Streptomyces Spinosus Sp. Nov. And Streptomyces Shenzenensis Subsp. Endophyticus Subsp. Nov. Endophytic Actinobacteria Isolated From Jasmine Rice and Their Genome Mining Correlate With Potential as Antibiotic Producers and Plant Growth Promoters

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Abstract

Two endophytic actinobacteria, strains SBTS01^T and W18L9^T, were isolated from leaf sheath tissue and leaf sample of Jasmine rice (*Oryza sativa* KDML 105), respectively, grown in a rice paddy field in Roiet Province, Thailand. The polyphasic study showed that both strains belonged to the genus *Streptomyces*; they are aerobe, with well-developed substrate mycelia and aerial mycelia to form long chains of spores. Strain SBTS01^T shared the highest 16S rRNA gene sequence similarity with *Streptomyces rochei* NRRL B-2410^T (99.0%) and *Streptomyces naganishii* NRRL ISP-5282^T (99.0%). Strain W18L9^T shared the highest 16S rRNA gene sequence similarity with *Streptomyces shenzhenensis* DSM 42034^T. The genotypic and phenotypic data of strains SBTS01^T and W18L9^T distinguished these two strains with the closely related species with valid names. The genome analysis showed the dDDH, ANIb and ANIm values of the draft genome between strain SBTS01^T and its related species; *S. rochei* NRRL B-2410^T (29.4, 81.1, 86.5%) and W18L9^T and its closest species; *S. shenzhenensis* DSM 42034^T (72.5, 95.1, 97.0%). The name proposed for the new species of type strain SBTS01^T is *Streptomyces spinosus* (=NRRL B-65636^T =TBRC 15052^T). The name proposed for the novel subspecies of strain W18L9^T is *Streptomyces shenzenensis* subsp. *endophyticus* (=NRRL B-65635^T =TBRC 15051^T). Both strains could produce antibiotics to inhibit pathogens and contained plant growth promoting (PGP) traits. Genome mining of these two strains revealed that they comprised many Biosynthesis Gene Clusters (BGCs); terpene, type 1, 2 and 3 polyketide synthase, Non-ribosomal peptide synthetase (NRPS), and lanthipeptide including genes encoding PGP traits; nitrogen fixation, ACC (1-aminocyclopropane-1-carboxylate) deaminase and siderophore production.

Introduction

The genus *Streptomyces* belongs to the family *Streptomycetaceae* which was proposed by Waksman and Henrici (1943). The genus contains more than 900 validly published species and 50 validly published subspecies (<https://lpsn.dsmz.de/genus/streptomyces>) (Parte et al. 2020). Most of them were isolated from soil as well as from various environments such as marine sediments, seawater, fresh water, caves, animals and plant tissues (Kämpfer 2012). *Streptomyces* spp. are well recognized as producers of bioactive compounds, especially antibiotics, as more than 70 percent of currently known antibiotics are produced by this genus. Also, there are many reports of its application as plant growth promoters by producing phytohormones, solubilize phosphate, inhibiting plant pathogens and inducing the plant immune system (Conn et al. 2008; Kämpfer 2012). There have been many studies of isolation and application of endophytes belonging to the genus *Streptomyces* as biocontrol agents and plant growth promoters (Barnett et al. 2019; Xu et al. 2019). Recently, genome mining is a high throughput method for discovering new drugs and searching for beneficial strains to use as plant growth promoters (Luo et al. 2014; Abdelmohsen et al. 2015). Also, many BGCs are known as “silent” or “cryptic” which may not express to produce bioactive compounds in a laboratory or require some inducers (van Bergeijk et al. 2020).

Jasmine rice (*Oryza sativa* KDML 105) or Khao Dawk Mali of Thailand containing aroma compound, 2-acetyl-1-pyrroline (2AP) has expanded wide popularity in Asia and the Middle East (Wakte et al. 2017). However, this rice variety is susceptible to blast causing by *Pyricularia oryzae* and bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (Kampapongsa and Kaewkla, 2016). Recently, there were some studies of endophytic actinobacteria from rice and their application to inhibit rice pathogens and promote rice growth (Naik et al. 2009; Rungin et al. 2012; Kampapongsa and Kaewkla 2016; Xu et al. 2019; Gao et al. 2021; Saikia and Bora, 2021). Currently, there are two valid species of the genus *Streptomyces* isolated from rice tissue; *Streptomyces oryza* (Mingma et al. 2015) and *Streptomyces roietensis* (Kaewkla and Franco, 2017).

In this study, the taxonomic position of endophytic actinobacteria isolated from Jasmine rice, strains SBTS01^T and W18L9^T were determined by using a polyphasic taxonomic approach, including morphological, physiological, chemotaxonomic, phylogenetic and genomic characteristics. Antibiotic production against rice and human pathogens and plant growth promoting studies were conducted. Genome data mining of these two strains was also reported and showed the correlation with these properties.

Materials And Methods

Isolation of strains SBTS01^T and W18L9^T

Strains SBTS01^T and W18L9^T were obtained from a project which focused on the biodiversity of endophytic actinobacteria from Jasmine rice plants and their application as plant growth promoters and biocontrol agents to inhibit plant pathogens. Jasmine rice (*Oryza sativa* KDML 105) was collected from the grounds of the Rice Research Centre paddy field, Roi Et province, Thailand (15.54937N 103.80975E) and surface sterilized by applying the previously described method (Kampapongsa and Kaewkla 2016). Surface-sterilized leaf, leaf sheath, stem and root tissue were placed onto three different media (Kampapongsa and Kaewkla 2016). Plates were kept in sealed plastic boxes, which were moistened with wet paper towels, and incubated at 27 °C for 12 weeks. Actinobacterial strains were purified by using half strength potato dextrose agar (HPDA) and stored on HPDA slants at 4 °C and in 20% glycerol at -80 °C.

Phylogenetic analysis of 16S rRNA gene

Genomic DNA of strains SBTS01^T and W18L9^T was extracted by using **GenElute™** (Sigma), and the 16S rRNA gene was amplified and sequenced as described previously (Kaewkla and Franco 2013). The 16S rRNA gene sequence of these two strains was examined by using EzbioCloud server (Yoon et al. 2017), and subsequently aligned with the 16S rRNA gene sequences (available from GenBank/EMBL) of the closely related members of the genus *Streptomyces* with validly published names, using CLUSTAL X (Thompson et al. 1997) with *Nocardia africana* DSM 44491^T as the out-group.

The phylogenetic trees were constructed by the neighbor-joining (NJ) and maximum parsimony (MP) algorithms using the software package *MEGA* version X (Kumar et al. 2018). The Jukes-Cantor method (Jukes and Cantor 1969) was applied to the analysis with the NJ algorithm (Saitou and Nei 1987). The MP tree was obtained using the min-mini heuristic algorithm with a search factor of 1 (Nei and Kumar 2000). The topology of the tree was evaluated by performing

a bootstrap analysis based on 1000 replications (Felsenstein 1985).

Sequencing, assembly, annotation

Genomic DNA of strain W18L9^T for whole genome sequencing was extracted by using GenElute™ (Sigma). For genome sequencing of this strain, library was prepared by using short insert size library and samples were sequenced by using Illumina HiSeq X-ten platform (Illumina) (2x150 bp paired-end reads) at the Beijing Genome Institute (BGI), Hongkong.

For whole genome sequencing of strain SBTS01^T and the type strain, *S. shenzhenensis* DSM 42031^T, genomic DNA was extracted according to the previously described protocol (Saito and Miura 1963). A library was prepared by using microbial short insert size library (PCR free library)

and samples were sequenced by using the Novaseq 6000 systems, HiSeq PE (Illumina) (2x150 bp paired-end reads) at the Singapore Joint Venture and Sequencing Center, Novogene AIT.

The reads were De novo assembled by using Unicycler (0.4.8) (Wick et al. 2017). The draft assemblies of genomes of strains SBTS01^T, W18L9^T and *S. shenzhenensis* DSM 42031^T have been submitted to GenBank as accession number JAINRC000000000, JACBWY000000000, and JAJONF000000000, respectively. Genomes of strains SBTS01^T and W18L9^T were annotated by using Prokka version 1.14.5 (Seemann 2014) and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al. 2016).

Genome comparison

The phylogenomic tree of strains SBTS01^T, W18L9^T and related taxa was constructed by using the Type (Strain) Genome Server (TYGS) (Meier-Kolthoff et al. 2013; Meier-Kolthoff and Göker 2019). The tree inferred with FastME 2.1.6.1 (Lefort et al. 2015) from Genome BLAST Distance Phylogeny (GBDP) distances calculated from genome sequences. The branch lengths were scaled in terms of GBDP distance formula *d*₄. Digital DNA-DNA hybridization (dDDH) values

between strains SBTS01^T and W18L9^T and their related type strains were calculated by using the Genome-to-Genome Distance calculator (GGDC 2.1; BLAST + method) applying formula 2 (identities/HSP length) (Meier-Kolthoff et al. 2013).

ANI-BLAST (ANiB) and ANI-MUMmer (ANIm) algorithms within the JspeciesWS web service were applied to calculate Average Nucleotide Identity values (ANI) between strain SBTS01^T and W18L9^T and its related species with pairwise genome alignment (Richter and Rosselló-Móra 2009; Richter et al. 2016).

Chemotaxonomic characterization

Chemotaxonomic characterization of strain SBTS01^T and W18L9^T was done according to the standard procedures. Whole-cell sugars were analyzed by the TLC method (Hasegawa et al. 1983) and diaminopimelic acid (DAP) was detected by Thin Layer Chromatography (TLC) (Bousfield et al. 1985). Extraction and purification of isoprenoid quinones were performed using the previously

described method (Minnikin et al. 1984). The purified menaquinones were analyzed by reverse phase LC-MS and electrospray mass spectrometry (ESI) (Shimadzu LCMS-8030). The column was InertSustain® C18 (2.1 × 150 mm, 3 µm), and the solvent system was isopropanol:methanol (1:1) at a flow rate of 0.4 mL/min (Kaewkla and Franco 2019). The polar lipid was analyzed as described previously by using 5% ethanolic molybdophosphoric acid, Dragendorff and molybdenum blue reagents, anisaldehyde, α-naphthol and ninhydrin sprays (Minnikin et al. 1984; Komagata and Suzuki 1987).

The fatty acid methyl esters (FAMES) of strains SBTS01^T and W18L9^T and their closest type strains were analyzed for FAMES under the same conditions by following the protocol described by Microbial Identification Inc. (MIDI) (Sasser 2001). The two closest type strains of strain SBTS01^T were *S. rochei* NRRL B-2410^T, and *S. naganishii* NRRL ISP-5282^T and the four closest types strains of strain W18L9^T were *S. shenzhenensis* DSM 42034^T, *S. graminisoli* NBRC 108883^T, *Streptomyces jiujiangensis* DSM 42115^T, and *Streptomyces hyaluromycini* DSM 100105^T. These type strains were obtained from ARS type culture collection, USA (NRRL strain), German Culture Collection (DSMZ), Germany (DSM strains) and Biological Resource Center, National Institute of Technology and Evaluation (NITE), Japan (NBRC strains). All these cultures were grown for 7 days at 27 °C in Tryptic Soya Broth (Oxoid) in an Erlenmeyer flask at 150 rpm and harvested by centrifugation. Washed cells were saponified, methylated and extracted for FAME analysis. The Sherlock software version 6.4 was used for analysis.

Phenotypic Characterization

Based on 16S rRNA gene similarity, the two closest type strains of strain SBTS01^T and four closest type strains of strain W18L9^T as stated above, were studied in a side-by-side comparison for phenotypic characterization. Morphological characteristics of strains SBTS01^T, W18L9^T and their closest type strains were studied on 8 different media: ISP 2, ISP 3, ISP 4, ISP 5, ISP 7

(ISP; International Streptomyces Project) (Shirling and Gottlieb 1966; Atlas and Parks 1993), Bennett's agar, HPDA and Nutrient agar (NA) (Atlas and Parks 1993). ISP 7 was used to test for melanin pigment production. Color determination was based on Methuen Handbook of Color (Kornerup and Wanscher 1978). Catalase production, assimilation of six organic acids and utilization of four phenolic compounds as sole carbon source, hydrolysis of skim milk and

starch were tested according to the previously described procedures (Kurup and Schmitt 1973). Decomposition of L-tyrosine, adenine, xanthine, urea, esculin, hippurate and acid production from eighteen carbohydrates were carried out according to the previously described methods (Gordon et al. 1974). Growth at different NaCl concentrations (1, 3, 5, 10, 15 and 20 %, w/v), temperatures (4, 15, 27, 37, 45 and 55°C), and pH between 4 and 10 (in 1 pH unit intervals and pH was adjusted aseptically after autoclaving) were evaluated after incubation for 14 days on ISP 2 medium (Kurup and Schmitt 1973).

Scanning Electron Microscopy (SEM)

Strains SBTS01^T and W18L9^T were cultured on HPDA at 27 °C for 7 days and cells and spores were visualized by Scanning Electron Microscopy (The Carl Zeiss version AURIGA) at the Center for Scientific Equipment and Technological Equipment, Suranaree University of Technology, Thailand. The sample preparation was carried out according to a previously described protocol (Kaewkla and Franco 2019).

In vitro antibacterial and antifungal activity

The bacteria used for the antibacterial bioassay were a rice pathogen; *Xanthomonas oryzae* pv. *oryzae* (Xoo) PXO 86, human pathogens; *Staphylococcus aureus* ATCC 29213 and Methicillin Resistant *Staphylococcus aureus* (MRSA) 03120385. The bacterial assay was modified from the dual culture method (Williston et al. 1947; Kaewkla and Franco et al. 2021). There were four fungal pathogens; *Curvularia lunata* BCC 15558, *Helminthosporium oryzae* DOAC 1570, *Pyricularia grisea* BRIP 61689 and *Rhizoctonia solani* AG8. Xoo PXO 86 and *R. solani* AG8 were obtained from Department of Medical Biotechnology, Flinders university. *S. aureus* ATCC 29213 and MRSA 03120385 were obtained from the Clinical Microbiology Department, the Flinders University, South Australia. *P. grisea* BRIP 61689 were obtained from Plant Pathology

Herbarium, Department of Agriculture and Forestry, Queensland, Australia. Strain DOAC 1570 and BCC 15558 were obtained from the Plant Protection Research and Development office, Thailand and the Thailand Biological Resource Center (TBRC), respectively. The fungal assay was carried out according to the dual culture method previously described protocol (Kampapongsa and Kaewkla 2016).

Antibiotics production assay

Strain SBTS01^T and W18L9^T were tested for antibiotics production by agar diffusion assay. Both strains were cultured on ISP 2 agar and HPDA for 7 days and whole agar plate was cut to small pieces and extracted with 100% methanol and shaken for three hours. The methanol extract was evaporated by using an evaporator. Then, extracted compound was freeze dried by using a lyophilizer. The dried samples were dissolved with 70% methanol and tested for antibiotics production against *S. aureus* ATCC 29213, MRSA 03120385 and Xoo PXO 86 according to the agar diffusion assay described by Kaewkla and Franco (2021).

For antifungal assay against *P. grisea* BRIP 61689, a fungi was grown on Potato Dextrose agar (PDA, Oxoid) at pH 6.0 for 7 days. A plug was cut at the edge of the radial growth of fungi by using a 6 mm cork borer and placed at the centre of a new PDA plate at pH 6.0 and incubated at 27 °C for 4 days or until the diameter of the fungi was about 2.5 cm. Then, six wells were made by using a 6 mm cork borer with the centre of the wells approximately 1.5 cm away from the edge of the PDA plate. Fifty microliters of each extracted compound were put into the wells. Each sample was repeated in triplicate. Methanol was used as a negative control. Also, plate of fungi without making wells was used as a negative control. The growth of fungi was measured toward the direction of each well in 4 days after the test.

Indole acetic acid (IAA) production

The production of IAA was studied according to the method of Khamna et al. (2009) with some modifications. One actinobacterium disc (8 mm) of 7-day growth on HPDA was inoculated into ISP 2 broth supplemented with 0.2% (w/v) L-tryptophan, on a rotary shaker at 150 rpm for 7 days in the dark at 28 °C. The cultures were centrifuged at 6000 g for 10 min. The supernatant was analyzed for IAA production according to the method of Glickmann and Dessaux (1995) by using Salkowski's reagent.

Cytokinin production

The cytokinin production of strain SBTS01^T and W18L9^T was carried out according to the previously described method (Hussain and Husnain 2009). One actinobacterium disc (8 mm) was inoculated into Yeast malt extract casamino acid broth (Malt extract 10 g, Yeast extract 1 g, Glucose 4 g, casamino acid 3 g, Na₂HPO₄ 0.5 g, DO water 1 L) on a rotary shaker at 150 rpm for 7 days in the dark at 28 °C. The cultures were centrifuged at 10000 g for 10 min. The supernatant was analyzed for cytokinins by using reverse phase HPLC (Prontosil, Hypersorb ODS 5.0 µm, photodiode array; 265 nm), eluted with a flow rate of 1 ml/min with gradient elution of acetonitrile and 0.1 M acetic acid in 5% acetonitrile, at the Central Laboratory, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand. Total cytokinins were analyzed as free cytokinins; zeatin, zeatin riboside, *N*⁶ (D²-isopentenyl) adenine and *N*⁶ (D²-isopentenyl) adenosine.

Siderophore production

Siderophore production of strain SBTS01^T and W18L9^T was tested according to the previously described method (Pérez-Miranda et al. 2007). Briefly, actinobacterium was streaked on HPDA and grown for 7 days. Chrome azurol S (CAS) medium added agarose

as a solidified agent was poured at the top of the HPDA plate and left for 15 min. The positive result with changing from blue to purple indicated catechol type or from blue to orange indicated hydroxamates type.

Phosphate solubilization

The ability of strain SBTS01^T and W18L9^T for phosphate solubilization was tested by applying the standard method (Castagno et al. 2011). NBRIP agar was used and a positive result was observed by measuring the halo zone around colonies of these two strains after incubation for 14 days.

1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production

The ability of strains SBTS01^T and W18L9^T to produce ACC deaminase was tested by using the DF agar (Penrose and Glick 2003). Growth on DF medium added ACC as nitrogen source was compared to growth on DF medium with and without nitrogen source as negative and positive controls, respectively.

Nitrogen fixation assay

The method of nitrogen fixation of strains SBTS01^T and W18L9^T *in vitro* was applied by the following methods (Baldani et al. 1997). Nitrogen-free semi solid (NFB) agar was used and a positive result was detected by the color of the indicator changing from light blue to dark blue.

Hydrogen cyanide production

The method of hydrogen cyanide production of two strains was applied by the following procedure (Bakker and Schippers, 1987).

Cellulase production

Strains SBTS01^T and W18L9^T were tested for cellulase production on carboxymethyl cellulose (CMC) agar plates and the positive result was detected by a congo red method according to the previously described protocol (Islam and Roy 2018).

Secondary metabolite and biosynthesis gene cluster prediction

Secondary metabolite Analysis Shell (anti-SMASH) version 6.0 (Blin et al. 2021) was used to predict biosynthetic gene clusters (BGCs) for secondary metabolite synthesis of strains SBTS01^T and W18L9^T. The genomes of strains SBTS01^T and W18L9^T were examined by *in silico* approach to search genes encoding metabolite products relating to various properties: bioactive compound production, plant growth promoting traits, degradable enzymes and bioremediation. The closest similarity of microorganisms was acquired by applying blastp on the UniProt database with matrix; blosum62 (The UniProt 2021).

Results & Discussion

Isolation of strains SBTS01^T and W18L9^T

Strain SBTS01^T was isolated from Jasmine rice (*Oryza sativa* KDML 105) and arose as a dark green colony on leaf sheath tissue after incubation for nine weeks on Humic acid vitamin B agar (HVA) (Hayakawa and Nonomura 1987). Strain W18L9^T was also isolated from Jasmine rice and emerged as a white colony on leaf tissue after incubation for two weeks on Tap Water Yeast extract agar (TWYA) (Coombs and Franco 2003).

Phylogenetic analysis of the 16S rRNA gene

The phylogenetic evaluation of strains SBTS01^T and W18L9^T with the closely related members of the genus *Streptomyces* showed that both strains belonged to genus *Streptomyces* and were encompassed by other members of this genus (Fig 1 and Fig S1). Strain SBTS01^T shared 16S rRNA gene similarity at 98.6% with strain W18L9^T. Both strains were placed in different clusters in both trees. Based on these results, both strains were considered as different species.

The closest type strains which shared the highest 16S rRNA gene sequence similarity with strain SBTS01^T were *S. rochei* NRRL B-2410^T and *S. naganishii* NBRC 12892^T, both at 99.0%. However, strain SBTS01^T formed a different cluster with these type strains and formed a single phylogenetic lineage in both trees (Fig 1 and Fig S1). The type strains which shared the highest 16S rRNA gene sequence similarity with strain W18L9^T were *S. shenzhenensis* DSM 42034^T, *S. graminisoli* NBRC 108883^T, *S. jiujiangensis* DSM 42115^T, and *S. hyaluromycini* DSM 100105^T at 99.7, 99.2, 98.9, and 98.9%, respectively. The closest neighbor of strain W18L9^T positioning in the same clade was *S. shenzhenensis* DSM 42034^T in both trees with high bootstrap support at 81 and 67, respectively (Fig. 1 and Fig. S1).

Based on the percentage of 16S rRNA gene similarity and phylogenetic position of strains SBTS01^T and W18L9^T, two closely related type strains of strain SBTS01^T and four closely related type strains of strain W18L9^T as mentioned above were selected for a side-by-side comparison study.

Genome analysis

Genome comparison between strains SBTS01^T and W18L9^T

The phylogenetic tree based on TYGS revealed that strain SBTS01^T was placed in the different species cluster with strain W18L9^T (Fig S2). The genome analysis showed the dDDH, ANIb and ANIm values of the draft genome between strain SBTS01^T and strain W18L9^T being 28.3%, 81.6% and 87.1%, respectively which were well below the threshold for species delineation (Meier-Kolthoff et al. 2013; Chun et al. 2018). Based on a genome comparison study, both strains were in different species. Genome features of strains SBTS01^T, W18L9^T and their closest type strains were shown on Table S1.

Genome comparison of strain SBTS01^T

The draft genome sequence of strain SBTS01^T was 9.17 Mb with DNA G+C content determined by in *silico* genome sequence as 72.5 mol%. The phylogenetic tree based on TYGS revealed the relationship between strain SBTS01^T and the related type strains. The phylogenomic tree showed that strain SBTS01^T was placed in the different species cluster from all related type strains (Fig. S2). Strain SBTS01^T formed a single phylogenetic cluster with the closest neighbour, *Streptomyces corchorusii* DSM 40340^T, while it positioned away from the closest type strains, *S. rochei* NRRL B-2410^T and *S. naganishii* NBRC 12892^T.

The genome analysis showed the dDDH, ANIb and ANIm values between strain SBTS01^T and two related species which shared 16S rRNA gene similarity at 99.0%; *S. rochei* NRRL B-2410^T were 29.4, 81.1, 86.5%, and *S. naganishii* NBRC 12892^T were 26.9, 82.1, 86.9%, respectively. Strain SBTS01^T and its closest neighbour on the phylogenomic tree; *S. corchorusii* DSM 40340^T had the highest dDDH, ANIb and ANIm values at 54.1, 92.6, 94.3%, respectively. However, strain SBTS01^T and *S. corchorusii* DSM 40340^T shared a 16S rRNA gene similarity of only 97.0%. Based on the previous report, the cutoff value at the species level was evaluated at 98.7% (Stackebrandt and Ebers 2006). Therefore, strain SBTS01^T and this type strain belonged to different species. Hence, there is no requirement to compare physiological and biochemical characteristics between strain SBTS01^T and this type strain. According to Richter and Rosselló-Móra (2019), the species delineation should have ANI values cut off lower than 95-96% and dDDH values were lower than the threshold of 70% used to define species level (Meier-Kolthoff et al. 2013; Chun et al. 2018). Based on the genome comparison, strain SBTS01^T is a novel species of the genus *Streptomyces*.

Genome comparison of strain W18L9^T

The draft genome sequence of strain W18L9^T was 10.13 Mb with DNA G+C content determined by in *silico* genome sequence as 70.9 mol%. The phylogenetic tree based on TYGS revealed that strain W18L9^T was placed in the same species cluster as its closest neighbour, *S. shenzhenensis* DSM 42034^T but in a different subspecies cluster (Fig. S2). The genome analysis showed the dDDH, ANIb and ANIm values of the draft genome between strain W18L9^T and its related species, *S. shenzhenensis* DSM 42034^T being 72.5, 95.1, 97.0% and *S. hyaluromycini* DSM 100105^T being 44.1, 88.8, 92.4%, respectively. The ANIb, ANIm and dDDH between strain W18L9^T and the closest type strain *S. shenzhenensis* DSM 42034^T were higher than 95-96% for ANI values (Richter and Rosselló-Móra 2009) and dDDH value was higher than the threshold of 70% used to define species delineation (Meier-Kolthoff

et al. 2013; Chun et al. 2018). Based on these data, strain W18L9^T belonged to the same species of *S. shenzhenensis* DSM 42034^T. According to Nouioui et al. (2018), dDDH values between 70 and 79% defined subspecies delineation. Based on the dDDH value between strain W18L9^T and *S. shenzhenensis* DSM 42034^T at 72.5% and the position at different subspecies clusters on the phylogenomic tree, strain W18L9^T belonged to the same species of *S. shenzhenensis* DSM 42034^T but it was defined as a novel subspecies.

Chemotaxonomic characteristics

The *LL*-isomer of DAP was detected in both strains SBTS01^T and W18L9^T. The whole-cell sugars of strain SBTS01^T contained galactose, glucose and mannose and strain W18L9^T contained galactose, glucose, mannose and xylose. The polar lipids of strain SBTS01^T contained phosphatidylethanolamine (PE), phosphatidylinositol (PI), three aminoglycolipids and one glycolipid and strain W18L9^T contained phosphatidylethanolamine (PE), phosphatidylinositol (PI), six glycolipids, one aminolipid, and four unknown lipids which correspond to phospholipid type II (Lechevalier et al. 1977) (Fig. S3).

The major menaquinones of strains SBTS01^T and W18L9^T were MK-9(H₆) and MK-9(H₈). The major cellular fatty acids (more than 10%) of strain SBTS01^T were identified as *anteiso*-C_{15:0} (26.6%), *iso*-C_{16:0} (19.1%) and *anteiso*-C_{17:0} (19.1%) which were the same pattern with the closest type strains; *S. rochei* NRRL B-2410^T (Table 1). The major fatty acids of strain W18L9^T (more than 10%) were *anteiso*-C_{15:0} (21.8%), *anteiso*-C_{17:0} (20.9%) and *iso*-C_{16:0} (18.5%)

and which were the same pattern with the closest type strain; *S. shenzhenensis* DSM 42034^T (Table S2).

Phenotypic and physiological characterization

Strain SBTS01^T

The colony morphology of strain SBTS01^T is described in Table S3. Strain SBTS01^T showed morphological characteristics with well-developed substrate mycelium and aerial mycelia on most media used. Melanin pigment was produced on ISP 7. Electron micrographs showed that strain SBTS01^T formed round rod-shaped spores with spiny surface and spore chains in loop forms (approximately 1 micron length x 1 micron diameter) (Fig. 2A). The physiological properties of strain SBTS01^T and its closest type strain; *S. rochei* NRRL B-2410^T were different. Strain SBTS01^T could assimilate benzoate and tartrate and grow at NaCl 5% (w/v) but the closest type strain could not assimilate these compounds and could grow weakly at this salt concentration. Also, strain SBTS01^T could grow weakly at 45 °C and produce melanin pigment on ISP 7 but the type strain could not. On the other hand, the type strain could hydrolyze L-tyrosine and xanthine but strain SBTS01^T could not (Table 1).

Strain W18L9^T

The colony morphology of strain W18L9^T is described in Table S3. Strain W18L9^T showed morphological characteristics with well-developed substrate mycelia and aerial mycelia on most media used. No melanin pigment was observed on ISP 7. Electron micrographs showed that strain W18L9^T formed oval rod-shaped spores (approximately 1.5 micron length x 1 microns diameter) with warty surface and formed spore chains in loops (Fig. 2B). The physiological properties of strain W18L9^T and its closest type strain, *S. shenzhenensis* DSM 42034^T were different. Strain W18L9^T could assimilate maltose, hydrolyze urea, assimilate tartrate and could grow at 15 °C and pH 10 but the type strain could not. On the other hand, the type strain could hydrolyze hippurate and grow weakly at 5% NaCl (w/v) but strain W18L9^T could not (Table 2).

Based on the results of polyphasic study, strain SBTS01^T is proposed as a representative of a novel species of the genus *Streptomyces* named *Streptomyces spinosus* sp. nov. and strain W18L9^T is proposed as a novel subspecies of *Streptomyces shenzhenensis* DSM 42034^T named *Streptomyces shenzhenensis* subsp. *endophyticus* subsp. nov.

In vitro antibacterial and antifungal activities

Strains SBTS01^T and W18L9^T showed strong and moderate activity against a bacterial rice pathogen; *Xoo* PXO 86, respectively. Strains SBTS01^T and W18L9^T showed moderate and strong activity against *P. grisea* BRIP 61689, respectively. These results were obtained by the dual culture method. Both strains showed strong activity against *S. aureus* ATCC 29213 and MRSA 03120385 on both dual culture and agar extraction methods. The antifungal assay against other fungal pathogens was tested by using only a dual culture method. Strain SBTS01^T showed weak activity against *R. solani* AG8 while strain W18L9^T had strong activity against this fungus. Both strains showed moderate activity against a rice fungus; *C. lunata* BCC 15558. Strains SBTS01^T and W18L9^T showed moderate and weak activity against *H. oryzae* DOAC 1570, respectively (Table 3). HPDA was the best medium for antimicrobial activity of both strains tested by dual culture and agar extraction methods. HPDA and ISP 2 contained 10 and 4 g of dextrose, respectively. Then, it was not an effect of carbon catabolite regulation (CCR) which is a regulatory mechanism that limits production due to a negative effect applied by high concentrations of dextrose (Sanchez and Demain 2002). ISP 2 contained malt extract and yeast extract at 10 and 4 g/L while HPDA comprised potato infusion. It was reported that some nitrogen sources may decrease antibiotic production in different microorganisms including *Streptomyces* (Rafieenia 2013). Based on the result of antibiotic production, it is necessary to select at least two media with different compositions to screen for antimicrobial activity.

Plant growth promoting traits

Only strain W18L9^T could grow on DF agar with ACC deaminase as a nitrogen source, fix nitrogen on NFB medium, solubilize inorganic phosphate and produce cellulase enzyme (Table 4). Strain SBTS01^T had negative results for these tests. Both strains could not produce HCN but they could produce siderophore (hydroxamate type). Strains SBTS01^T and W18L9^T produced IAA at 71 and 150 ug/mL and produced cytokinins at 0.165 and 0.169 mg/L, respectively (Table 4).

Secondary metabolites and biosynthesis gene cluster prediction

There were many BGCs of strains SBTS01^T and W18L9^T derived from “antiSMASH” database. There were six groups of metabolites; terpene, type 1 polyketide synthase (T1PKS), type2 PKS (T2PKS), type3 PKS (T3PKS), Non-ribosomal peptide synthetase cluster (NRPS), lanthipeptide cluster and other compounds (Table 5). Both strains contained terpene clusters; geosmin and albaflavenone (100% similarity). Geosmin is recognized as an earthy odor that was generally produced by many genera of actinobacteria including *Streptomyces* species (Jiang et al. 2007). Albaflavenone, a tricyclic sesquiterpene antibiotic, which was produced by many *Streptomyces* sp. possessed an antibacterial activity (Moody et al. 2011). Also, strains SBTS01^T and W18L9^T contained gene clusters encoding hopene at 76 and 96% similarity, respectively. Hopene is a precursor for producing bioactive hopanoids, a group of pentacyclic triterpenoids such as diploterol which is toxic to mouse leukemia cells (Schmerk et al. 2015; Sohlenkamp and Geiger 2016). Strain SBTS01^T contained isorenieratene (62% similarity) which is a carotenoid light- harvesting pigment produced by green sulfur bacteria; *Chlorobium* spp. There were some reports of isorenieratene biosynthesis by actinobacteria but the function of this compound is still unknown and it may relate to blue or UV light resistance (Maresca et al. 2008). Strain SBTS01^T comprised T1PKS cluster; sceliphrolactam and antimycin (92 and 93% similarity). Sceliphrolactam, a macrocyclic lactam exhibited antifungal activity against amphotericin B-resistant *Candida albicans* (Oh et al. 2011). *Antimycin is produced by many strains of Streptomyces sp.* and it was reported that the antimycin A family possessed effective antifungal, insecticidal, nematocidal and anticancer activities (Ondrejčková et al. 2016). Also, Nakayama et al. (1956) reported the activity of this compound against rice blast fungus which correlated to this study in which strain SBTS01^T showed moderate activity against this pathogen in vitro. Moreover, strain SBTS01^T contains T2PKS cluster; lugdunomycin (40% similarity). Lugdunomycin is an angucycline-derived molecule containing a heptacyclic ring system and represents a novel subclass of aromatic polyketides. It was reported that this compound had antimicrobial activity against the Gram-positive *Bacillus subtilis* 168 (Wu et al. 2019). As strain SBTS01^T had low similarity at 40% with lugdunomycin gene cluster, it has a possibility to produce a novel compound of aromatic polyketides. Furthermore, strain SBTS01^T contained a RiPP: Lanthipeptide cluster;

informatipeptin (57% similarity). It was stated that microorganisms containing a RiPP: Lanthipeptide clusters are likely to produce novel bioactive compounds (Repka et al. 2017). Strain SBTS01^T comprised NRPS, ladderane cluster; ishigamide (100% similarity), a polyene-containing amide (Du et al. 2016). Furthermore, strain SBTS01^T comprised gene cluster, alkaloid; anisomycin (61% similarity), a pyrrolidine antibiotic containing a variety of activities. It was reported that anisomycin can block peptide bond

formation and showed potential against pathogenic protozoa and fungi. This compound can induce cell cycle apoptosis and activate the c-Jun N-terminal kinases (JNKs) signal pathway to exhibit potential antitumor activity (Shen et al. 2019).

In correlation with the genome data mining, strain SBTS01^T also showed good activity against *Xoo* PXO 86, *S. aureus* ATCC 29213 and MRSA 03120385 in which this strain may be likely to produce novel antibiotics. Finally, strain SBTS01^T contained a gene cluster, melanin (71% similarity). Melanin pigment produced from microorganisms could help to resist chemicals and oxidizing agents, thermal, and biochemical stresses (El-Naggar and El-Ewasy, 2017). This gene finding correlated with in vitro study in which strain SBTS01^T produced melanin pigment on ISP 7. In addition, this strain contained a gene cluster encoding spore pigment which benefits bacteria to tolerate UV and other environmental stresses (El-Naggar and El-Ewasy, 2017). As strain SBTS01^T was isolated from a rice paddy field which applied many pesticides such as fungicide, herbicide, and insecticide, this strain tended to adapt for resisting to survive from chemicals (personal communication with Dr. Ronnachai Changsri, a researcher at rice department, Thailand).

Strain W18L9^T comprised a variety of BGCs. This strain contained T1PKS clusters; rhizomide A, B and C (100% similarity). Rhizomide A, B and C showed weak antitumor activity against several human tumor cells lines (IC₅₀ 34 µM to 96 µM). It was reported that rhizomide A showed protective activity against cucumber downy mildew (*Pseudoperonospora cubensis*), *Staphylococcus aureus* and *Bacillus subtilis* (Wang et al. 2018). Strain W18L9^T contained T2PKS cluster; curamycin (57% similarity), a polyketide antibiotic produced by *Streptomyces curaco* which could inhibit *S. aureus* (Galmarini and Deulofeu 1961). Strain W18L9^T contained T3PKS clusters; germicidin and feglymycin (100 and 47% similarity). Germicidin was the auto regulative inhibitor of spore germination in the genus *Streptomyces* (Petersen et al. 1993).

Feglymycin, a novel peptide antibiotics was reported to inhibit the replication of the human immunodeficiency virus (HIV) in vitro (Vértesy et al. 1999). Furthermore, strain W18L9^T comprised NRPS/NRPS like clusters; xenotetrapeptide, tolaasin I / tolaasin F (100 and 50% similarity). The novel xenotetrapeptides were nonribosomally synthesized peptides which have been still unknown function produced from *Xenorhabdus nematophila*, bacteria reside as endosymbionts in the foreguts of soil nematodes in genus *Steinernema* (Khandelwal and Banerjee-Bhatnagar 2003). In correlation with the genome data mining, strain W18L9^T showed good activity against *S. aureus* ATCC 29213, MRSA 03120385, *P. grisea* BRIP 61689 and *R. solani* AG8 in vitro. The properties of strain W18L9^T to inhibit rice pathogens are correspondent with its ecophysiology inside rice tissue which benefits plant to inhibit pathogens.

In addition, the result showed that both strains contained gene cluster to synthesize siderophore, desferrioxamine E which plays an important role in the growth and development of *Streptomyces* (Codd et al. 2018). It was correspondent with the result of PGP traits of both strains which could produce siderophore (hydroxymate type) in vitro.

***In silico* gene prediction**

Other genes of strains SBTS01^T and W18L9^T encoding other bioactive compounds relating to plant growth promoting, degradable enzymes and bioremediation were scanned. The result showed that these strains contained a variety of genes encoding drought tolerant and stress response proteins and iron uptake protein relating to plant growth promoting properties (Table S4 and S5). For drought and salt tolerant proteins, both strains contained genes encoding ectoine production, glycine betaine/L-proline transporter, glycine, betaine and proline production, Ca²⁺: H⁺ antiporter and chloride channel protein. Only strain SBTS01^T contained sodium solute symporter and potassium channel family protein which can support plants to tolerate salt stress (Horn et al. 2006).

Only strains W18L9^T contained the gene encoding ACC deaminase which supports plants in stress condition such as drought, flooding, nutrient starvation, temperature, salt and oxidative stresses including toxic agents (Gupta and Pandey 2019). Strain W18L9^T contained genes relating to nitrogen fixation; ADP-ribosyl-dinitrogen reductase. These genes correlated with the results in which this strain could produce ACC deaminase and had ability to fix nitrogen in vitro. Also, the gene encoding indole-3-glycerol phosphate synthase relating to indole acetic acid (IAA) production was detected in the genome of strain SBTS01^T. IAA is a derivative of a plant hormone, auxin which promotes plant growth and support plant growth in salt stress condition (Keswani et al. 2020). In correlation with this gene finding, strain SBTS01^T and W18L9 were able to produce IAA in vitro. Furthermore, there were

many genes encoding universal stress proteins (*usp*) which were detected in both genomes. It was reported that bacteria containing *usp* genes play a role in maintaining internal and external protection mechanisms in biotic and abiotic stresses (Kim et al. 2012). Strain W18L9^T and strain SBTS01^T comprised genes encoding exopolyphosphatase and inorganic pyrophosphatase while strain SBTS01^T also contained phosphatase and alkaline phosphatase. However, only strain W18L9^T showed an ability to solubilize inorganic phosphate *in vitro*. From this result, it can conclude that strain SBTS01^T contained cryptic genes which required gene induction to express phosphate solubilization. The rice paddy field which plant source collected is in Suvannabhum district, Roiet province. This area is known as Thug Gura Ronghai which contains poor soil, fine-loamy soil type and moderate salt (EC 4-8 dS/m). The subsoil contains a lot of rock salt and potash (Sahunalu 2003). In correlation with the ecophysiology, strains SBTS01^T and W18L9^T contained genes encoding plant growth promoting traits to promote rice growth in poor soil and salinity condition.

Strain SBTS01^T and W18L9^T comprised many genes encoding beneficial enzymes which can be applied in a variety of industries. There were several types of enzymes; alpha-N-arabinofuranosidase, amylase, β -glucosidase, β -xylosidase, and β -xylanase which could degrade hemicellulose, polysaccharide, or plant residue to obtain fermentable sugars for applying in bioethanol production (Podkaminer et al. 2012). Genes encoding chitinase were also detected in both strains. It was reported that bacteria producing chitinolytic enzymes could inhibit fungal pathogens by degrading fungal cell walls and degrading waste containing chitin (Brzezinska et al. 2014). Also, these two strains contained genes encoding lipase and various types of proteases which can be applied in many industries such as detergent, food and feed additive, leather and textile industries.

Genes encoding proteins in bioremediation were also mined. Strain W18L9^T contained five genes encoding xenobiotic degradable enzymes; alkanesulfonate monooxygenase, nitroalkane oxidase, nitroreductase, epoxide hydrolase and phenol 2-monooxygenase. Alkanesulfonate monooxygenase participates in alkanesulfonate degradation. Nitroalkane oxidase relates to nitroalkane degradation in which nitroalkane compounds are used as solvents and explosive compounds and as herbicide and pesticide agents in agriculture and 2-nitropropane was reported as a carcinogen (Li et al. 2008). Nitroreductase can reduce pollutants such as 2,4-dinitrotoluene and 2,4,6-trinitrotoluene, as well as key chemical intermediates, 3-nitrophthalimide, 4-nitrophthalimide and 4-nitro-1,8-naphthalic anhydride. Also, strain W18L9^T contained gene encoding tellurium resistance protein. Gene encoding a latex clearing protein was detected in this strain which can apply to degrade waste containing rubber such as used gloves and tires. This will be valuable to degrade xenobiotic waste in the environment. As stated above, the rice paddy field was contaminated with many pesticides applied in the area for many years. Strain W18L9^T may adapt to survive in the environment contaminated with these compounds and contains genes encoding enzymes for degrading pesticides.

Strain SBTS01^T contained genes encoding aldehyde dehydrogenase family protein, 2-nitropropane dioxygenase and haloacid dehalogenase type II. This strain also contained genes encoding ion detoxification protein; selenium-binding protein, tellurium resistance protein,

and arsenate reduction. Gene encoding mycoredoxin which plays an important role in arsenic resistance was also detected in the genome of strain SBTS01^T (Mourenza et al. 2019).

For anticancer compound, strain W18L9^T comprised gene encoding immune checkpoint inhibitors (ICIs) which are monoclonal antibodies that block inhibitors of T-cell activation and function. The ICIs have potential effects on more than 14 different cancers which were reported to have effects in some patients for more than 5 years (20% - 40%; 5-year survival rate in patients with melanoma) (Johnson et al. 2018). Based on the genome data mining, strain W18L9^T has the potential to produce anti-HIV and anticancer compounds which will be fruitful to study this strain in-depth for novel bioactive compounds in the future.

Conclusion

In conclusion, strains SBTS01^T and W18L9^T can be differentiated from other species of the genus *Streptomyces* by ANI and dDDH values, a phylogenetic tree of the genome, and their phenotypic and chemotaxonomic features. The name, *Streptomyces spinosus* is proposed for this novel species and *Streptomyces shenzhenensis* subsp. *endophyticus* is proposed for this novel subspecies. These strains could produce antibiotics to inhibit bacterial pathogens, *X. oryzae* pv. *oryzae* PXO 86, *S. aureus* ATCC 29213, MRSA

03120385 and a fungal pathogen, *P. grisea* BRIP 61689. Strain W18L9^T could solubilize inorganic phosphate, produce ACC deaminase, produce cellulase enzyme and fix nitrogen. Both strains could produce siderophore, IAA and cytokinin. Genome mining revealed that these strains have the potential to produce novel metabolites. The genome data mining of these strains comprised several genes relating to plant growth promotion under stress conditions. Genome mining correlated with properties of these strains in vitro. Also, both strains contained versatile genes encoding beneficial enzymes and xenobiotic degradation which can be used in many industries and remedy polluted environments for further study.

Description of *Streptomyces spinosus* sp. nov.

Streptomyces spinosus (spi.no'sus. L. masc. adj. *spinosus*, spiny, referring to the spiny spore surface).

Gram stain-positive, aerobic, catalase positive. Cells grow between 15 and 45°C but grow well at 27 °C. Cells grow between pH 5.0 and 10.0 but grow well between pH 7.0 and pH 8.0. Cells cannot grow in the presence of 10% (w/v) NaCl. Substrate mycelium develops well on all the media used with light brown color and produces dark green spore on all media used. This strain produces melanin pigments on ISP 7. The mycelium is extensively branched and forms loop spore chains. A rod-shaped spore (1 µm diameter x 1 µm length) is observed with a spiny surface. Strain SBTS01^T produces acid from cellobiose, fructose, galactose, glucose, maltose, mannose, *myo*-inositol, mannitol, rhamnose, ribose, trehalose, xylose, arabinose, salicin, raffinose and sucrose but not from dulcitol and sorbitol. This strain can hydrolyze esculin, urea, adenine, starch, skim milk but not hippurate, *L*-tyrosine and xanthine. Strain SBTS01^T cannot use pyridine, toluene, phenol and benzene as sole carbon sources. This strain can assimilate acetate, benzoate, malate, tartrate, citrate and propionate. Whole-cells contain *LL*-diaminopimelic acid in its peptidoglycan and galactose, glucose and mannose as whole-cell sugars. Polar lipids are phosphatidylethanolamine (PE) and phosphatidylinositol (PI). MK-9(H₆) and MK-9(H₈) are predominant menaquinones. Major cellular fatty acids are *anteiso*-C_{15:0}, *iso*-C_{16:0} and *anteiso*-C_{17:0}. The DNA G+C content of the type strain is 72.5%.

The type strain, SBTS01^T (=NRRL B-65636^T =TBRC 15052^T), is an endophytic actinobacterium isolated from leaf sheath tissue of Jasmin rice (*Oryza sativa* KDML 105) grown at rice paddy field, Roiet province, Thailand. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the whole genome shotgun project of strain SBTS01^T are MZ901362 and JAINRC000000000, respectively. Strain SBTS01^T can strongly inhibit *Xanthomonas oryzae* pv. *oryzae* PXO 86, *Staphylococcus aureus* ATCC 29213 and Methicillin Resistant *Staphylococcus aureus* 03120385 in vitro.

Description of *Streptomyces shenzhenensis* subsp. shenzhenensis

Streptomyces shenzhenensis subsp. shenzhenensis (**shen.zhen.en'sis. N.L. masc. adj. shenzhenensis, pertaining to Shenzhen, China, from where the strain was isolated**)

The description is as given for *Streptomyces shenzhenensis* (HU et al. 2011) with the following modification. The G+C content of the type-strain genome determined by in silico approach is 71.4%, its approximate size 10.64 Mbp, its GenBank deposit JAJONF000000000. Hu et al. (2011) deposited 16S rRNA gene sequence of this strain as GenBank accession number HQ660226. The type strain is =CCTCC AA2011001^T =DSM 42034^T. This strain was isolated from a composite mangrove sediment sample collected in Shenzhen, China.

Description of *Streptomyces shenzhenensis* subsp. endophyticus subsp. nov.

Streptomyces shenzhenensis subsp. endophyticus (*en.do.phy'ti.cus*. Gr. pref. *endo*, within; Gr. neut. n. phyton, plant; L. fem. suff. -*ica*, adjectival suffix used with the sense of belonging to; N.L. masc. adj. *endophyticus* within plant, endophytic, pertaining to isolation of the type strain from plant tissues).

Cells grow between 15 and 37°C but grow well at 27 °C. Cells grow between pH 5.0 and 10.0 but grow well between pH 7 and pH 8.0. Cells cannot grow in the presence of 5% (w/v) NaCl. Substrate mycelium develops well on all the media used with yellow color and cells produce light gray spores on all media used. This strain cannot produce melanin pigments on ISP 7. The mycelium is extensively branched and forms loop spore chains. A rod-shaped spore (1 µm diameter x 1.5 µm length) is observed with a warty surface. Strain W18L9^T produces acid from cellobiose, fructose, galactose, glucose, mannose, *myo*-inositol, mannitol, ribose,

sucrose, trehalose, arabinose, maltose, raffinose, rhamnose, xylose but not dulcitol, salicin and sorbitol. Strain W18L9^T can hydrolyze starch, urea, skim milk but not xanthine, esculin, hippurate, L-tyrosine and adenine. They could assimilate benzoate, malate, tartrate, acetate, citrate and propionate. This strain cannot use phenol, pyridine, toluene and benzene as sole carbon source. Whole-cells contain

LL-diaminopimelic acid in its peptidoglycan and galactose, glucose, mannose and xylose as whole-cell sugars. Polar lipids are phosphatidylethanolamine(PE) and phosphatidylinositol (PI). MK-9(H₆) and MK-9(H₈) are predominant menaquinones. Major cellular fatty acids are *anteiso*-C_{15:0}, *anteiso*-C_{17:0} and *iso*-C_{16:0}. The DNA G+C content of the type strain is 70.9%.

The type strain, W18L9^T (NRRL B-65635^T = TBRC 15051^T), is an endophytic actinobacterium isolated from leaf tissue of Jasmin rice (*Oryza sativa* KDML 105) grown at rice paddy field,

Roiet province, Thailand. The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequence and the whole genome shotgun project of strain W18L9^T are MZ901363 and JACBWY000000000, respectively. Strain W18L9^T can strongly inhibit *Staphylococcus*

aureus ATCC 29213 and Methicillin Resistant *Staphylococcus aureus* 03120385, *Pyricularia grisea* BRIP 61689 and *Rhizoctonia solani* AG8 in vitro.

Declarations

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Author contribution statement

OK planned the experiments, carried out the experimental work, analyzed all data and prepared the draft manuscript. SS did the technician works for physiological and morphological study. CS and NC extracted and analyzed FAMEs. CS extracted genomic DNA of strain SBTS01^T and *S. shenzhenensis* DSM 42034^T for whole genome sequence. TC analyzed menaquinones by using LC-MS. CF proofed the manuscript and provided the facilities for conducting the research.

Declaration of Competing interest

The authors report no declarations of interest.

Ethical statement

This article does not contain any studies with human participants or animal experiments by any of the authors.

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Tables

Table 1. Differential characteristics between *Streptomyces* SBTS01^T and related species of *Streptomyces*.

Strain: 1, *Streptomyces spinosus* SBTS01^T; 2, *Streptomyces rochei* NRRL B-2410^T; 3, *Streptomyces naganishii* NRRL ISP-5282^T.

+ positive or present; w, weakly positive; -, negative or absent. Catalase was positive for all strains. All strains could produce acid from cellobiose, fructose, galactose, glucose, maltose, mannose, *myo*-inositol, mannitol, rhamnose, ribose, sucrose, trehalose and xylose, but not from dulcitol and sorbitol. All strains could hydrolyze adenine, starch, skim milk but not hippurate. They could not use pyridine, toluene, phenol and benzene. They could assimilate acetate, citrate, malate and propionate. All strains could grow at 1% and 3% but not at 10%, 15% and 20% NaCl (w/v). They could grow at 27°C and 37 °C and between pH 5 and pH 10 but they could not grow at 4 °C

and 55 °C.

Characteristics	1	2	3	Characteristics	1	2	3
Acid production from:				Organic acid assimilation:			
Arabinose	+	+	-	benzoate	+	-	+
salicin	+	+	-	tartrate	+	-	-
Raffinose	+	+	-	Growth at:			
Hydrolysis of:				NaCl 5%((w/v)	+	w	+
L-tyrosine	-	+	+	15 °C	+	+	w
xanthine	-	+	+	45 °C	w	-	-
Esculin	+	+	-	pH 4	-	-	w
				Melanin pigment production	+	-	-

Table 2. Differential characteristics between *Streptomyces* W18L9^T and related species of *Streptomyces*.

Strain: 1, *Streptomyces* W18L9^T; 2, *Streptomyces shenzhenensis* DSM 42034^T;

3, *Streptomyces graminisoli* NBRC 108883^T; 4, *Streptomyces jiujiangensis* DSM 42115^T; 5, *Streptomyces hyaluromycini* DSM 100105^T. + positive or present; w, weakly positive; -, negative or absent. Catalase was positive for all strains. All strains could produce acid from cellobiose, fructose, galactose, glucose, mannose, *myo*-inositol, mannitol, ribose, sucrose, trehalose, but not dulcitol, salicin and sorbitol. All strains could hydrolyze skim milk but not L-tyrosine and adenine. They could assimilate acetate, citrate and propionate. They could not use phenol. All strains could grow at 1% but not at 10%, 15% and 20% NaCl (w/v). They could grow at 27°C but could not grow at 4°C, 45°C and 55°C. They could grow between pH 7 and pH 9 but not at pH 4.

Characteristics	1	2	3	4	5	Characteristics	1	2	3	4	5
Acid production from:						Organic acid assimilation:					
Arabinose	+	+	+	-	+	benzoate	+	+	+	-	+
Maltose	+	-	-	-	-	malate	+	+	+	-	-
Raffinose	+	+	+	-	+	tartrate	+	-	+	-	+
Rhamose	+	+	+	-	+	Use of phenolic compounds:					
xylose	+	+	+	-	+	Pyridine (sigma)	-	-	-	-	+
Hydrolysis of:						Toluene	-	-	-	-	+
xanthine	-	-	-	-	+	benzene	-	-	-	-	+
Esculin	-	-	-	+	+						
Hippurate	-	+	-	+	-	Grow at:					
Starch	+	+	+	-	+	NaCl 3% (w/v)	+	+	+	+	-
urea	+	-	-	-	+	NaCl 5% (w/v)	-	w	+	w	-
						15°C	+	-	+	-	w
						37°C	+	+	+	+	-
						pH 5	+	+	+	w	+
						pH 6	+	+	+	w	+
						pH10	+	-	+	+	+

Table 3: Antimicrobial activity of strains SBTS01^T and W18L9^T to inhibit pathogens

Strain/ methods	Inhibition against pathogens						
	<i>Xoo</i> PXO 86	<i>P. grisea</i> BRIP 61689	<i>S. aureus</i> ATCC 29213	MRSA 03120385	<i>R. solani</i> AG8	<i>H. oryzae</i> DOAC 1570	<i>C. lunata</i> BCC 15558
SBTS01^T							
Dual culture; HPDA	3+	2+	3+	3+	1+	2+	2+
Dual culture; ISP 2	1+	2+	1+	1+	1+	2+	2+
Methanol extraction; HPDA (mm)	12	0	18.5	17	-	-	-
Methanol extraction; ISP2 (mm)	0	0	8	0	-	-	-
W18L9^T							
Dual culture; HPDA	2+	3+	3+	3+	3+	1+	2+
Dual culture; ISP 2	ni	2+	2+	2+	3+	1+	2+
Methanol extraction; HPDA (mm)	0	15	17.5	15	-	-	-
Methanol extraction; ISP 2 (mm)	0	8.5	15	13	-	-	-
Vancomycin 500 ug/ml (mm)	-	-	19	18	-	-	-
Streptomycin 500 ug/ml (mm)	20	-	-	-	-	-	-

: 3+; strong inhibition, 2+; moderate inhibition, 1+; weak inhibition, ni; no inhibition, -; not done.

Table 4: Plant growth promoting (PGP) traits of *Streptomyces spinosus* SBTS01^T and *Streptomyces shenzhenensis* subsp. *endophyticus* W18L9^T

Strain	PGP traits							
	ACC deaminase	Nitrogen fixation	Phosphate solubilization	Cellulase production	HCN Production	Siderophore production	IAA production (µg/mL)	Cytokinin production (mg/L)
SBTS01 ^T	-	-	-	-	-	+	71	0.165
						(hydroxymate type)		
W18L9 ^T	+	+	+	+	-	+	150	0.169
						(hydroxymate type)		

: -; negative result, +; positive result.

Table 5. The distribution of BGCs of *Streptomyces spinosus* SBTS01^T and *Streptomyces shenzhenensis* subsp. *endophyticus* W18L9^T

Gene type	product	Span (nt)	Gene similarity	Most similar biosynthetic gene cluster	Similarity
SBTS01^T					
Terpene					
	geosmin	524,641 - 546,794	100%	<i>Streptomyces</i> sp. jing01	94%
	albaflavenone	183,689 - 204,612	100%	<i>Streptomyces hygroscopicus</i> subsp. <i>limoneus</i>	94%
	hopene	1 - 21,432	92%	<i>Streptomyces dengpaensis</i> strain XZHG99	75%
	isorenieratene	18,402 - 38,308	62%	<i>Streptomyces</i> sp. jing01	100%
Type 1 PKS (T1PKS)	sceliphrolactam	89,955 - 200,758	92%	<i>Streptomyces hygroscopicus</i> subsp. <i>jinggangensis</i>	81%
	antimycin	1 - 48,820	93%	<i>Streptomyces hygroscopicus</i> subsp. <i>jinggangensis</i>	42%
T2PKS	spore pigment	195,492 - 268,003	83%	<i>Streptomyces hygroscopicus</i> subsp. <i>limoneus</i>	91%
	lugdunomycin	1 - 45,736	40%	<i>Streptomyces</i> sp. jing01	62%
RiPP :Lanthipeptide	informatipeptin	302,814 - 380,368	57%	<i>Streptomyces corchorusii</i> DSM 40340	87%
NRPS, ladderane	ishigamide	1 - 49,528	100%	<i>Streptomyces corchorusii</i> DSM 40340	53%
Others:					
Siderophore	desferrioxamin B / desferrioxamine E	227,349 - 239,118	83%	<i>Streptomyces hygroscopicus</i> subsp. <i>limoneus</i>	100%
	ectoine	88,957 - 99,361	100%	<i>Streptomyces</i> sp. jing01	100%
Alkaloid	anisomycin	185,220 - 206,614	61%	Candidatus <i>Streptomyces philanthi</i>	50%
Pigment	melanin	333,875 - 344,378	60%	<i>Streptomyces hygroscopicus</i> subsp. <i>limoneus</i>	92%
	melanin	240,779 - 251,126	71%	<i>Streptomyces</i> sp. jing01	100%
W18L9^T					
Terpene					
	hopene	1 - 20,148	76%	<i>Streptomyces scabiei</i>	77%
	geosmin	1 - 10,288	100%	<i>Streptomyces</i> sp. 1-11 strain 1- 11	31%
	albaflavenone	1 - 10,143	100%	<i>Streptomyces</i> sp. jing01	52%
Type 1 PKS (T1PKS)	rhizomide A / rhizomide B / rhizomide C	1 - 4,771	100%	No hit	
	rhizomide A / rhizomide B / rhizomide C	1 - 3,488	100%	No hit	

	rhizomide A / rhizomide B / rhizomide C	1 - 1,700	100%	No hit	
T2PKS	curamycin	1 - 11,119	57%	<i>Streptomyces</i> sp. DSM 40868	15%
T3PKS	germicidin	1 - 15,140	100%	<i>Streptomyces</i> sp. BK141	8%
	feglymycin	1 - 16,377	47%	<i>Streptomyces</i> sp. TLI 053	22%
NRPS	xenotetrapeptide	1 - 2,316	100%	No hit	
NRPS like					
Lipopeptide	tolaasin I / tolaasin F	1 - 1,056	50%	<i>Streptomyces</i> sp. ADI96-15	18%
Other:					
Siderophore	desferrioxamin B /desferrioxamine E	8,458 - 20,227	83%	<i>Streptomyces ambofaciens</i> ATCC 23877	88%
	flaviolin	1 - 21,999	50%	<i>Streptomyces hawaiiensis</i> ATCC 12236	58%
	ectoine	1 - 4,466	100%	<i>Streptomyces</i> sp. BK387	66%
Butyrolactone	A-factor	1 - 2,461	100%	<i>Streptomyces</i> sp. BK674	5%

Figures

Figure 1

16S rRNA gene-based neighbour-joining tree showing the phylogenetic relationships between *Streptomyces spinosus* SBTS01^T and *Streptomyces shenzhenensis* subsp. *endophyticus* W18L9^T with other related strains in genus *Streptomyces* and *Nocardia africana* DSM 44491^T as the out-group. Bootstrap values (>50%) based on 1000 replicates are shown at the branch nodes. The scale bar represents 0.010 changes per nucleotide.

Figure 2

Scanning electron microscope showed hyphae and spores of two strains grown on HPDA for 7 days at 27°C. A) Strain SBTS01^T B) Strain W18L9^T. Bar represents 1 µm.

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