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Identification and genomic analyses of a novel actinobacterium *Streptomyces shaowuensis* sp. nov. with biocontrol potential for rice bacterial blight

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Abstract

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is one of the most severe bacterial diseases in rice. The current BLB-prevention strategy depends on chemical antimicrobials. The biological control methods have gained considerable attention. Among them, *Streptomyces* are particularly promising candidates due to their ability to produce diverse natural antimicrobial and plant-growth-promoting metabolites. In this study, we isolated a *Streptomyces* strain HSW2009 from the rice rhizosphere. This strain displayed significant antagonistic activity against Xoo. The antagonistic metabolite was extracted and purified from the HSW2009 culture. High-performance liquid chromatography-mass chromatography and nuclear magnetic resonance analyses revealed that the active compound is Piericidin A1, a member of the piericidin family metabolites containing a 4-pyridinol core linked with a methylated polyketide side chain. Piericidin A1 was shown to protect rice from Xoo infection in the microclimate chamber. The strain HSW2009 produced pale yellow aerial mycelia on the agar plate of the International *Streptomyces* Project-2 medium. Its cellular morphology conformed to that typically observed in the genus *Streptomyces*. Phylogenetic analysis of 16S rRNA gene sequences showed that HSW2009 was closely related to *Streptomyces zagrosensis*, *S. youssoufiensis*, and *S. varsoviensis*. HSW2009 displayed a unique DNA profile in BOX-PCR fingerprinting analysis and had a genome size of 8,806,972 bp, with a 72.83% G + C content. Average nucleotide identity analysis and digital DNA–DNA hybridization using the Type Strain Genome Server supported HSW2009 as a novel *Streptomyces* sp. It was therefore proposed as *Streptomyces shaowuensis* sp. nov., type strain HSW2009.

Keywords Biological control, New species, Piericidin A1, *Streptomyces*, *Xanthomonas oryzae* pv. *oryzae*

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Background

Rice is one of the world's most important staple foods, serving as a dietary foundation for more than half of the global population. As a crucial commodity in international trade, rice farming supports millions of livelihoods and significantly contributes to many developing nations' agricultural gross domestic product (GDP). Similar to many other crops, rice is affected by diseases, such as leaf blight and rice blast (Khan et al. 2023). *Xanthomonas oryzae* pv. *oryzae* (Xoo), the causal agent of rice bacterial leaf blight (BLB), infects rice plants primarily through wounds or natural openings such as hydathodes and stomata. Once inside, the bacteria multiply and spread through the vascular system, particularly the xylem vessels (Nino-Liu et al. 2006). They exploit variant virulence factors, including extracellular polysaccharides, extracellular enzymes, Type II and Type III secretion systems and their effectors, and diffusible signal factor (DSF) family signal-dependent quorum sensing signaling systems, to facilitate their movement and colonization (Shen and Ronald 2002; Buettner and Bonas 2010). The infection reduces the plant's photosynthetic capability and overall health, and severe infections can lead to significant yield losses, resulting in substantial economic losses and posing threats to food security in regions reliant on rice production (Khan et al. 2023). The application of chemical pesticides and antibiotics generally can manage BLB diseases, but effectiveness is often strain-dependent due to the emergence of antimicrobial resistance issues (Shi et al. 2021). Moreover, these chemical methods have been limited due to concerns regarding their toxicity (Lindsey et al. 2020). Therefore, biological control applications employing microbial antagonists or microbial metabolites to control crop diseases represent an environmentally friendly substitute for chemical pesticides or antibiotics (Lahlali et al. 2022).

Streptomyces is a significant genus of actinobacteria, responsible for producing over 70% of the natural antibiotics currently being used in medicine and agriculture (Alam et al. 2022; Dow et al. 2023). *Streptomyces* spp. are Gram-positive bacteria producing metabolites with significant bioactivity, such as antibacterial, antifungal, antimalarial, anticancer, antioxidant, and neuroprotection activities (Isaka et al. 2002; Shaaban et al. 2017; Sivalingam et al. 2019; Chen et al. 2021; Fahmy and Abdel-Tawab 2021). *Streptomyces* remains a leading genus in the phylum Actinobacteria for exploring novel species and bioactive compounds (Lee et al. 2020). To date, there are 1200 *Streptomyces* spp. with validly published names (https://bacterio.net/) (Parte et al. 2020). Despite the abundance of known species, the potential for uncovering novel *Streptomyces* spp. remains undiminished.

There is extensive documentation on the use of *Streptomyces* spp. in the agriculture industry due to their plant growth-promoting, antimicrobial, and biocontrol properties (Pacios-Michelena et al. 2021; Dow et al. 2023; Khan et al. 2023; Al-Quwaie 2024; Wang et al. 2024). *Streptomyces* spp. have been adopted in agricultural applications due to their favorable attributes as potential endophytes and their proficiency in colonizing the rhizosphere and rhizoplane (Ayswaria et al. 2020; Pang et al. 2022). *Streptomyces* spp. are producers of bioactive metabolites that are effective in the control of rice diseases. Blasticidin S and Kasugamycin are metabolites initially produced by *Streptomyces griseochromogenes* and *Streptomyces kasugaensis,* respectively, which have been commercially utilized for the control of rice blast disease (Misato et al. 1959; Huang et al. 1964; Umezawa et al. 1965; Adaskaveg et al. 2011; Qi et al. 2021; Slack et al. 2021; Dow et al. 2023). Recent studies have revealed several secondary metabolites from various *Streptomyces* isolates that exhibit strong antagonistic activity against Xoo in various assays, including in vitro inhibition zone tests, greenhouse-based cutting leaf tests, and field-based leaf spraying tests. These metabolic compounds include aloesaponarin II (Donghua et al. 2013), carbazomycin B (Shi et al. 2021), aureonuclemycin (Wang et al. 2022), and 3,4-dimethoxyphenol (Lai et al. 2024). These reports underscore the importance of *Streptomyces* as a resource reservoir for controlling BLB.

This study aimed to isolate, identify, and characterize new microorganisms with potential applications in BLB prevention. We identified an anti-Xoo *Streptomyces* strain HSW2009 from a rhizosphere soil sample in Hubei Province, China. A polyphasic taxonomic study revealed that HSW2009 represents a novel species of the genus *Streptomyces* and was proposed as *Streptomyces shaowuensis* sp. nov. HSW2009. The antimicrobial metabolite against Xoo in HSW2009 was demonstrated to be Piericidin A1.

Results

Identification of a rice rhizosphere isolate with strong antagonistic activity against Xoo

The isolated strains and the corresponding culture supernatants of the bacterial isolates collected from the rice paddy field were rated for their antagonistic activity against Xoo. One of the collected isolates, HSW2009, showed the most significant inhibition zone (4.0 mm) towards Xoo compared with *Escherichia coli* (*E. coli*) strain DH5α (Fig. 1a, b). Similarly, the supernatant of the HSW2009 NA culture also showed a strong inhibitory growth effect on Xoo (4.2 mm) (Fig. 1c). These results indicate that HSW2009 produced and secreted an anti-Xoo metabolite. The HSW2009 strain was deposited at

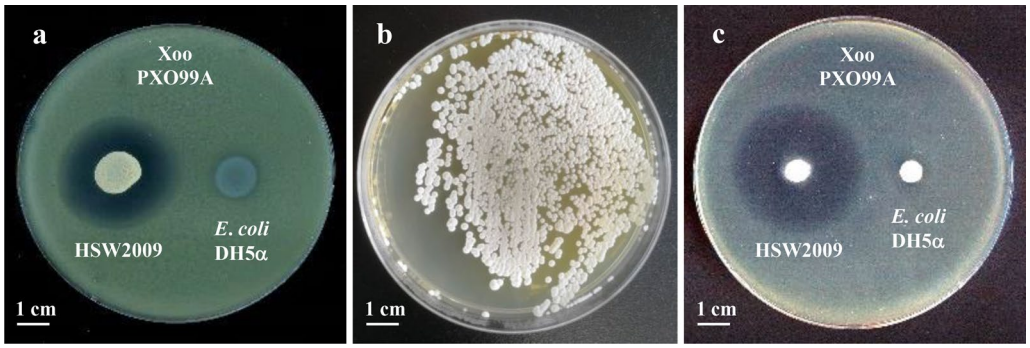

Fig. 1 Identification of a *Streptomyces*-like isolate HSW2009 with strong antagonistic activity against Xoo. **a** Antagonistic activity of the HSW2009 colony on a nutrient agar (NA) plate. **b** Phenotype of HSW2009 colonies on an NA plate. **c** Antagonistic activity of the HSW2009 NB culture supernatant observed by the clear inhibition zone. Culture with the supernatant of *Escherichia coli* strain DH5α had no inhibitory effect on Xoo growth, as indicated by the absence of an inhibition zone. Xoo: *Xanthomonas oryzae* pv. *oryzae* strain PXO99A

the China Center for Type Culture Collection (CCTCC) under registration number CCTCC M 2020069.

Phylogenetic, genotypic, and genomic analyses of HSW2009

HSW2009 was first identified using the 16S rRNA gene sequence (1516 bp; GenBank/EMBL/DDBJ accession number OR826630). The sequence was manually aligned with the corresponding partial 16S rRNA gene sequences of the type strains of representative members of the *Streptomyces* genus. HSW2009 had the highest sequence similarity to *Streptomyces zagrosensis* HM 1154 ^{T} (99.7%), *Streptomyces youssoufiensis* X4^{T} (99.5%), and *Streptomyces varsoviensis* NRRL ISP-5346 ^{T} (99.2%). A phylogenetic tree was constructed based on the 16S rRNA gene sequences to determine the phylogenetic position of HSW2009 (Fig. 2). Phylogenetic analysis revealed that the most closely related strain was *Streptomyces zagrosensis* HM 1154 ^{T} with the shortest evolutionary distance, which was in accordance with the results obtained from the 16S rRNA gene sequence analysis. BOX-PCR fingerprint analysis revealed that HSW2009 exhibited a unique DNA profile compared to the closest related type strains (Fig. 3a).

After adapter and sequencing raw read trimming at Q20, HSW2009 generated 4,890,326 reads that were used for whole genome assembly. The length of three k-mers was used for whole genome assembly, namely 21, 33, 55, and 77. By setting the minimum contig length at 200 bp, this assembly generated 1334 contigs, comprised of 8,806,972 bp, with a DNA G+C content of 72.83 mol% (Fig. 4a). The calculated sequencing coverage was 62.5 times. The N50, N70, and N90 were 12,255, 7986, and 3387 bp, respectively. The whole genome Shotgun project for HSW2009 was deposited at DDBJ/EMBL/GenBank under accession JBEHZD000000000. Based on the Prokaryotic Genome Annotation Pipeline, 7162 protein-coding genes with 70 tRNA and 8 rRNA genes were predicted in the strain HSW2009 genome (Fig. 4a). They were assigned to 1262 subsystems by Rapid Annotation using Subsystem Technology (RAST). Most genes were involved in amino acids and derivatives metabolism (4.74%), carbohydrate metabolism (3.12%), and protein metabolism (2.85%) (Additional file 1: Figure S1). AntiSMASH analysis revealed the presence of various biosynthetic gene clusters in the strain HSW2009 genome, such as a type-I polyketide synthetase for the production of antibiotic compounds, including Piericidin A1 (91% known cluster similarity) (Fig. 4b) and nigericin (77% known cluster similarity).

The whole genome of HSW2009 was compared to the retrieved genome of its closely related strain *S. zagrosensis* HM 1154 ^{T}/CECT 8305 ^{T}, resulting in an average nucleotide identity (ANI) value of 83.50% (Table 1). Furthermore, phylogenomic analysis of the whole genome sequences with the Type Strain Genome Server (TYGS) showed that HSW2009 was closely related to *S. buecherae* AC541^{T}, *S. youssoufiensis* JCM 18307 ^{T}, and *S. zagrosensis* CECT 8305 ^{T} (Additional file 1: Figure S2), with digital DNA–DNA hybridization (dDDH) values (formula d_4) of 30.2, 30.2, and 27.5%, respectively (Additional file 2: Table S1).

Phenotypic characteristics of HSW2009

The phenotypic study showed that HSW2009 grew robustly on International *Streptomyces* Project (ISP) 2 and AIA, moderately on ISP 4, ISP 5, ISP 7, NA, and LBA, and poorly on ISP 6, SA, SCA, and TSA. No growth was observed on ISP 3. The aerial and substrate mycelium colors of HSW2009 were recorded (Additional file 2: Table S2). On an NA plate, HSW2009 produced yellow diffusing pigment and white spores, resembling

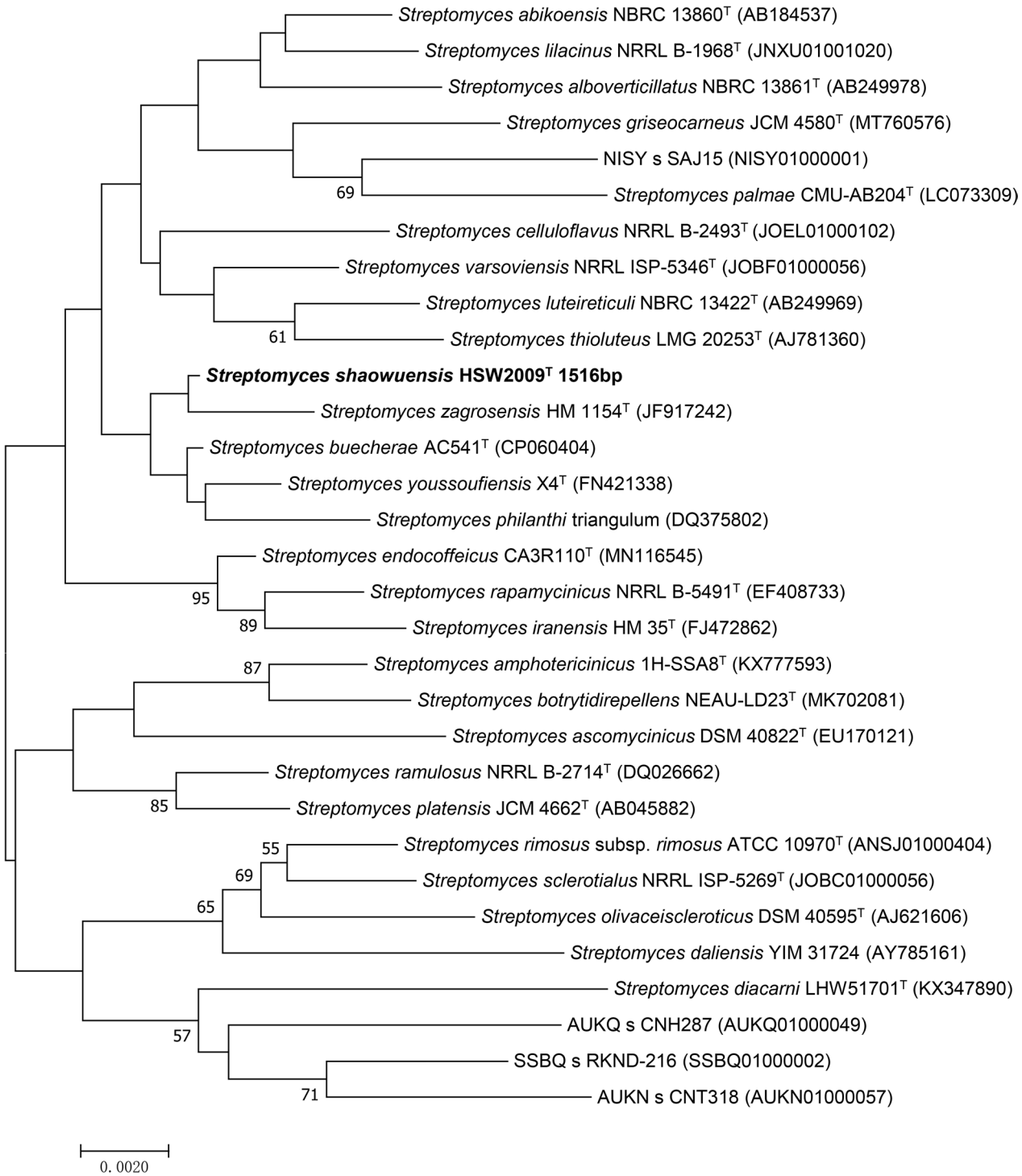

Fig. 2 Neighbor-joining phylogenetic tree based on nearly complete 16S rRNA sequences illustrating the relationship between *Streptomyces shaowuensis* strain HSW2009 (1516 bp) and representatives of related taxa. Numbers at nodes indicate percentages of 1000 bootstrap re-samplings; only values above 50% are displayed. Bar, 0.002 substitutions per site

the phenotype of a typical *Strepotomyces* strain (Fig. 1b). HSW2009 did not produce a melanoid pigment on ISP 7. Additionally, HSW2009 exhibited NaCl tolerance ranging from 0 to 6%, with optimal growth observed at 0–2% NaCl. Scanning electron microscopy observation of HSW2009 demonstrated typical features of *Streptomyces* genus (Fig. 3b).

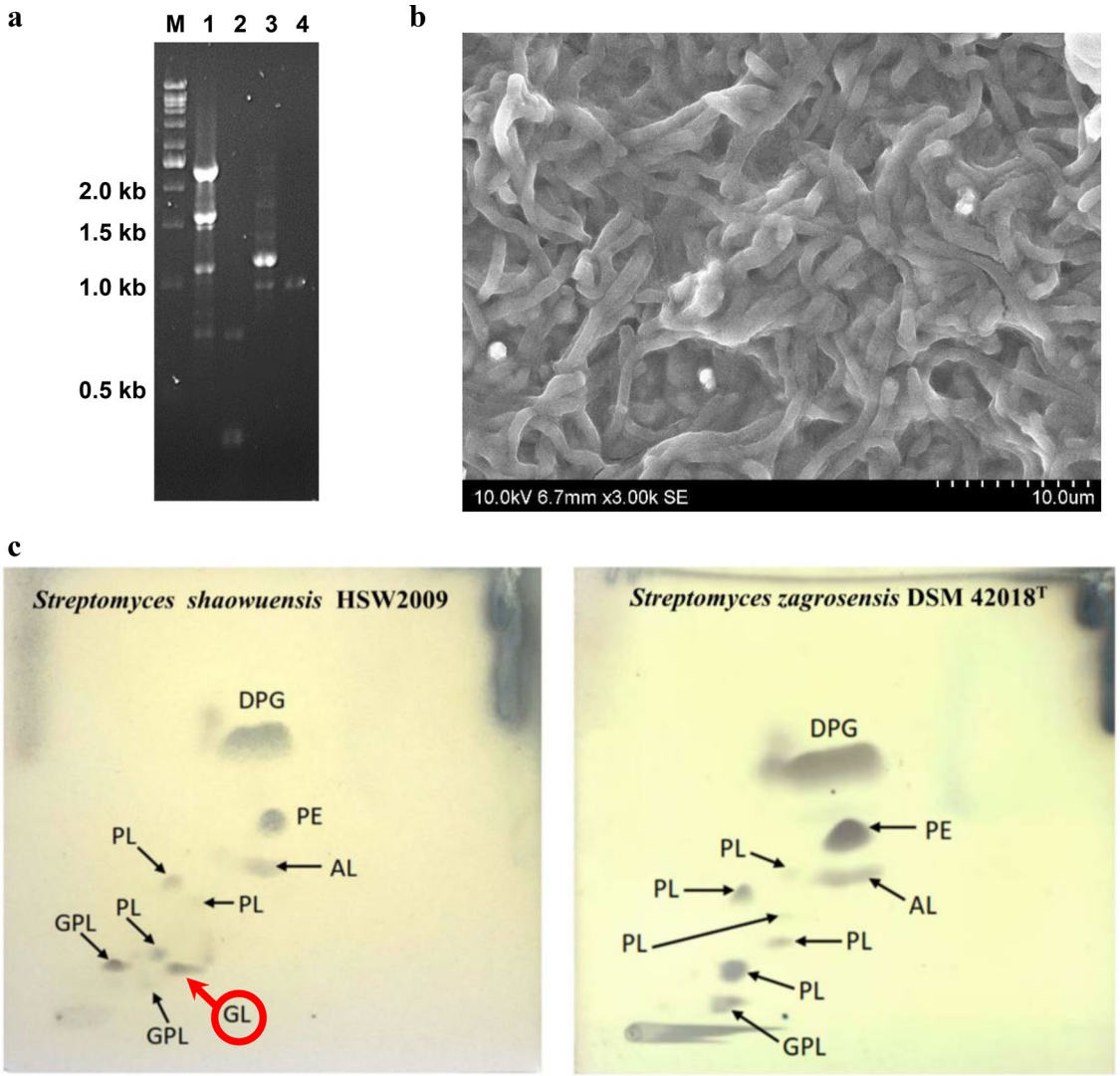

Fig. 3 Genotypic, morphologic, and chemotaxonomic characteristics of HSW2009. **a** BOX-PCR comparison of *Streptomyces shaowuensis* HSW2009 and the closest related type strains. Lanes: M, GeneRuler 1 kb DNA ladder marker; 1, *Streptomyces varsoviensis* DSM 40346 ^{T}; 2, *Streptomyces youssoufiensis* DSM 41920 ^{T}; 3, *Streptomyces zagrosensis* DSM 42018 ^{T}; 4, *Streptomyces shaowuensis* HSW2009. **b** Scanning electron microscopy observation of *Streptomyces shaowuensis* strain HSW2009 grown on an ISP 2 agar plate at 28°C. **c** Two-dimensional total polar lipid profiles of *Streptomyces shaowuensis* HSW2009 and its closely related type strain *Streptomyces zagrosensis* DSM 42018 ^{T}. DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; AL, aminolipid; GL, glycolipid; GPL, glycophospholipid; PL, phospholipid

Chemotaxonomic features of HSW2009

HSW2009 had a type I cell wall containing LL-diaminopimelic acid. The major menaquinones (MK) of HSW2009 were identified as MK-9(H_4) (66.6%) and MK-9(H_2) (18.9%). The whole-cell sugars detected were glucose and ribose. The primary cellular fatty acids present in HSW2009 were determined as $C_{16:0}$ (17.1%), iso-$C_{16:0}$ (14.7%), iso-$C_{15:0}$ (14.2%), anteiso-$C_{15:0}$ (13.0%), and $C_{16:1}$ Cis 9 (11.4%) (Additional file 2: Table S3). The fatty acid profile of HSW2009 showed a quantitative difference in the fatty acid composition compared to its closely related type strains, particularly for $C_{16:0}$, which was the most abundant fatty acid in HSW2009 and significantly higher than the other strains (Additional file 2: Table S3). However, some similarities were observed. For example, HSW2009, *S. zagrosensis* DSM 42018 ^{T}, *S. youssoufiensis* DSM 41920 ^{T}, and *S. varsoviensis* DSM 40346 ^{T}, all contained anteiso-$C_{15:0}$ (13.0%–22.4%) and iso-$C_{16:0}$ (13.7%–16.4%) as their major fatty acids (Additional file 2: Table S3). For polar lipids, HSW2009 possessed diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), aminolipid (AL), glycolipid

a

	***Streptomyces shaowuensis* HSW2009**
Genome size (bp)	8,806,972
Contigs	1334
Contigs N_{50} (bp)	12255
G + C content	72.83 mol%
CDS	7162
tRNA	70
rRNA	2(5S), 2(16S), 4(23S)

b

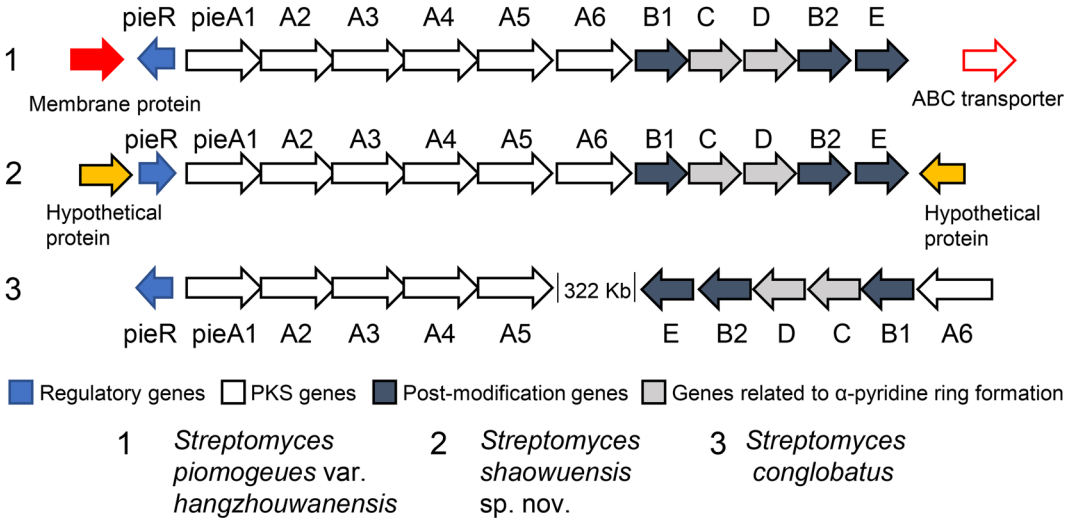

Fig. 4 Bioinformatics analysis of HSW2009 genome and piericidin A1 biosynthetic gene cluster. **a** General features of the genome of *Streptomyces shaowuensis* strain HSW2009. **b** Comparative analysis of piericidin A1 biosynthetic gene clusters in three *Streptomyces* strains. 1: *Streptomyces piomogeues* var. Hangzhouwanensis; 2: *Streptomyces shaowuensis* sp. nov; 3: *Streptomyces conglobatus*

Table 1 FastANI generated ANI values for *Streptomyces shaowuensis* HSW2009 and closely related strains from genus *Streptomyces*

No	Species	Type strain	Accession NO.	ANI value
1	*Streptomyces zagrosensis*	HM 1154^T	JACHJL010000000	83.5
2	*Streptomyces alboverticillatus*	NRRL B-24281^T	MUFU01000000	81.1
3	*Streptomyces varsoviensis*	NRRL ISP-5346^T	JOBF00000000	80.9
4	*Streptomyces botrytidirepellens*	NEAU-LD23^T	RIBZ00000000	80.9
5	*Streptomyces griseocarneus*	JCM 4580^T	BNBL00000000	80.9
6	*Streptomyces rapamycinicus*	NRRL B-5491^T	QYCY00000000	80.8
7	*Streptomyces iranensis*	HM 35^T	JAGGLR000000000	80.8
8	*Streptomyces angustmyceticus*	JCM 4053^T	CP082945.1	80.7
9	*Streptomyces rimosus* subsp. *rimosus*	ATCC 10970^T	CP048261.1	80.6
10	*Streptomyces abikoensis*	NBRC 13860^T	BMRT00000000	80.6

(GL), glycophospholipid (GPL), and phospholipid (PL) (Fig. 3c). The polar lipid profile of HSW2009 was compared to that of its closely related type strain, *S. zagrosensis* DSM 42018 ^{T}. HSW2009 contained glycolipids, which were not detected in *S. zagrosensis* DSM 42018 ^{T}. The resulting differences in polar lipid profiles indicate that HSW2009 differs from *S. zagrosensis* DSM 42018 ^{T}.

HSW2009 produces piericidin A1 and inhibits Xoo growth

To identify the putative anti-Xoo metabolite produced by HSW2009, the total metabolites in the condensed extracts were separated and enriched using preparative high-performance liquid chromatography (HPLC) analysis (Fig. 5a). The resultant fractions were verified using the in vitro antagonistic assay against Xoo. Fractions with stronger antagonistic activity than the crude exacts (CE) (Fig. 5b) were further diluted 10 and 100 folds and again subjected to the antagonistic assay. Fractions 16 and 17 had comparable anti-Xoo activity with the crude exacts after 100-fold dilution (Fig. 5c); therefore, they were purified and analyzed using Ultra-Performance Liquid Chromatography-Time of Flight-Mass Spectrometry (UPLC-Q-TOF–MS). In the active compounds of fractions 16 and 17, $[M+H]^+$ ions were present at m/z 416.2804 (Fig. 5d, e); the corresponding molecular formula was $C_{25}H_{37}NO_4$. This compound closely resembles the metabolite Piericidin A1 (Molar mass: 415.57 g/mol), which is known to be produced by *Streptomyces piomogeues* var. Hangzhouwanensis (Liu et al. 2012; Li et al. 2018). The gene cluster for Piericidin A1 was identified in the genome of strain HSW2009 (Fig. 4b), suggesting that Piericidin A1 is the anti-Xoo metabolite produced by HSW2009. Further support from Nuclear Magnetic Resonance (NMR) data, including ^{1}H and ^{13}C spectra, heteronuclear single quantum coherence (HSQC), and correlation spectroscopy (COSY), confirmed that the active compound purified from fractions 16 and 17 was Piericidin A1 (Fig. 6). This result was chemically validated through UPLC-Q-TOF–MS analysis of a commercially available standard for Piericidin A1.

HSW2009 contains a *pie* gene cluster for piericidin A1 biosynthesis

The biosynthetic gene clusters for Piericidin A1 in *Streptomyces piomogeues* var. Hangzhouwanensis and *Streptomyces conglobatus* have been previously reported (Liu et al. 2012; Li et al. 2018, 2022). Anti-SMASH analysis of the HSW2009 genome also revealed a 52.343 Kb gene cluster, containing the regulatory gene *pieR*, the polyketide synthase genes *pieA1-pieA6*, the genes related to α-pyridine ring formation (*pieB1*, *pieB2*, *pieE*), as well as the post-modification genes *pieC* and *pieD* (Fig. 4b). The *pie* gene cluster in HSW2009 is phylogenetically closer to that in *Streptomyces piomogeues* var. Hangzhouwanensis (Fig. 4b). However, the flanking genes of the *pie* gene clusters are different in both HSW2009 and *Streptomyces piomogeues* var. Hangzhouwanensis (Fig. 4b). These results further support the conclusion that HSW2009 represents a novel species within the *Streptomyces* genus.

Piericidin A1 protects rice from xoo infection under microclimate conditions

To further investigate the potential of Piericidin A1 for the BLB control, the commercially available Piericidin A1 standard was diluted with methanol to reach a final concentration ranging from 0.1 to 1000 mg/L, and the resultant solutions were tested for antagonistic activity against Xoo. A 1 mg/L concentration of Piericidin A1 exhibited an inhibitory effect on Xoo growth (Fig. 7a). Further Xoo growth analysis in NB medium in the presence of Piericidin A1 resulted in a half maximal effective concentration (EC_{50}) of 6.363 mg/L (Fig. 7b). Based on these results, we further evaluated the effects of Piericidin A1 on Xoo infection in a controlled microclimate chamber. Using the leaf clipping method, we inoculated Xoo strain PXO99A onto Nipponbare rice leaves and then sprayed them with 100 mg/L of purified Piericidin A1. The rice disease incidence in the presence and absence of Piericidin A1 was 87% and 93%, respectively, suggesting that the application of Piericidin A1 had no significant effect on the disease incidence. However, Piericidin A1 application effectively inhibited Xoo infection by reducing the average lesion length from 4.3 cm to 0.6 cm, representing an approximately sevenfold reduction (Fig. 7c, d). These results indicate that Piericidin A1 strongly suppresses the progression of rice bacterial blight under microclimate conditions.

Discussion

The discovery and identification of novel taxa involves a multifaceted method incorporating different techniques required to classify the microbe of interest. This approach collects data from diverse sources, including genomics information, phenotypic traits, and biochemical characteristics. The combination of these data offers a comprehensive understanding of the microorganism's identity, which facilitates precise classification of new taxa. In this study, HSW2009 was most closely related to *Streptomyces zagrosensis* HM 1154 ^{T} (99.7% sequence similarity) based on 16S rRNA gene sequence identification using the EzBioCloud server and phylogenetic analysis (Fig. 2). Despite the high 16S rRNA gene sequence similarity, the whole genome comparison of HSW2009 and *Streptomyces zagrosensis* HM 1154 ^{T} via FastANI analysis resulted in an ANI value of 83.5% (Table 1). FastANI has emerged as an alternative approach to traditional laboratory-based

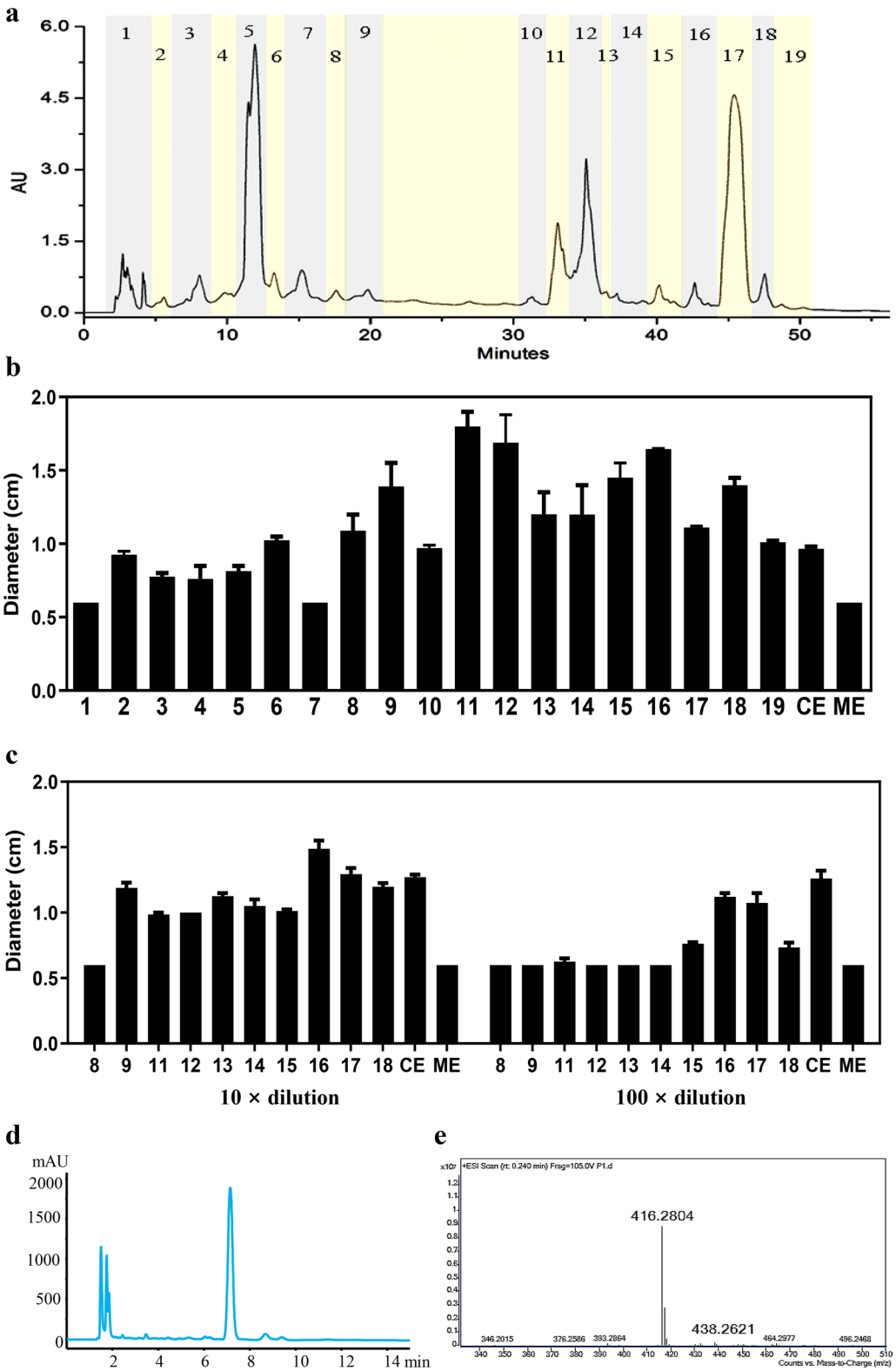

Fig. 5 Identification and characterization of the metabolite with antagonistic activity against Xoo in the ethyl acetate extracts of the NB culture of HSW2009. **a** HPLC analysis of the active fractions derived from the preparative HPLC analysis of the crude ethyl acetate extract. **b** Relative antagonistic activities of the separated fractions. **c** Relative antagonistic activities of the 10- and 100-fold diluted fractions. **d** HPLC analysis of the compound in fractions 16 and 17. **e** MS analysis of the purified compound in fractions 16 and 17. mAU, milli absorbance unit; CE, crude extracts; ME, methanol

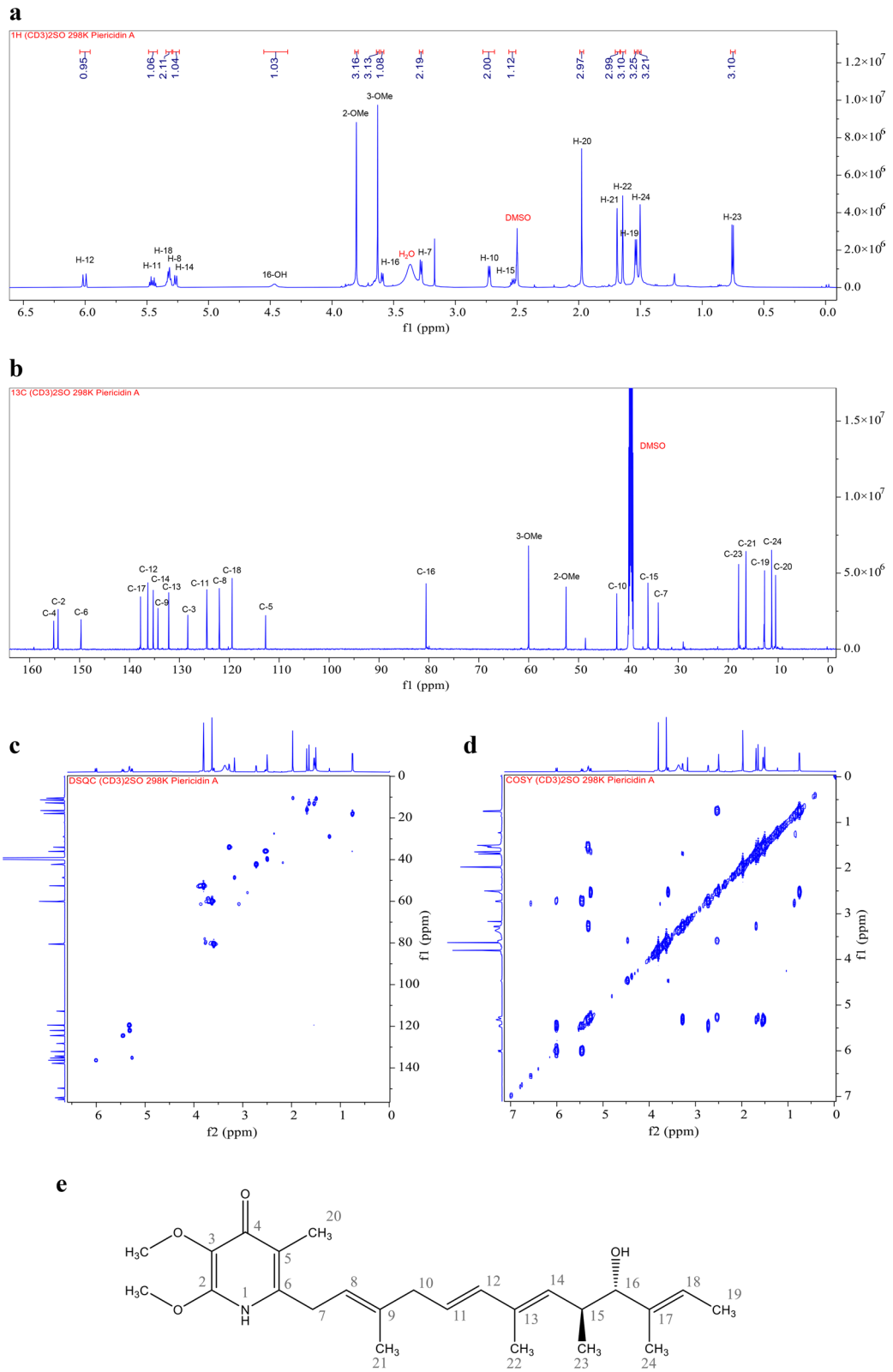

Fig. 6 Nuclear magnetic resonance (NMR) analysis of the purified active compound confirming it as Piericidin A1. **a** ^{1}H NMR spectrum. **b** ^{13}C NMR spectrum. **c** Heteronuclear singular quantum correlation (HSQC) spectra. **d** H–H correlation spectroscopy (COSY) spectra. **e** Proposed chemical structure of fractions 16 and17

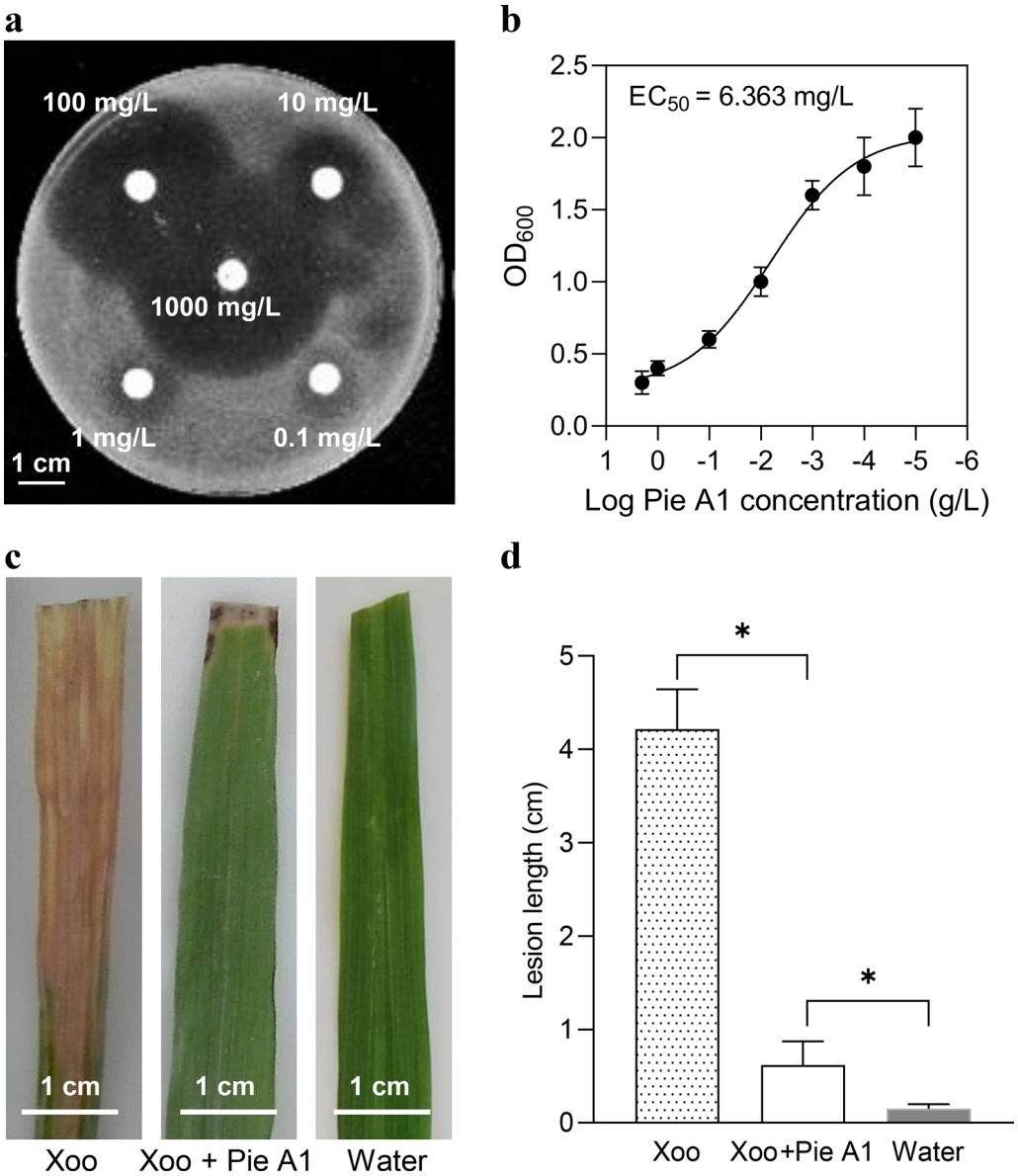

Fig. 7 Piericidin A1 has the potential to prevent rice bacterial blight. **a** Xoo growth inhibitory zone with 0.0001–1 g/L Piericidin A1 standard on an NA plate. **b** OD_{600} of Xoo NA culture in the presence of 0.0001–1 g/L Piericidin A1; the EC_{50} of Piericidin A1 to inhibit Xoo growth was identified as 0.006363 g/L. **c** Xoo-infected rice leaves in the absence and presence of 100 mg/L Piericidin A1 (Pie A1). **d** Lesion length of Xoo-infected rice in the absence and presence of 100 mg/L Piericidin A1 (Pie A1), alongside a control leaf treated with stile water only (water). Shown are the averages for 15 rice leaf lesions with standard deviations. Statistically significant differences are indicated by one asterisk ($P \leq 0.05$)

DDH, enabling ANI estimation based on an alignment-free sequence mapping technique. FastANI offers a rapid analysis comparable to alignment-based ANI approaches, expediting the investigation of novel species by comparing the query genome sequence to all available genomes in the database (Jain et al. 2018). ANI values of 95% and 69% conserved DNA are equivalent to the 70% DNA–DNA hybridization threshold typically used for species delineation (Goris et al. 2007). The ANI value between HSW2009 and its closely related strain *S. zagrosensis* HM 1154 ^{T} was 83.5%, significantly below the recommended 95% cutoff for species definition. In addition, the digital DDH values estimated by TYGS for HSW2009 and its closely related type strains were below 30.2% (Additional file 2: Table S1), significantly below the 70% threshold (Wayne et al. 1987). The TYGS web server integrated whole-genome-based methods for phylogeny and taxonomic classification, which also substitute

laboratory-based DDH (Meier-Kolthoff and Göker 2019). Based on the results obtained from FastANI and TYGS, HSW2009 represents a novel species in the genus *Streptomyces*. The high discriminative power of the BOX-PCR fingerprint technique revealed that HSW2009 had a distinct BOX pattern (Fig. 3a), which further demonstrated its uniqueness when compared to its closely related type strains based on the 16S rRNA gene sequence analysis (Lanoot et al. 2004).

The phenotypic and chemotaxonomic features of HSW2009 served as supplementary data to confirm that HSW2009 belonged to the *Streptomyces* genus. For instance, HSW2009 produced yellowish-white aerial mycelia and light greenish-yellow substrate mycelia, followed by the development of white spores on NA plates (Fig. 1b; Additional file 2: Table S2). The formation of aerial and substrate mycelia, as well as sporulation, are typical colony characteristics of the *Streptomyces* genus (Law et al. 2019a). Moreover, HSW2009 possessed LL-diaminopimelic acid, which is an amino acid often found in *Streptomyces* spp. (Lechevalier and Lechevalier 1970; Lee et al. 2014; Ser et al. 2016). The quantitative difference in the fatty acid compositions of HSW2009 and its unique polar lipid profile supported that HSW2009 is a distinct strain from the closely related type strains within the genus *Streptomyces* (Fig. 3c; Additional file 2: Table S3). Overall, the results of phylogenetic, genomic, phenotypic, and chemotaxonomic analyses strongly supported that HSW2009 is qualified to be assigned as a novel species in the *Streptomyces* genus, for which the name *Streptomyces shaowuensis* sp. nov. is proposed.

This study also demonstrated that HSW2009 exhibited promising in vitro antibacterial activity against the rice blight pathogen Xoo (Fig. 1a). The antagonistic compound was identified as Piericidin A1 (Fig. 6), a member of the piericidin family of microbial metabolites. Piericidin A1 was first isolated as an insecticide against houseflies, green caterpillars, spider mites and rice stem borers (Tamura et al. 1963; Zhou and Fenical 2016; Azad et al. 2022). It also demonstrated efficacy in controlling potato soft rot disease caused by *Erwinia carotovora* subsp. *atroseptica* (Kang et al. 2016). Glycosylated piericidins were shown to be effective against rice bacterial leaf streak caused by *Xanthomonas oryzae* pv. *oryzicola* (Shang et al. 2018). In the present study, Piericidin A1 effectively alleviated the symptoms of rice bacterial blight caused by Xoo, under controlled microclimate conditions (Fig. 7c, d). These findings highlight the biocontrol potential of Piericidin A1 and its producer, the *Streptomyces* strain HSW2009.

Piericidins are composed of an α-pyridone ring linked to an unsaturated linear polyketide chain, showing high structural similarities to coenzyme Q (Zhou and Fenical 2016; Azad et al. 2022). These resemblances enable these metabolites to exhibit insecticide activity by acting as NADH-ubiquinone oxidoreductase (complex I) inhibitors (Liu et al. 2012). However, the mechanism of the antagonistic activity of Piericidins against microbes has rarely been studied. Although both the phytopathogens *Xanthomonas* and *E. coli* produced coenzyme Q_8 (Wang et al. 2016; Zhou et al. 2019), Piericidin A1 strongly inhibited the growth of Xcc, Xoo, and Xoc while it had no significant effect on *E. coli* growth (Shang et al. 2018; Fig. 1c). These findings suggest that Piericidin A1 might have alternative target in the sensitive microorganism. Indeed, Piericidin A1 was found to suppress the type III secretion system *of Yersinia pseudotuberculosis* and *Yersinia enterocolitica* by blocking the assembly of the YscF needle and the secretion of the T3SS substrates (Duncan et al. 2014; Morgan et al. 2017). Additionally, Piericidin A1 showed strong quorum-sensing inhibitor activities (Ooka et al. 2013; Kang et al. 2016). Therefore, the molecular target of Piericidin A1 in *Xanthomonas* strains remains to be explored in the future.

Conclusions

In this study, we identified and characterized the *Streptomyces* strain HSW2009, which exhibited strong anti-Xoo activity. The anti-Xoo effect of HSW2009 was attributed to the production of the metabolite Piericidin A1. Application of Piericidin A1 effectively protected rice plants from Xoo infection under microclimate conditions. Comprehensive genomic, phylogenetic, phenotypic, and chemotaxonomic analyses revealed that HSW2009 represents a novel species within the *Streptomyces* genus, for which we propose the name *Streptomyces shaowuensis* sp. nov., with HSW2009 as the type strain.

Methods

Bacterial strains, media, and culture conditions

Unless specifically stated, HSW2009 was grown on nutrient agar (NA) plates or in nutrient broth (NB) at 28°C for antagonistic strain screening against Xoo. The NA medium was prepared with 5 g of peptone, 3 g of beef extract, 10 g of sucrose, 1 g of yeast extract, and 15 g of agar powder per liter of distilled water (pH 7.0–7.2), and the NB medium contained the same medium composition as NA except the agar powder. The Xoo strain PXO99A and *Escherichia coli* strain DH5α were the laboratory stock strains. They were grown in NB medium or on nutrient agarose plates at 28°C. The nutrient agarose medium contained the same components as the NA medium except that 15 g of agar powder was replaced with 8 g of agarose powder per liter of distilled water. When PXO99A was cultured, 20 μg/mL cephalexin was constantly added to the medium. The growth of Xoo

PXO99A or *E. coli* DH5α in NB medium was determined by measuring the optical density at a wavelength of 600 nm (OD_{600}) using the UV–Visible spectrophotometer Genesys 150 (ThermoScientific, Shanghai, China).

Isolation of the *Streptomyces* strain HSW2009

The HSW2009 strain was isolated from the rhizosphere of hybrid rice (*Oryza sativa* L. ssp. *indica* 'Gui-nongzhan') grown in a paddy field at He-Shang-Wu, Anshan village, Kuzhu Town, Huangmei County, Hubei Province, China. Briefly, the collected rice root sample was gently washed with sterile water and cut into smaller pieces using sterilized scissors. Ten grams of the sample were weighed, ground, and suspended in 10 mL phosphate buffer solution (PBS). The solution was shaken until homogenized, then filtered and diluted 100 times with PBS before spreading onto NA plates. The plates were incubated at 28°C for 3 d. A total of 1152 single colonies were selected and used by inoculating each of them into 300 µL of NB medium in the 96-well plates. The plates were placed in a shaker (ZQZY-75CN, Shanghai, China) at 200 rpm for 3 days at 28°C. All isolated strains were cryopreserved by adding glycerol into each well to a final concentration of 20% (v/v) and were subsequently subjected to in vitro antagonist assay against Xoo.

In vitro antagonistic assay against xoo

The antagonist activities of the isolated bacterial strains were tested against Xoo strain PXO99A following the previously described method (Zhou et al. 2016). Briefly, the isolated colonies were used to inoculate a 96-well plate containing 300 µL of NB medium and incubated for 72 h at 28°C. The cultures were centrifuged at 5000 rpm for 10 min to obtain the supernatant. In a laminar flow hood, 10 µL of the supernatant of each strain was absorbed on a 0.5 cm sterile filter paper. Each anti-Xoo screening plate was prepared by adding 2 mL of NB culture of PXO99A ($OD_{600}=1.0$) to 18 mL of nutrient agarose medium, pre-warmed to 40°C. When the screening plates were set, the dried discs were transferred to the plates. The colony or culture supernatant of *E. coli* DH5α was used as the negative control. Piericidin A1 standard, purchased from Aladdin Biochemical Technology (Shanghai, China), was diluted to specified concentrations for the assay. The test plates were incubated at 28°C for 3 days. The diameter of the inhibition zones was observed and measured by rulers.

A turbidimeter test was used to determine the half-maximal effective concentration (EC_{50}) of Piericidin A1 against Xoo. Piericidin A1 was diluted in methanol to create a concentration gradient, which was then added to a 96-well plate. The Xoo suspension, cultured in NB medium to an OD_{600} of approximately 1.0, was diluted 1:1000 (v/v) with fresh NB medium and introduced into the 96-well labeled plate containing varying concentrations of Piericidin A1. The plate was incubated at 28°C with continuous shaking at 180 rpm for 48 h until the bacteria in the wells containing NB medium and methanol only (negative control) reached the logarithmic growth phase. Bacterial growth was monitored by measuring the absorbance at 600 nm using a microplate reader (Perkin Elmer, Massachusetts, USA). The concentration-effect curve was fitted using a three-parameter logistic equation (GraphPad Prism, San Diego, CA, USA). EC_{50} value is expressed as geometric means with 95% confidence intervals.

Phylogenetic and genotypic analyses

Genomic DNA extraction and 16S rRNA PCR amplification were performed according to previously described protocols (Lee et al. 2014; Law et al. 2019b). A nearly complete 16S rRNA gene sequence for HSW2009 was obtained. The 16S rRNA sequence of HSW2009 was aligned with representative sequences of related type strains within the *Streptomyces* genus retrieved from the GenBank/EMBL/DDBJ database using CLUSTAL-X software. Manual alignment and verification of the sequences were conducted, and a phylogenetic tree was constructed based on the neighbor-joining algorithm using MEGA 7.0 software. The evolutionary distances were calculated using Kimura's two-parameter model. The tree topologies were analyzed via bootstrap based on the 1000 resampling method according to Felsenstein (Felsenstein 1985). The level of sequence similarity was estimated using the EzBioCloud server (https://www.ezbiocloud.net/).

To generate DNA fingerprinting profiles of HSW2009 based on the presence of repetitive BOX elements in the genomes, BOX-PCR analysis was conducted according to the established protocol to characterize HSW2009 and the closely related type strains, utilizing primer BOX-A1R (5'-CTACGGCAAGGCGACGCTGACG-3') (Lee et al. 2014; Law et al. 2017).

Whole genome sequencing and bioinformatic analyses

Whole genome sequencing was performed using the Identification Service of DSMZ (Braunschweig, Germany) (https://www.dsmz.de/). Briefly, genomic DNA extraction for whole genome sequencing was performed using a MasterPure™ Gram Positive DNA Purification Kit (LGC Biosearch Technologies, London, UK) according to the manufacturer's instructions. Libraries were prepared by using a Nextera XT DNA Library Preparation Kit (Illumina®, San Diego, CA, USA). Samples were sequenced on a NextSeq 550 Sequencing System using a NextSeq 500/500 High Output Kit v2.5 (Illumina®, San Diego, CA, USA). The sequencing quality for HSW2009

was evaluated using FastQC (version 0.11.9). Subsequently, the raw reads were trimmed at Q20 using Trim Galore (version 0.6.7) along with the adapter sequences. The trimmed raw reads were then de novo assembled using a St. Petersburg genome assembler (SPAdes) (version 3.15.2). The assembled genome was evaluated using QUAST (version 5.2.0) and BUSCO (version 5.4.3). The sequencing coverage was calculated using the Burrow–Wheeler Aligner (BWA) (version 0.7.17) and Samtools (version 1.16.1). The assembled genomic sequence was annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) and analyzed by Rapid Annotation using the Subsystem Technology (RAST) Web server (https://rast.nmpdr.org/) for annotation with the following settings: default pipeline for RASTtk, with domain bacteria and automatically fixed error options turned on. The assembled genomic sequence was compared with other *Streptomyces* spp. genomes (retrieved from the NCBI database) using FastANI (version 1.33). The genome sequence was uploaded to the Type Strain Genome Server (https://tygs.dsmz.de) for phylogenomic analysis. Digital DNA–DNA hybridization (dDDH) was calculated with a Genome-to-Genome Distance Calculator (GGDC) v3.0. AntiSMASH (https://antismash.secondarymetabolites.org) was used to detect the biosynthetic gene clusters related to secondary metabolites.

Phenotypic analysis

Colony morphology and growth of HSW2009 was examined on the following media agar plates (HiMedia, Mumbai, India) (Law et al. 2019b): ISP 2, ISP 3, ISP 4, ISP 5, ISP 6, ISP 7, actinomycetes isolation agar (AIA), *Streptomyces* agar (SA), starch casein agar (SCA), NA, Luria Bertani agar (LBA), and trypticase soy agar (TSA). The plates were incubated at 28°C for 14 days. The Inter-Society Color Council-National Bureau of Standards (ISCC-NBS) color charts were utilized to determine the colony color of HSW2009. After incubation on an ISP 2 agar plate for 14 days, the cellular morphology of HSW2009 was observed using light microscope (80i, Nikon, Tokyo, Japan) and scanning electron microscope (JSM 6400, JEOL, Tokyo, Japan). The salt tolerance of HSW2009 was evaluated in this study using tryptic soy broth (TSB) with 1%–10% NaCl (w/v) at intervals of 2% and incubated at 28°C for 7–14 days. The phenotypic assays were performed concurrently for HSW2009 and the control strains: *Streptomyces zagrosensis* DSM 42018 ^{T}, *Streptomyces youssoufiensis* DSM 41920 ^{T}, and *Streptomyces varsoviensis* DSM 40346 ^{T}.

Chemotaxonomic analysis

To obtain the detailed and specific chemical information of HSW2009, chemotaxonomic analyses were conducted by the Identification Service of the DSMZ (Braunschweig, Germany) (https://www.dsmz.de/). Basically, analyses of cell wall peptidoglycans, whole cell sugars, respiratory quinones, fatty acids, and polar lipids were investigated.

Fermentation of HSW2009 and extraction of antagonistic metabolites from the fermentation culture

For the preparation of seed cultures, HSW2009 was cultured on NA plates at 28°C for 3 days. An NA plug (1 cm × 2 cm) containing the colonies was cut using a sterile scalpel blade and transferred into 50 mL of TSBY broth medium (30 g/L trypticase soy broth, 10 g/L yeast extract, 103 g/L sucrose, pH 7.2) and incubated at 28°C and shaken at 200 rpm for 24 h to obtain the seed culture. HSW2009 was fermented in a 250-mL flask by pipetting 5 mL of seed culture into 50 mL of fermentation medium (FM; 45 g/L sucrose, 25 g/L soybean powder, 1 g/L NaCl, 3 g/L $CaCO_3$, 0.2 g/L K_2HPO_4, 0.2 g/L KNO_3, 0.05 g/L $FeSO_4$, 0.1 g/L Na_2SO_4, pH 7.0). The culture was incubated at 28°C at 250 rpm for 48 h.

The supernatant of the fermented culture was extracted with an equal volume of ethyl acetate for 30 min. The upper organic phase of ethyl acetate was collected and subjected to a rotary vacuum evaporator to remove the extracting solvent. The final concentrated extract was collected and dissolved in 1 mL of methanol.

Enrichment of the active components from the fermentation extract by preparative HPLC

A preparative C18HCE column (20 × 250 mm, Acchrom, Beijing, China) was used to enrich the active components from the above-mentioned culture extract. The mobile phases were composed of water (A) and methanol (B). A linear gradient was set as 10%–35% B within 40 min. The flow rate was 15 mL/min, and the injection volume was 3 mL. The wavelength of the UV detector was set at 267 nm (Waters, Massachusetts, USA). The collected fractions were evaporated and then dissolved in methanol for the in vitro antagonistic assay against Xoo. The active fractions were pooled and submitted for the next round of purification.

HPLC for the purification of the active compounds

An Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm, Agilent, California, USA) was used to purify the active fractions obtained from preparative HPLC. The mobile phases were composed of water containing 0.5% formic acid (FA) (A) and methanol (B). The isocratic elution was 25% B, and the flow rate was 1 mL/min. The UV detector was set at 267 nm (Agilent, California, USA). A total of 19 fractions were collected by in vitro antagonistic assays against *Xoo*. Fractions with highest antagonistic activities were submitted for further analysis.

UPLC-Q-TOF–MS analysis

The pooled residues were subsequently dissolved in 0.1 mL of methanol and analyzed using UPLC-Q-TOF–MS (Agilent, California, USA) with a C18 reverse-phase column (Zorbax XDB; 5 µm, 4.6×150 mm; Agilent, California, USA). Methanol and water containing 0.1% formic acid (80/20, v/v) were used to elute the sample at a 0.4 mL/min rate. MS was operated in the positive Electrospray Ionization (ESI) mode.

NMR analysis for the structural characterization

^{1}H, ^{13}C, ^{1}H-^{1}H COSY, and HSQC nuclear magnetic resonance spectra in dimethylsulfoxide (DMSO)-*d6* solution were obtained using Bruker Avance III 600 MHz (Rheinstetten, Germany) at the Instrumental Analysis Center at Shanghai Jiao Tong University.

Evaluation of piericidin A1 in xoo-infected rice leaves

Xoo strain PXO99A was grown in NB medium at 250 rpm for 36 h at 28°C until $OD_{600}=1.5$. The culture was suspended in modified Millers' minimal medium (10.5 g/L K_2HPO_4, 4.5 g/L KH_2PO_4, 1 g/L $(NH_4)_2SO_4$, 0.5 g/L sodium citrate, 200 mg/L $MgSO_4{\cdot}7H_2O$, 5 µg/L thymine hydrochloride, 100 mg/L L-methionine, 100 mg/L nicotinic acid, 10 g/L glucose, and 1 g/L L-glutamic acid) until it reached $OD_{600}=0.1$ (Kelemu and Leach 1990). Leaves, approximately 25 cm in length and 1 cm in width, of rice plants (cultivar 'Nipponbare') at the booting stage were inoculated with a PXO99A suspension using the leaf clipping method (Ke et al. 2017). Inoculated rice leaves were divided into three treatment groups: one group was sprayed with a methanol solution as a solvent control, another group was sprayed with a methanol solution containing 0.1 g/L of purified Piericidin A1 from HSW2009, and a third group was treated with sterile water as an additional control. A total of 15 inoculated rice leaves were included in each treatment group. The rice leaves were covered with transparent plastic wrap and placed in an illuminated incubator at 28°C with 90% humidity, under a photoperiod of 12 h light and 12 h dark, with a light intensity of approximately 200 µmol m^{-2} s^{-1}, until the appearance of bacterial leaf blight symptoms. Lesion lengths of the blighted areas were measured 14 days post Xoo inoculation, as the indication of Piericidin A1's protective effect.

Statistical analysis

Experiments involved the investigation of antagonistic activities and evaluation of virulence were performed in triple duplicates. One-way analysis of variance (ANOVA) was performed using JMP software version 5.0 (SAS Institute Inc., Cary, NC, USA). Significant effects of different treatments were determined by the F value ($P=0.05$). When the F test was significant, means were separated using Fisher's protected least significant difference (LSD) test at $P<0.05$.

Abbreviations

AIA	Actinomycetes isolation agar
AL	Aminolipid
ANI	Average nucleotide identity
BLB	Bacterial leaf blight
BWA	Burrow–wheeler aligner
CCTCC	China center for type culture collection
CE	Crude exacts
COSY	Correlation spectroscopy
DDBJ	DNA data bank of japan
dDDH	Digital DNA–DNA hybridization
DMSO	Dimethylsulfoxide
DPG	Diphosphatidylglycerol
DSF	Diffusible signal factor
E. coli	*Escherichia coli*
EC_{50}	Half maximal effective concentration
EMBL	European molecular biology laboratory
ESI	Electrospray ionization
FM	Fermentation medium
GBDP	Genome BLAST Distance Phylogeny
GL	Glycolipid
GPL	Glycophospholipid
HPLC	High-performance liquid chromatography
ISCC-NBS	Inter-society color council-national bureau of standards
ISP	International *Streptomyces* project
LBA	Luria bertani agar
MK	Menaquinone
NA	Nutrient agar
NADH	Nicotinamide adenine dinucleotide (NAD) + Hydrogen (H)
NB	Nutrient broth
NCBI	National center for biotechnology information
NMR	Nuclear magnetic resonance
OD	Optical density
PBS	Phosphate buffer solution
PCR	Transcription polymerase chain reaction
PE	Phosphatidylethanolamine
PGAP	Prokaryotic genome annotation pipeline
Pie A1	Piericidin A1
PKS	Polyketide synthase
PL	Phospholipid
RAST	Rapid annotation using subsystem technology
SA	*Streptomyces* Agar
SCA	Starch casein agar
SPAdes	St. Petersburg genome assembler
T3SS	Type III secretion system
TSA	Trypticase soy agar
TSB	Tryptic soy broth
TYGS	Type strain genome server
UPLC-Q-TOF-MS	Ultra-high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry
Xoo	*Xanthomonas oryzae* Pv. *Oryzae*

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-025-00313-9.

Additional file 1: Figure S1. Subsystem coverage and category distribution of *Streptomyces shaowuensis* strain HSW2009 retrieved from the RAST annotation server. **Figure S2.** The whole genome sequence tree constructed using the TYGS web server for *Streptomyces shaowuensis* strain HSW2009 and closely related type strains.

Additional file 2: Table S1. Pairwise dDDH values between genome of *Streptomyces shaowuensis* strain HSW2009 and the selected type strain genomes. **Table S2.** Colony morphology of *Streptomyces shaowuensis* strain HSW2009 on different culture media at 28^{0}C after 7–14 days of incubation. **Table S3.** Cellular fatty acid composition of Streptomyces shaowuensis strain HSW2009 and its closely related *Streptomyces* type strains.

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Author contributions
YH, LL, and LZ conceived the experimental design, interpreted the results, and wrote the manuscript. JWL, YJ, and KC participated in the interpretation of the results and writing of the manuscript. JWL, SMA, WH, LJ, and KS performed the experiments. All authors read and approved the final manuscript.

Availability of data and materials
The 16S rRNA sequencing data generated from this study have been deposited in GenBank/EMBL/DDBJ under the accession OR826630. The whole genome shotgun project of HSW2009 was deposited at DDBJ/EMBL/GenBank under the accession JBEHZD000000000.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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