position.

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

References


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