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BACTERIAL, FUNGAL AND VIRUS MOLECULAR BIOLOGY - RESEARCH PAPER

Metabolic and genomic analysis deciphering biocontrol potential of endophytic *Streptomyces albus* RC2 against crop pathogenic fungi

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Abstract

Plant diseases caused by phytopathogenic fungi are one of the leading factors affecting crop loss. In the present study, sixty-one *Streptomyces* strains were screened for their antifungal activity against relevant wide range fungal pathogens prominent in Vietnam, namely *Lasiodiplodia theobromae*, *Fusarium fujikuroi*, and *Scopulariopsis gossypii*. Endophytic strain RC2 was the most effective strain in the mycelial inhibition of the tested fungi. Based on phenotypic characteristics, 16S rDNA gene analysis, and genomic analysis, strain RC2 belonged to *Streptomyces albus*. An ethyl acetate extract of *S. albus* RC2 led to the strong growth inhibition of *S. gossypii* Co1 and *F. fujikuroi* L3, but not *L. theobromae* N13. The crude extract also suppressed the spore germination of *S. gossypii* Co1 and *F. fujikuroi* L3 to 92.4 ± 3.2% and 87.4% ± 1.9%, respectively. In addition, the RC2 extract displayed potent and broad-spectrum antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and the phytopathogenic bacteria *Ralstonia solanacearum* and *Xanthomonas oryzae*. The genome of strain RC2 was sequenced and revealed the presence of 15 biosynthetic gene clusters (BGCs) with similarities ≥ 45% to reference BGCs available in the antiSMASH database. The UPLC-HRMS analysis led to the identification of 8 other secondary metabolites, which have not been reported in *S. albus*. The present study indicated that RC2 could be a potent biocontrol agent against phytopathogenic fungi. Further attention should be paid to antifungal metabolites without functional annotation, development of product prototypes, and greenhouse experiments to demonstrate effective control of the plant diseases.

Keywords Antifungal activity · Biocontrol agent · Phytopathogenic fungi · *Streptomyces albus* · Biosynthetic gene clusters

Introduction

Plant pathogens are recognized as an increasing threat to crop production as well as global food security. It is estimated that around 14% of the global crop production is lost due to plant diseases, in which phytopathogenic fungi accounted for 70–80% [1]. Dieback originated from *Lasiodiplodia theobromae* and rice bakanae disease caused by *Fusarium fujikuroi* are prevalent in various agricultural countries such as Vietnam, Thailand, and India [2, 3]. In addition, *Scopulariopsis gossypii* combined with *Verticillium dahliae* lead to leaf interveinal chlorosis and vascular browning of cotton plants [4]. Due to their broad host range and resistance to plethora of stressors, it is very difficult to control them. Despite the efficiency in preventing phytopathogenic fungi, the overuse of synthetic pesticides results in severe environmental and health problems [5]. Biocontrol using bacteria has been expected to be a sustainable and economical alternative for controlling various fungal phytopathogens. However, efforts are still being investigated to make bacterial inoculants commercially available.

Streptomyces spp. are Gram-positive filamentous bacteria that produce a plethora of new and innovative secondary metabolites with biological activities [6, 7]. They have been gaining attention as potential biocontrol agents to date.

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Streptomyces sp. SCA3-4 isolated from soil was shown to inhibit mycelial development and spore germination of *Fusarium oxysporum* f. sp. *cubense* causing banana *Fusarium* wilt [8]. The phytopathogenic fungus *Sclerotinia sclerotiorum* causing stem rot of soybean, rapeseed oil, and sunflower was effectively controlled by *Streptomyces* sp. NEAU-S7GS2 isolated from the root of *Glycine max*. Comprehensively, antagonistic activity against fungal pathogens attributes to secondary metabolites produced by *Streptomyces*. Blasticidin S produced from *Streptomyces griseochromogenes* was used as a commercial fungicide to control rice blast [9]. Given that the identification of antifungal compounds is time-consuming, laborious, and expensive, whole-genome sequencing of bacteria offers the possibility to rapidly identify secondary metabolite BGCs and understand their evolution in different environments [10, 11]. *Streptomyces* spp. isolated from saline soils were recently reported to be producers of antimicrobial compounds using genome mining [12]. In addition, five BGCs encoding for hopene, elaiophylin, coelichelin, nigericin, and geldanamycin and other unknown BGCs could be involved in biocontrol potential of endophytic *Streptomyces malaysiensis* 8ZJF-21 against *F. oxysporum* f. sp. *cubense* tropical race 4. Literature review reveals that most of the genomic studies have been only focusing on soil-derived *Streptomyces* and neglecting other isolation sources such as plants. It is evident that the same *Streptomyces* species isolated from different environments have distinct metabolic and genomic profiling [13, 14]. For this reason, endophytic *Streptomyces* can still be an interesting source of novel antifungal and biocontrol agents.

In Vietnam, crop infection by *S. gossypii*, *F. fujikuroi*, and *L. theobromae* results in severe loss in the food production and extensive usage of chemical fungicides. A few studies showed potential of *Streptomyces* to control phytopathogenic fungi. Our earlier works proved that endophytic *Streptomyces* spp. are a prolific source of antioxidant and anticancer compounds [11, 13, 15]. In this study, screening antifungal activity of 61 endophytic *Streptomyces* was performed. One of these strains, showing the strongest inhibitory effects against fungal pathogens, was identified as *Streptomyces albus* RC2. Genomic and metabolic analyses were further employed to decipher relevant secondary metabolites. This study highlights the potential of endophytic *Streptomyces albus* as a promising candidate to control microbial phytopathogens causing plant diseases in Vietnam.

Materials and methods

Screening of antifungal *Streptomyces* spp.

Sixty-one *Streptomyces* strains previously isolated from *Aegiceras corniculatum*, *Bruguiera gymnorrhiza*, and *Oryza sativa* and three phytopathogenic fungi (*S. gossypii* Co1, *F. fujikuroi* L3, and *L. theobromae* N13) were provided by VAST-Culture Collection of Microorganisms, Vietnam Academy of Science and Technology. They were screened for their antifungal activity against fungal pathogens using a plate confrontation method on the potato dextrose agar (PDA) (HiMedia, India) [8]. Briefly, a phytopathogenic fungal block with a 5-mm diameter was placed in the center of the PDA plates. After that, each *Streptomyces* isolate was inoculated at four symmetrical points 25 mm from the center of the PDA plate. Plates without *Streptomyces* isolates were used as controls. All plates were incubated at 30 °C for 5–7 days, and the inhibition zones represented as distance between the fungal mycelium edge and the *Streptomyces* colony were measured. The percentage of fungal growth inhibition was calculated using the following formula:

$$\mathrm{FGI} = [(D_0 - D_1)/D_0] \times 100\%$$

In which, D_0 and D_1 represented the diameters of fungal mycelium growth in the control and treated plates, respectively.

Morphological characteristics and 16S rRNA analysis of potent strain

Strain RC2 was cultivated on International Streptomyces Project (ISP) 1–7 agar plates at 30 °C for 4–5 days to observe the growth, the color of aerial mycelia, and diffusible pigments as described previously [16]. Scanning electron microscopy (SEM) was used to determine the spore and aerial mycelium of strain RC2. Physiological characteristics of strain RC2 were examined by changes in temperature (16–48 °C), pH (4–11), and NaCl concentration (0–5%). The growth ability of strain RC2 in the presence of sole carbon sources was assessed according to the previous procedure [15].

Genomic DNA of *Streptomyces* sp. RC2 was extracted using the G-spin Total DNA Extraction Mini Kit (Intro Bio, Korea) according to the manufacturer's instructions. PCR amplification of 16S rRNA gene was performed using the primer pairs 27F and 1429R as described previously [15]. The PCR products were purified with a DNA purification kit (Promega, USA) and sent for sequencing at First BASE Laboratories Sdn. Bhd (Malaysia). The resulting 16S rRNA sequence was compared with related type strains accessible on GenBank (NCBI) (http://www.ncbi.nlm.nih.gov/) and EzTaxon server (http://www.eztaxon.org/). The phylogenetic tree was constructed by the neighbor-joining method with 1000 bootstrap using Kimura 2-parameter distances in MEGA v7.0 [17], and *Enterococcus faecalis* ATCC 19433 ^{T} (NR_115765) was used as the outgroup branch.

Effect of the RC2 extract on mycelial growth and spore germination of fungal phytopathogens

Streptomyces sp. RC2 was freshly aerobically cultured on PDA plates at 30 °C for 5 days. Chucks of agar with fully grown strain were inoculated into a 250-mL Erlenmeyer flask containing 50 mL of the potato dextrose broth (PDB) medium (HiMedia, India). After 3 days of incubation at 30 °C with 180 rpm, the seed culture was transferred to 4 flasks (1000 mL), each containing 200 mL of PDB medium and incubated at 30 °C, 180 rpm for 8 days. The culture broth was combined and centrifuged at 6000 rpm for 20 min to obtain the cell-free supernatant. About 600 mL of the cell-free supernatant was retrieved and mixed with an equal volume of ethyl acetate [8]. The resulting organic layers were separated and evaporated to dryness at 45 °C to yield a crude extract. The working solution (10 mg/mL) of dried ethyl acetate crude extract of RC2 was prepared in 5% (v/v) dimethyl sulfoxide (DMSO). The crude extract of RC2 was added to the PDA agar plates to final concentrations of 0, 22.5, 45, 90, and 180 µg/mL. A 5-mm fungal block of *S. gossypii* Co1, *F. fujikuroi* L3, and *L. theobromae* N13 was put in the center of the plates. The PDA medium supplemented with 5% (DMSO) was used as a control. These plates were incubated at 30 °C for 5–7 days, and mycelial inhibition was calculated according to the abovementioned formula.

The effect of RC2 extract on spore germination of tested fungi was carried out based on the previous protocol with a minor modification [9]. *S. gossypii* Co1 and *F. fujikuroi* L3 were cultured on the PDA at 30 °C for 7 days in order to collect spores. About 90 µg/mL of extract solution was added to fungal spore suspension at the ratio of 1:1 (v/v), which was then placed on a sterile glass slide. After incubation in a moist chamber at 30 °C for 24 h, 100 spore germination of each slide was counted by an optical microscope. The inhibition efficiency (IE) of RC2 extract on spore germination of tested fungi was calculated following the formula: $IE = (A - B)/A$, where A and B stand for the spore germination rate of control and treatment samples, respectively.

Antibacterial spectrum

Antibacterial activity of RC2 extract was evaluated using the agar well-diffusion method [18]. Six pathogenic bacteria including five Gram-negative (*Escherichia coli* ATCC 11105, *Pseudomonas aeruginosa* ATCC 9027, *Xanthomonas oryzae* R1, *Ralstonia solanacearum* CC8, *Salmonella typhimurium* ATCC 14028) and one Gram-positive (*Bacillus cereus* ATCC 11778) bacteria were utilized as test pathogens. Among them, *X. oryzae* R1 isolated from infected rice foliar and *R. solanacearum* CC8 recovered from wilted tomato were provided by VAST-Culture Collection of Microorganisms. About 50 µL of RC2 extract (90 µg/mL) was used. And the experiment was performed in triplicate, and the antibacterial activity was evaluated through measurement of the inhibition zone around 6-mm wells.

Genome sequencing and bioinformatic analysis

Genome of strain RC2 was sequenced using the Illumina MiSeq platform (Illumina, CA, USA) as described previously [13]. Quality control and read trimming were performed by FastQC and Trimmomatic 3.0. The draft genome was assembled de novo with SPAdes 3.15 using default parameters and annotated with RASTtk at Rapid Annotation using Subsystem Technology (RAST) as well as the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [19, 20]. The completeness of the assembled genome was evaluated by Benchmarking Universal Single-Copy Orthologous (BUSCO) 3 (https://gitlab.com/ezlab/busco). Genome sequence of *S. albus* RC2 was deposited at DDBJ/ENA/GenBank under the accession number JASCYL000000000.

Functional annotation of the predicted coding sequences of *S. albus* RC2 in comparison with *S. albus* G153 (AP025687), *S. albus* NRRL B-1811 (NZ_JODR00000000), and *S. albus* NRRL:B-2445 (NZ_JOED00000000) was analyzed using the SEED-viewer analysis of genome sequences by RASTtk [19]. Secondary metabolite biosynthetic gene clusters (BGCs) of *S. albus* RC2 were predicted with antiSMASH 7.0.0 with default parameters [12, 21]. Genes related to potential BGCs responsible for antifungal activity of strain RC2 were identified and compared with reference genes using BLASTp (e-value cutoff = 1e − 5) and TBLASTN tools (e-value cutoff = 1e − 5) against a database of enzymes, transcriptional regulators reported in UniProtKB-Swiss-Prot. An e-value cutoff $< 10^{-10}$, identity > 25%, and coverage > 50% were used to filter the outcome.

UPLC-HRMS analysis and dereplication

The samples were analyzed on an LCTM—X500R QTOF (SCIEX, USA) using a Hypersil GOLD column (150 × 2.1 mm, 3 µm) at 40 °C. The mobile phase consists of phase A (0.05% formic acid in water) and phase B (0.05% formic acid in acetonitrile) at the flow rate of 400 µL/min. A gradient of 20–98% B in 25 min followed by 98% B in 5 min was used. The MS spectra were detected in the positive mode. Mass spectrometry parameter was set as following: air curtain gas CUR: 35psi; IS voltage: + 5500, source temperature: 500 ℃; cone voltage: + 80 V, atomizing gas GAS1: 55psi. Spectra were scanned over the range of 50–2000 Da, and raw data of LC–MS analysis were converted and processed by the software MZmine 3.0. Dereplication of metabolites from the analytes was conducted using high-resolution mass spectrometry (HRMS) data. The exact

mass data of the detected metabolites were identified using representative adducts ($[M+H]^+$, $[M+Na]^+$, $[2\ M+H]^+$, $[2\ M+Na]^+$, $[M+H]^-$, $[M+HCOO]^-$) or simple losses ($[M\text{-}H_2O+H]^+$, $[M\text{-}H_2O+Na]^+$). The exact mass data were compared to those of databases from Reaxys, SciFinder, or DNP. The difference between exact mass and calculated mass should not be over 5 ppm. The identified results were confirmed by checking the published data of the compounds in previous studies.

Results

Screening of antifungal activity of Streptomyces isolates

In the present study, a total of 61 *Streptomyces* spp. were screened for their antifungal activity against phytopathogenic fungi on PDA plates using a dual culture assay. It turned out that 22 (36.1%) out of 61 *Streptomyces* isolates displayed inhibition activity against at least one fungal strain (Table S1). All bioactive strains showed robust activity against *S. gossypii* Co1 than *L. theobromae* N13 and *F. fujikuroi* L3. Among them, *Streptomyces* sp. RC2 isolated from the rice roots was most effective in reducing mycelium growth of *S. gossypii* Co1 (72.6 ± 0.5%), *L. theobromae* N13 (52.2 ± 0.7%), and *F. fujikuroi* L3 (57.0 ± 0.6%), which was selected for further identification.

Phenotypic characteristics and molecular identification of strain RC2

Strain RC2 grew well on all ISP media at 30 °C after 4 days, and morphological colonies varied depending on media. On ISP2 agar plates, colonies produced white to gray aerial mycelium and pale-yellow substrate mycelium with no diffusible pigment (Fig. 1A and Table S2). The aerial mycelium under SEM was spiral with a smooth spore surface (Fig. 1B). Regarding physiological characteristics, strain RC2 could grow at temperatures of 22–45 °C (optimum at 30 °C), pH 5–10 (optimum at pH 7.0), and NaCl concentration of 0–4 (optimum at 2% NaCl). The strain utilized glucose, sucrose, fructose, raffinose, and galactose, but not mannitol and xylose (Table S2). Moreover, extracellular enzymes such as

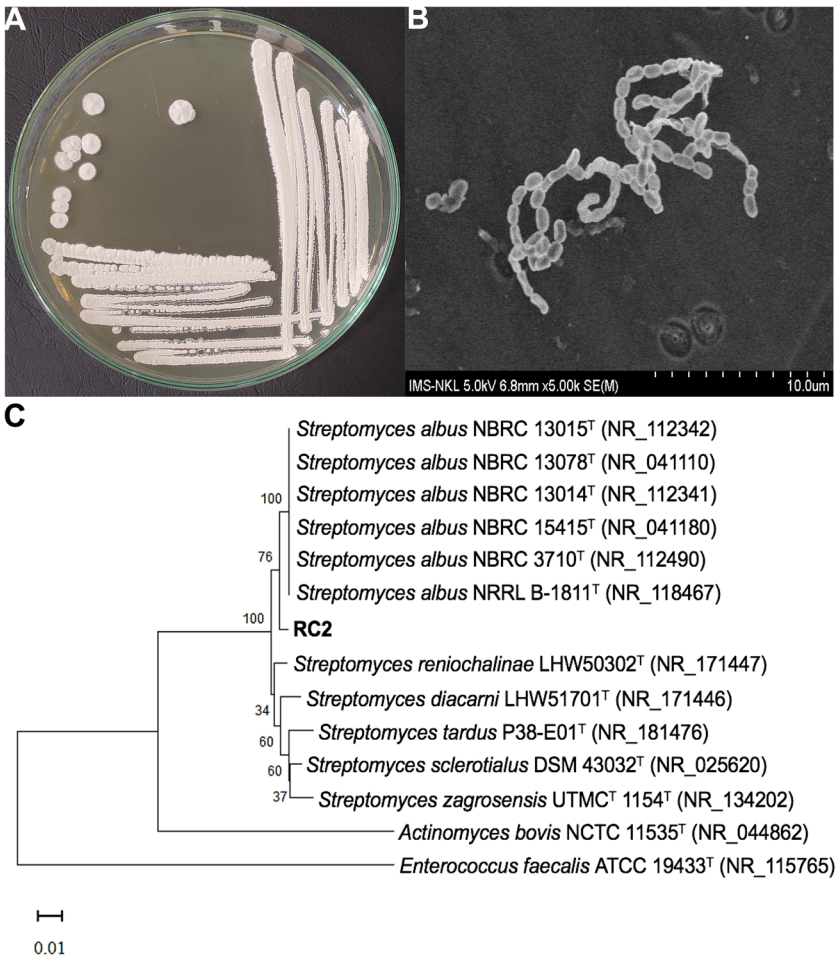

Fig. 1 Identification of endophytic strain RC2. **A**, **B** Morphological characteristics of endophytic *Streptomyces* sp. RC2. **C** Phylogenetic tree of type strains closely related to *Streptomyces* sp. RC2 based on 16S rRNA gene sequences

amylase, CMCase, chitinase, protease, and xylanase were detected. In support of morphological identification, the full-length 16S rRNA gene sequence of RC2 showed 100% pairwise similarities with *Streptomyces albus* NBRC 13015 ^{T} and *Streptomyces albus* NRRL B-1811 ^{T}. Phylogenetic analysis revealed that *Streptomyces* sp. RC2 was referred to as *S. albus* (Fig. 1C).

Antagonistic activity of RC2 extract against phytopathogenic fungi

To know whether secondary metabolites of strain RC2 were responsible for antifungal activity, the culture was extracted by the ethyl acetate solvent. The results revealed that the RC2 extract inhibited the growth of 3 tested fungi at different levels. The RC2 extract displayed significant inhibition activity against *S. gossypii* Co1 (70.8 ± 0.6%) and *F. fujikuroi* L3 (55.8 ± 0.3%) when being treated with 90 µg/mL, while *L. theobromae* N13 was not affected (Fig. 2A, B, C). An increase in concentration to 180 µg/mL led to strong mycelium inhibition of *L. theobromae* N13 to 50.0 ± 0.4%. In line with these results, exposure to 90 µg/mL of the RC2 extract resulted in remarkable suppression of spore germination of *F. fujikuroi* L3 (87.4% ± 1.9%) and *S. gossypii* Co1 (92.4 ± 3.2%) (Fig. 2D, E, F, G). In terms of positive controls, the percentage of spore germination reached 92.4–95.6%.

Broad-spectrum antibacterial activity of the RC2 extract

Further tests on the antibacterial activity showed that Gram-negative and Gram-positive bacteria were strongly inhibited by 90 µg/mL of the crude extract with inhibition zones ranging from 12.0 to 34.0 mm (Table 1). The highest antibacterial activity was recorded for *P. aeruginosa* ATCC 9027 (34.0 ± 0.1 mm) and *E. coli* ATCC 11105 (20.1 ± 0.2 mm). Of note, the RC2 extract also showed antagonistic activity against phytopathogenic bacteria such as *X. oryzae* R1 (12.4 ± 2.4 mm) and *R. solanacearum* CC8 (19.5 ± 2.7 mm).

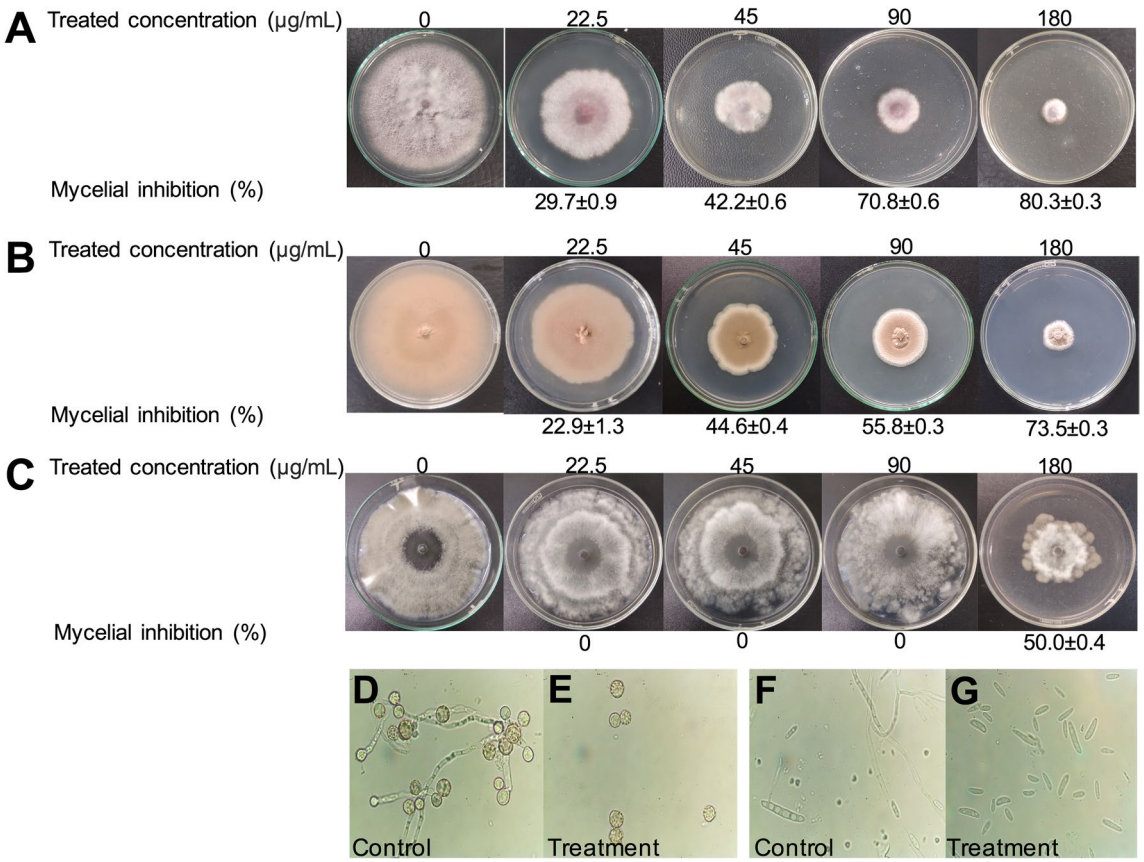

Fig. 2 Antifungal activity against phytopathogenic fungi of RC2 extract. RC2 extract suppressing mycelial growth of *F. fujikuroi* L3 (**A**), *S. gossypii* Co1 (**B**), and *L. theobromae* N13 (**C**). Spore germination of *S. gossypii* Co1 (**D**, **E**) and *F. fujikuroi* L3 (**F**, **G**) inhibited by 90 µg/mL of RC2 extract

Table 1 Inhibitory effects of the RC2 extract against pathogenic bacteria

Pathogenic bacteria	Inhibition zones (mm)
Gram-positive bacteria	
Bacillus cereus ATCC 11778	15.1 ± 0.1
Gram-negative bacteria	
Xanthomonas oryzae R1	12.4 ± 2.4
Ralstonia solanacearum CC8	19.5 ± 2.7
Escherichia coli ATCC 11105	20.1 ± 0.2
Pseudomonas aeruginosa ATCC 9027	34.0 ± 0.1
Salmonella typhimurium ATCC 14028	12.0 ± 0.1

Genomic characteristics of strain RC2

Based on the raw data from whole-genome sequencing by Illumina MiSeq, de novo genome assembly by Unicycler led to a draft genome of 7,847,620 bp, with a GC content of 72.7% (Table S3). The obtained genome was assembled into 52 contigs with N50 of 464,302 including 6483 coding gene sequences, 58 tRNA, and 3 rRNA genes and showed 99.2% BUSCO completeness. In agreement with 16S rRNA sequence analysis, the average nucleotide identity for *S. albus* RC2 with *S. albus* subsp. *albus* NRRL B-1811 (GCF_000725885.1) was 99% using the taxonomic assignment of the genome by GTDB-Tk.

Analysis of *S. albus* RC2 features revealed all annotated proteins were classified into 28 Clusters of Orthologous Groups (COGs). According to the COG annotations, the five largest groups were the following: amino acids and derivatives (370 genes), carbohydrates (312 genes), protein metabolism (216 genes), and cofactors, vitamins, prosthetic groups, and pigments (192 genes) (Fig. 3A). Comparison to those of *S. albus* G153, *S. albus* NRRL B-1811, and *S. albus* NRRL:B-2445 revealed very different predicted protein content and function toward *S. albus* strains.

Genome mining of secondary metabolite gene clusters and metabolic profiling

Genomic analysis revealed the presence of 30 clusters, in which 5 clusters did not exhibit similarities to reference

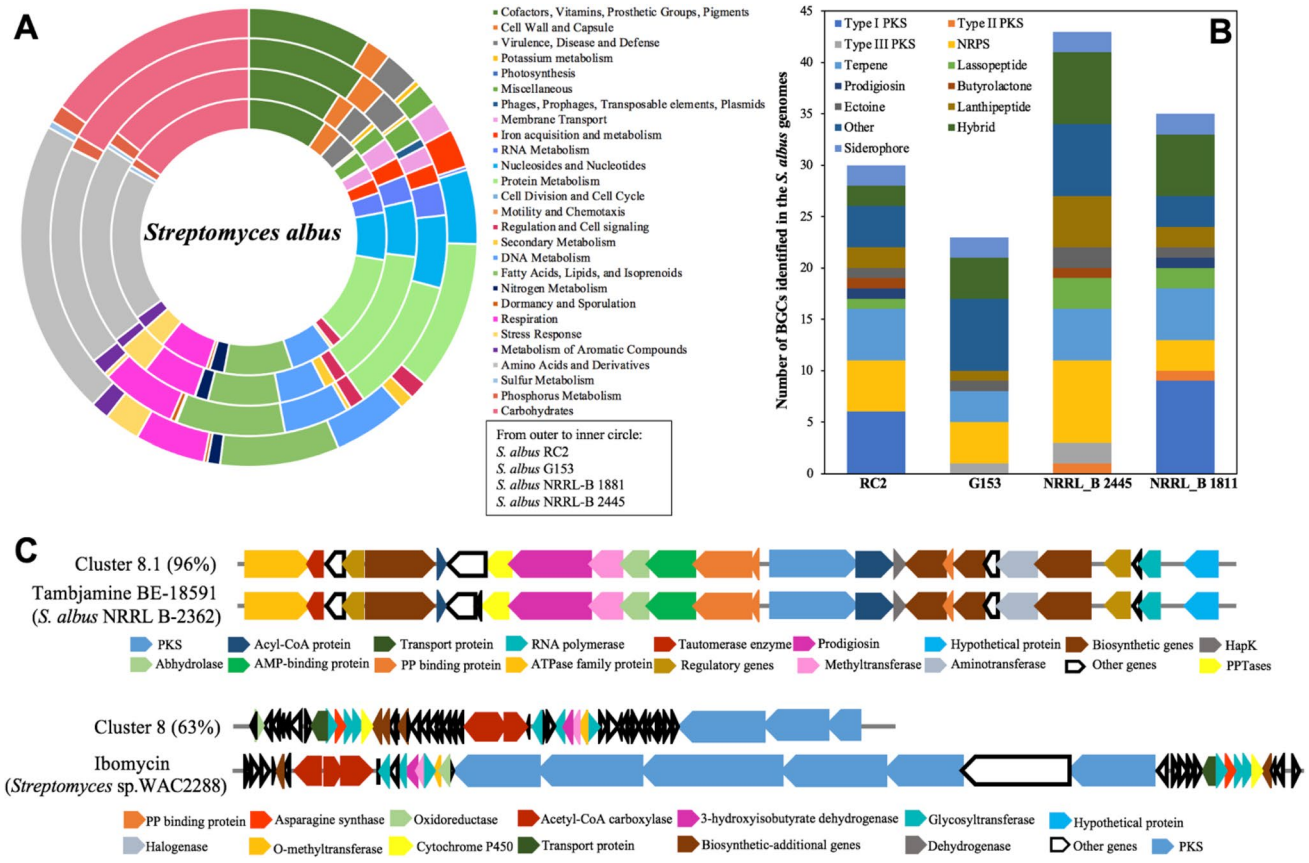

Fig. 3 Functional annotation and prediction of BGCs present in the *S. albus* RC2 genome. **A** The functional categories predicted in *S. albus* RC2 and 3 other *S. albus* genomes using RASTtk. **B** Comparison of number of BGCs in the *S. albus* genomes. **C** Identification of BGCs responsible for antifungal activity

clusters in the antiSMASH database and 15 BGCs exhibited similarities ≥ 45% (Table S4). RC2 had an average number of BGCs compared to *S. albus* G153 (23 BGCs), *S. albus* NRRL B-1811 (35 BGCs), and *S. albus* NRRL B-2445 (43 BGCs) (Fig. 3B). Among 15 BGCs, terpene and non-ribosomal peptide synthetases (NRPS) occupied predominantly with 4 BGCs. In addition, 7 other clusters included ectoine, type I polyketide synthase (T1PKS), prodigiosin, lanthipeptide, lasso peptide, butyrolactone, and nucleoside BGCs. Among them, BGCs encoding hopene, geosmin, sapB, desferrioxamine B, and ectoine were usually found within *Streptomyces* species.

Regarding bioactivities found in the RC2 extract, 2 BGCs encoding for tambjamine BE-18591 and ibomycin responsible for the production of antifungal agents were identified (Fig. 3C). Tambjamine BE-18591 gene cluster (cluster 8.1) showed 96% similarity with the corresponding genes in *S. albus* NRRL B-2362. Cluster 5.2 shared 63% of identity to ibomycin BGC from *Streptomyces* sp. WAC2288 as a large type I polyketide macrolactone. BGCs involved in the production of antibacterial compounds included xantholipin, tambjamine BE-18591, pseudouridimycin, thiazostatin, dudomycin A, and aborycin (Table S4).

To correlate genomic prediction, the ethyl acetate extract of RC2 was analyzed by UHPLC-HRMS/MS. A total of 14 compounds were detected in which 8 compounds were identified, including 1,2,3,4-tetrahydroquinazoline-2,4-dione, griseorhodin E, deamosaminylcytosamine, anthracimycin, sarubicin B, albonoursin, albofungin A, and indoxamycin D (Table 2). However, these compounds were not correlated with the BGCs predicted by antiSMASH.

Discussion

Plant diseases caused by phytopathogenic fungi are the leading causes of global crop production loss. Various studies have proved that *Streptomyces* strains are effective biocontrol agents to eliminate the threat of plant pathogens [15, 22]. In this study, among 61 isolates, 22 *Streptomyces* spp. (36.1%) demonstrated antifungal activity against at least one tested fungi. It was in agreement with a recent study reporting that about 35.7% of the Actinobacteria isolated from *Cinnamon basil*, *Ricinus communis*, *Epipremnum aureum*, *Citrus jambhiri*, and *Hibiscus rosa-sinensis* were active against fungal phytopathogens [23]. These results supported the assumption that *Streptomyces* spp. are excellent candidates for controlling fungal plant pathogens. Of note, endophytic *S. albus* RC2, as the most potent candidate, showed both remarkable antifungal and antibacterial activities. Different from our study, *S. albus* AN1 from honey only inhibited yeast *Candida albicans*, but not bacteria and fungi [14]. The latest report proved the biocontrol potential of *S. albus* CAI-21 recovered from herbal vermicompost in suppressing charcoal rot disease in sorghum caused by *Macrophomina phaseolina* [24]. There was a likelihood that the regulation of secondary metabolite produced by these *S. albus* strains is different from each other due to host adaptation.

Using the UHPLC-HRMS/MS, 8 compounds were identified in the RC2 extract, among which only albofungin A was previously reported to exhibit moderate inhibitory effects against *Curvularia lunata*, *Alternaria brassicicola*, *Colletotrichum capsici*, and *Colletotrichum gloeosporioides* [25, 26]. Albofungin A was discovered for the first time in *Streptomyces chrestomyceticus* BCC 24770 [27]. In addition, 8 compounds were not found in *S. albus* AN1 and *S. albus* J1074 isolated from beehives using the UHPLC-HRMS/MS [14]. In addition, 6 compounds with no functional annotation were also determined in the RC2 extract. Thus, albofungin A might not be responsible for the remarkable antifungal activity of *S. albus* RC2.

Recently, deciphering secondary metabolite BGCs involved in the production of antimicrobial agents has become more effective with the aid of whole-genome sequencing and genomic mining [7, 11]. Using antiSMASH, two BGCs encoding antifungal metabolites, tambjamine BE-18591 and ibomycin, were identified. Tambjamine BE-18591 is recently known as a new type of tambjamine antibiotic group which is considered a new source of antimicrobial and anticancer compounds [28]. In case of very

Table 2 Compounds identified in the ethyl acetate extract of strain RC2

No	Predicted compounds	Formula	Molecular weight	Biological activity
1	1,2,3,4-tetrahydroquinazoline-2,4-dione	$C_8H_6N_2O_2$	162	Antibacterial
2	Griseorhodin E	$C_{13}H_{12}O_6$	264	Antibacterial
3	De-amosaminyl-cytosamine	$C_{16}H_{25}N_3O_5$	339	Unknown
4	Anthracimycin	$C_{25}H_{32}O_4$	396	Antibacterial, anticancer
5	Sarubicin B	$C_{13}H_{10}N_2O_4$	258	Antibacterial, anticancer
6	Albonoursin	$C_{15}H_{16}N_2O_2$	256	Antibacterial
7	Albofungin A	$C_{26}H_{22}N_2O_9$	506	Antibacterial, antifungal
8	Indoxamycin D	$C_{22}H_{30}O_4$	358	Anticancer

high similarity, tambjamine BE-18591 BGC might contribute to the antifungal activity of *S. albus* RC2 if being expressed. The co-culture of *Streptomyces* sp. WAC2288 isolated from soil with the fungus *Cryptococcus neoformans* led to the production of ibomycin, which exerted antifungal activity against yeast pathogens [29]. But no report has proved that *Streptomyces* spp. are able to independently synthesize ibomycin. The pan-genome of *Streptomyces* is known to be open, in which various cryptic BGCs are only induced under defined conditions representing untapped sources of novel metabolites [11, 30]. Combining with the metabolomic analysis, 2 BGCs could not be induced in the PDB at 30 °C for 8 days. Since many compounds were undetectable using metabolomic and genomic analysis, *S. albus* RC2 might likely synthesize a novel compound with antifungal activity, which is an interesting subject for future investigations.

Besides antifungal activity, the capability of inhibiting pathogenic bacteria was also an outstanding biocontrol feature of endophytic *S. albus* RC2. Under laboratory condition, 6 antibacterial compounds, which were identified by the dereplication study of RC2 extract, were not found in its counterparts such as *S. albus* AN1 and *S. albus* J1074. Inhibitory effects against bacteria of AN1 and J1074 extracts were related to nocardamine, paolomycins, salinomycin, and fredericamycin A [14]. In support of antibacterial activity, genomic analysis of RC2 revealed the presence of 6 BGCs encoding for tambjamine BE-18591, xantholipin, aborycin, dudomycin A, thiazostatin, and pseudouridimycin with the similarity ranging from 46 to 96%. However, none of them was predicted in the genome of *S. albus* J1074 [31]. These shreds of evidence supported the assumption that the differences in the BGCs composition could be due to environmental selection pressure and horizontal gene transfer. Therefore, an opportunity to exploit a new antibacterial compound from *S. albus* RC2 remains intact.

Overall, this is the first study to exploit biocontrol potentials of *S. albus* against phytopathogenic fungi prominent in Vietnam, namely *Lasiodiplodia theobromae*, *Fusarium fujikuroi*, and *Scopulariopsis gossypii*. The combination of genome mining and metabolic profiling methods as a central player enabled us to prove the metabolic wealth of endophytic *S. albus* RC2 which was not completely identical to its counterparts such as AN1 and J1074 previously published [14, 31]. Novel and reported metabolites of RC2 might directly attack phytopathogenic fungi, which could lead to abnormal morphology, suppression of spore germination, and cytoplasmic organelles disorganization. A common phenomenon is that the capability of suppressing fungal pathogens under laboratory condition is not subjected to efficiency in fields as biocontrol agent. The successful model was *Streptomyces lydicus* WYEC108 which was formulated and commercialized as biocontrol products Actinovate® and Actino-Iron®. Therefore, the development of product prototypes and greenhouse experiments to demonstrate effective control of the plant diseases still needs to be further investigated.

Conclusion

In this study, endophytic *S. albus* RC2 was screened out as the most outstanding candidate with antifungal activity against phytopathogenic fungi including *S. gossypii* Co1, *L. theobromae* N13, and *F. fujikuroi* L3 causing plant diseases in Vietnam. The ethyl acetate extract of RC2 strongly inhibited both mycelium and spore germination of 3 fungal plant pathogens. In addition, treatment with RC2 crude extract resulted in the growth inhibition of 4 human pathogens and 2 phytopathogenic bacteria with inhibition zones ranging from 12.0 to 34.0 mm. In support of this phenomenon, genomic and metabolic studies highlighted the biocontrol potential of *S. albus* RC2 as an excellent bioresource able to produce various secondary metabolites. Thus, *S. albus* RC2 with an endophytic lifestyle might be a potent biocontrol agent protecting various crops in Vietnam.

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Author contribution NTQ and THNV designed this study; TTAN, PCL, GDH, TDN, TTLN, and THTP performed experiments; NTQ, HHC, and QTP wrote the paper. All authors approved this final manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability All data analyzed during this study are included in this published article. Data will be shared upon reasonable request.

Declarations

Ethics approval Not applicable.

Competing interests The authors declare no competing interests.

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