

Streptomyces chenzhouensis sp. nov., an endophytic actinobacterium from the root tissue of *Cathaya argyrophylla*

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Abstract

An endophytic actinobacterium, designated strain HUAS 5^T, was isolated from the root tissue of *Cathaya argyrophylla* collected in Chenzhou city of Hunan Province, PR China. This strain produced grey aerial mycelium that differentiated into spiral spore chains with smooth-surfaced round or oval spores on Gause's synthetic No. 1 medium. Strain HUAS 5^T grew well on Gause's synthetic No. 1, Reasoner's 2 and ISP serial media. This strain grew at 15–40°C (optimum, 28°C), pH 6.0–9.0 (optimum, pH 7) and in presence of 0–5.0% (w/v) NaCl. The predominant cellular fatty acids of strain HUAS 5^T (> 5.0%) were iso-C_{16:0}, iso-C_{14:0}, anteiso-C_{15:0}, iso-C_{15:0}, C_{16:0}, iso H-C_{16:1} and Sum in Feature 3 (C_{16:1} ω_{7C}/C_{16:1} ω_{6C}). Sequence analysis of the 16S rRNA gene indicated that this strain belonged to the genus *Streptomyces* and exhibited highest sequence similarity to *Streptomyces hirsutus* NRRL B-2713^T (97.3%), which is much less than 98.7% cut-off point of species definitions for bacteria and archaea. Phylogenetic analysis of 16S rRNA gene sequence indicated that strain HUAS 5^T formed an independent lineage, which suggested that it belonged to a potential novel species. Based on the morphological, cultural, physio-biochemical properties and chemotaxonomy, strain HUAS 5^T (= MCCC 1K08552^T = JCM 36055^T) is deemed to represent a novel *Streptomyces* species, for which we put forward the name *Streptomyces chenzhouensis* sp. nov.

INTRODUCTION

Endophytes are roughly defined as microorganisms, which exist in the internal tissues of the plant, and form a symbiotic or mutualistic relationship with host plants without causing obvious symptoms. Mehanni and Safwat (2010) have pointed out that endophytic microorganisms have ability to produce the same or similar compounds metabolites as their hosts plant. There may be five novel species in every kind of plant, and some of them can produce bioactive substances, accounting for 51% of the active substances found (Strobel 2010). In recent years, the increasing number of scientists believe (Shomura 1993) that novel microorganisms can be isolated from special environmental samples. Due to the particularity of living environment and the influence of natural selection pressure, microorganisms from special habitats may evolve new genes and enzymes, and then catalyze the production of new structural active compounds. In recent decades, many new actinomycetes have been isolated from plant samples (Pittayakhajonwut et al., 2016; Hu and Li et al., 2021; Li et al., 2020; Wang et al., 2023; Hu and Wang et al., 2021). Therefore, the exploration of plant endophytes, especially actinomycetes, is an important direction for us to develop potential microbial resources.

During investigating the diversity of endophytic actinobacteria from different plants, a novel *Streptomyces*, designated strain HUAS 5^T, was isolated from *Cathaya argyrophylla*. In the article, the classification of strain HUAS 5^T was described using a polyphasic approach.

METHODS

Isolation and maintenance

Strain HUAS 5^T was isolated from the root tissue of *Cathaya argyrophylla* collected in Chenzhou city of Hunan Province, PR China (26°23.59"N, 113°42'55.92"E). The root samples were disposed for surface sterilization as described by Qin et al. (2009) with minor modifications. The processed roots were cut into suitable size pieces and then sprinkled on modified Gause's synthetic medium (Mo et al. 2017) by adding K₂Cr₂O₇ (53 mg/L). After incubation of 14 days at room temperature, the like-*Streptomyces* strain was pick out obtained by repeated streaking on Gause's synthetic medium (Atlas 1993). The purified strains HUAS 5^T were maintained on ISP 3 (Shirling and Gottlieb 1966) agar slants at 4°C and was stored in freeze-drying using 18% non-fat powdered milk as a protective agent.

Morphological, cultural and physio-biochemical properties

The spore morphology of stain HUAS 5^T, including spore-chain morphology and surface ornamentation, were observed by light microscope (NE620, Ningbo Yongxin Optics CO., LTD, China) and scanning electron microscope (JSM-6610LV, JEOL, Japan) of 14-day-old cultures on Gause's synthetic medium at 28°C (Atlas 1993). The cultural characteristics of strain HUAS 5^T, including aerial mycelium, substrate mycelium pigmentation and the diffusible pigments, and then the colour of them was assessed by comparison with the colour standards and colour nomenclature (Ridgway 1912). The effects of different temperatures (4–45°C), pH values (pH 2.0–12.0 at intervals of 1.0 pH units) and NaCl concentrations (0–15%, w/v) cultured and monitored on ISP 3 plates for two weeks. The ability of strain HUAS 5^T to use different carbon and nitrogen sources was determined on ISP 9 (Shirling and Gottlieb) and medium (1.0% (w/v) d-glucose, 0.05% MgSO₄•7H₂O, 0.05% NaCl, 0.001% FeSO₄•7H₂O, 0.1% K₂HPO₄, 1.2% agar, pH 7.4.) (Williams et al. 1989) added with sterile carbon and nitrogen sources. Standard methods were used for the detection of gelatin liquefaction, starch and aesculin hydrolysis, urease activity, nitrate reduction, and Tweens (20, 40, 60, 80) hydrolysis (Ruan and Huang, 2011).

Chemotaxonomy

For chemical analysis, biomass for chemotaxonomic experiments was collected after incubation at 28°C for 7 days in shake flasks of trypticase soy broth medium. Isomers of diaminopimelic acid and sugars of whole-cell hydrolysates were detected and analysis according to the method of Hasegawa et al. (1983) and Lechevalier et al. (1970). The fatty acids were extracted, purified, methylated and quantified by using the Sherlock Microbial Identification system (MIDI system; <http://www.midi-inc.com/>) (MIDI 2005) and Microbial Identification software package (MADI 2005). Menaquinones and the polar lipids were analyzed according to the method of Ruan and Huang (2011).

Genome sequencing and phylogenetic analysis

Chromosomal DNA extraction and PCR amplification of the 16S rRNA gene from strain HUAS 5^T were completed following the methods as described by Mo et al. (2023), and then the sequenced directly using Sanger dideoxy sequencing. The EzTaxon server (Yoon et al. 2017) was used to identify the closely

related type strain with strain HUAS 5^T and calculate their sequence similarities. Phylogenetic trees based on 16S rRNA gene sequences were constructed with the neighbor-joining (NJ) (Saitou and Nei 1987), maximum-likelihood (ML) (Felsenstein 1981) and maximum-parsimony (MP) (Fitch 1971) methods using the mega version 7.0 (Kumar et al. 2016), with 1000 replications.

Whole-genome sequencing was performed on HiSeq 2500 Sequencing System according to the User Guide and assembling on MiSeq platform (Xie et al. 2014). The SEED and the Rapid Annotation of strain' genomes using Subsystems Technology (RAST) was described by Aziz et al. (2008). The secondary metabolism biosynthetic gene clusters of strain HUAS 5^T were performed using antiSMASH version 7.0.0 (Blin et al. 2021). Antibiotic resistance genes of strain HUAS 5^T were analysis using the CARD (Comprehensive Antibiotic Resistance Database, <https://card.mcmaster.ca>) (Alcock et al. 2020). Clustered regularly interspaced short palindromic repeat sequences (CRISPRs) and genomic islands were analysis by CRISPR-case Finder (Makarova et al. 2019) and the IslandViewer 4 webserver (Claire et al. 2017), respectively. The DNA G + C contents of this strain HUAS 5^T was extracted from whole genomic data using Bioscience's online Average Nucleotide Identity (ANI) calculator (Richter et al. 2016).

RESULTS AND DISCUSSION

Morphological and physio-chemical characteristics

Strain HUAS 5^T produced grey aerial mycelium that differentiated into spiral spore chains with smooth-surfaced round or oval spores on Gause's synthetic No. 1 medium (No. 1) at 28°C after incubation 14d, which indicated that it had typical morphological traits of the genus *Streptomyces* (Fig. 1). Strain HUAS 5^T grew well on No. 1, Reasoner' 2A (R2A), ISP 2–7 media without diffusible pigment. The strain grew at 15–40°C (optimum, 28°C), pH 6.0–9.0 (optimum, pH 7) and in presence of 0–5.0% (w/v) NaCl.

Strain HUAS 5^T was positive for nitrate reduction and hydrolysis of Tweens (20, 40 and 80), but negative for hydrolysis of starch and Tween 60. Strain HUAS 5^T could utilize d-fructose, d-glucose, d-ribose, d-xylose, malt sugar, lactose, l-rhamnose, raffinose, and saccharose as sole carbon sources, but could not utilize l-arabinose, d-cellobiose, d-galactose, d-mannose and *myo*-inositol. Aspartate, histidine, l-alanine, l-glycine, l-hydroxyproline, l-ornithine, l-phenylalanine, l-proline, l-serine, tyrosine and valine, were utilized as sole nitrogen sources, but not for arginine, l-cysteine and l-glutamic acid.

Chemotaxonomic characteristics

The diagnostic amino acid of stain HUAS 5^T contained *l*-diaminopimelic acid (*l*-DAP); Glucose, ribose and mannose were presented in whole-cell hydrolysates. The primary cellular fatty acids (> 5.0%) of strain HUAS 5^T were iso-C_{16:0} (30.3%), iso-C_{14:0} (14.4%), anteiso-C_{15:0} (11.7%), iso-C_{15:0} (9.9%), C_{16:0} (6.8%), iso H-C_{16:1} (5.5%), Sum in Feature 3 (C_{16:1} ω_{7C}/C_{16:1} ω_{6C}) (5.1%). Other fatty acids present in smaller amounts (> 1.0%) were anteiso-C_{17:0} (2.1%), C_{14:0} (1.5%), Sum in Feature 5 (C_{18:2} ω_{6,9C}/C_{18:0} ante) (1.5%), Sum in Feature 9 (10-methyl C_{16:0}) (1.5%), anteiso-C_{17:1} ω_{9C} (1.2%). The phospholipid profile consisted of

diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylinositol mannosides (PIM), phosphatidyl methyl ethanolamine (PME), phospholipids (PLS), phospholipids choline (PC), phospholipids of unknown structure containing glucosamine (NPG) and unidentified phospholipids (L1, L2, L3 and L4) (Fig. S1). All these results confirmed that strain HUAS 5^T should belong to the genus *Streptomyces* of (Williams et al., 1989).

Genomic characteristics and phylogenetic analysis

The result of Rapid Annotation by using Subsystems Technology indicated that, strain HUAS 5^T had 305 subsystems, consist of 23 categories, and the subsystem coverage was 18%. The identified subsystem features were 'amino acids and derivatives'(380 CDSs), 'carbohydrates'(331 CDSs), 'protein metabolism' (214 CDSs), 'cofactors, vitamins, prosthetic groups, pigments' (176 CDSs), 'fatty acids, lipids, and isoprenoids' (170 CDSs), 'respiration' (118 CDSs), 'nucleosides and nucleotides' (95 CDSs), 'DNA metabolism' (80 CDSs), 'stress response' (65 CDSs), 'metabolism of aromatic compounds' (52 CDSs), 'virulence, disease and defense' (50 CDSs), 'RNA metabolism' (50 CDSs), 'cell wall and capsule' (42 CDSs), 'membrane transport' (33 CDSs), 'nitrogen metabolism' (33 CDSs), 'miscellaneous' (30 CDSs), 'phosphorus metabolism' (27 CDSs), 'regulation and cell signaling' (26 CDSs), 'iron acquisition and metabolism' (22 CDSs), 'secondary metabolism' (18 CDSs), 'dormancy and sporulation' (9 CDSs), 'potassium metabolism' (6 CDSs) and 'sulfur metabolism' (6 CDSs). The 21 biosynthetic gene clusters were terpene, thiopeptide, LAP [linear azol(in)e-containing peptides], RiPP-like [post-translationally modified peptide product (RiPP) cluster], T1PKS (type 1 polyketide synthase), PKS-like (other types of PKS), NRPS-like (NRPS-like fragment), lanthipeptide-class-I, NI-siderophore, ladderane, arylpolyene, NRPS (non-ribosomal peptide synthetase), lassopeptide, crocagin, butyrolactone, redox-cofactor, ectoine, T2PKS (Type II PKS), betalactone, NAPAA (non-alpha poly-amino acids like e-polylysine) and RiPP-like [post-translationally modified peptide product (RiPP) cluster]. Meanwhile, *vanW* gene in *vanI* cluster, *HelR* and *Streptomyces venezuelae* *rox* were discovered by CRAD analysis, which might lead to resistance to glycopeptide antibiotic rifamycin antibiotic. Finally, 15 CRISPR repeats and 20 genetic islands with a size range from 6,001 to 39,486 bp were found in the genome of strain HUAS 5^T.

The blast analysis of the 16S rRNA gene sequence (1529 bp) of strain HUAS 5^T indicated that it belonged to the genus *Streptomyces* and exhibited highest sequence similarity to *Streptomyces hirsutus* NRRL B-2713^T (97.3%). A ML Phylogenetic analysis of 16S rRNA gene sequence indicated that strain HUAS 5^T formed an independent lineage, which suggested that it belonged to a potential novel species (Fig. 2). This relationship was recovered in the NJ and MP trees (Figs. S2 and S3). Stackebrandt and Ebers (2006) have pointed out that when the 16S rRNA gene similarity between a new isolate and its relatives was in the threshold range of 98.7–99%, DNA–DNA relatedness studies should be mandatory between them. However, in this work, the highest 16S rRNA gene similarity between strain HUAS 5^T and other type strains with validly published names was 97.3%, which far less than 98.7%, therefore there's no need to carry out average nucleotide identity (ANI) analysis and the digital DNA-DNA hybridization (dDDH) or DDH values for evaluating their DNA–DNA relatedness in delineating new species.

Based on all of these data above, strain HUAS 5^T is characteristically a *Streptomyces* and represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces chenzhouensis* sp. nov. is proposed.

DESCRIPTION OF STREPTOMYCES CHENZHOUENSIS SP. NOV.

Streptomyces chenzhouensis (chen.zhou.en'sis.N.L. fem. adj. chenzhouensis, pertaining to chenzhou, a city of Hunan Province in China).

The strain produces grey aerial mycelium that differentiated into spiral spore chains with smooth-surfaced round or oval spores on Gause's synthetic No. 1 medium. Grows well on No. 1, Reasoner' 2A, ISP 2–7 without diffusible pigment. Growth occurs at 15–40°C (optimum, 28°C), pH 6.0–9.0 (optimum, pH 7) and in presence of 0–5.0% (w/v) NaCl. Positive for nitrate reduction and hydrolysis of Tweens (20, 40 and 80), but negative for hydrolysis of starch and Tween 60. Utilizes d-fructose, d-glucose, d-ribose, d-xylose, malt sugar, lactose, l-rhamnose, raffinose, saccharose, as sole carbon sources, but not l-arabinose, d-cellobiose, d-galactose, d-mannose and *myo*-inositol. Utilizes aspartate, histidine, l-alanine, l-glycine, l-hydroxyproline, l-ornithine, l-phenylalanine, l-proline, l-serine, tyrosine and valine, as sole nitrogen sources, but not arginine, l-cysteine and l-glutamic acid. The diagnostic amino acid is *l*-diaminopimelic acid (*l*-DAP), glucose, ribose and mannose are present in whole-cell hydrolysates. The menaquinones are MK-9(H₄), MK-9(H₆) and MK-9(H₈). The phospholipids are diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylinositol mannosides (PIM), phosphatidyl methyl ethanolamine (PME), phospholipids (PLS), phospholipids choline (PC), phospholipids of unknown structure containing glucosamine (NPG) and unidentified phospholipids (L1, L2, L3 and L4). The DNA G + C content of the type strain is 71.3%.

The type strain, HUAS 5^T (= MCCC 1K08552^T = JCM 36055^T), was isolated from the root tissue of *Cathaya argyrophylla* collected in Chenzhou city of Hunan Province, PR China. The NCBI accession numbers for the full-length 16S rRNA gene sequence and genome sequence of strain HUAS 5^T are OR237570 and CP121682, respectively.

Declarations

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Author contributions L L and Y W contributed to conception and design of the study. C W, RN H, ZY C, YS L and P X performed the statistical analysis. P M and C W wrote the first draft of the manuscript. RN H, ZY C, YS L, P X, L L and Y W assisted with investigation and sampling. All authors contributed to manuscript revisions, read and approved the submitted version.

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Conflict of interest The authors declare no competing interests.

Code availability Not applicable.

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate Not applicable.

Consent for publication All authors are consent for publication in Antonie van Leeuwenhoek.

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Figures

Fig. 1

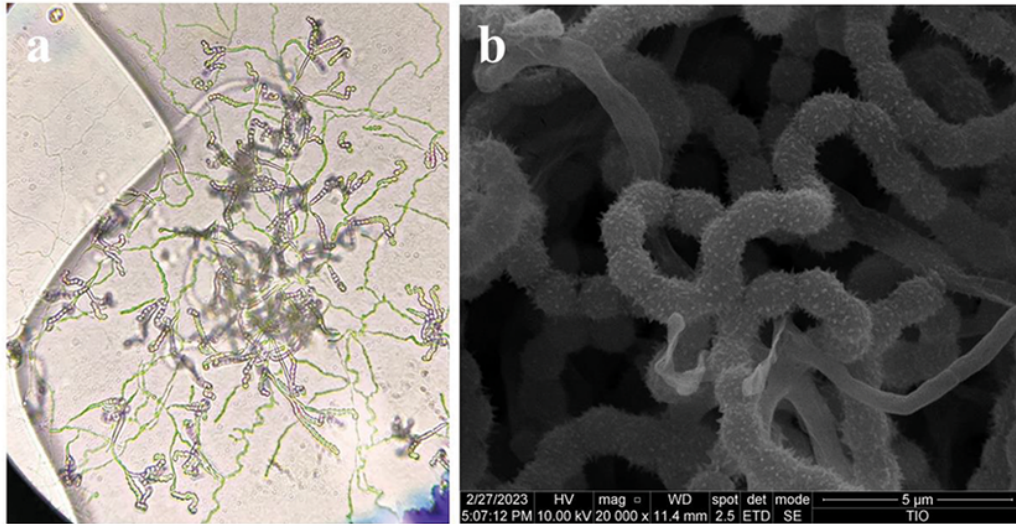


Figure 1

Optical micrograph (a) and scanning electron micrograph (b) of strain HUAS 5^T grown on Gause's synthetic medium at 28°C after incubation about 14d.

Fig. 2

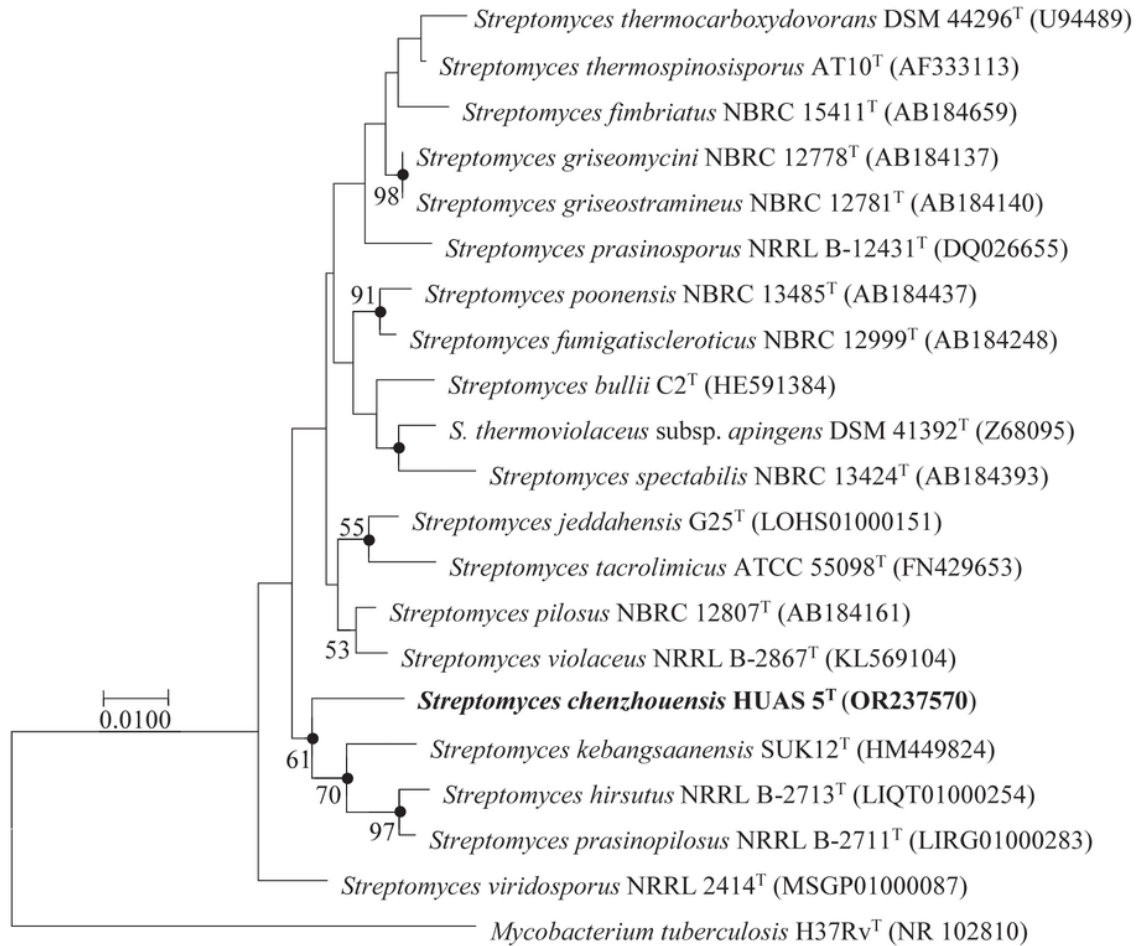


Figure 2

Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the relationship between selected species of the genus *Streptomyces*. *Mycobacterium tuberculosis* H37Rv^T was used as an outgroup. Bootstrap percentages over 50% derived from 1,000 replications are showed at the node. Dots indicate branches also recovered in the neighbor-joining and maximum-parsimony trees. Bar represents 0.0100.

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