Characterization of endophytic Streptomyces griseobrunneus Ahn75 and its potential for biocontrol and plant growth-promotion in rice

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Abstract

Plant endophyte *Streptomyces* are excellent candidates as biocontrol agents against the rice blast fungus, *Magnaporthe oryzae*. In this study, rice endophytic strain Ahn75 was isolated from healthy rice stem and identified as *Streptomyces griseobrunneus* by morphological and molecular characterization. Then the potential of strain Ahn75 for inhibition against *M. oryzae* and growth-promotion were evaluated using an in vitro assay, genome sequencing, and in vivo greenhouse pot experiments. Strain Ahn75 significantly inhibited the growth of *M. oryzae*, and its culture filtrate caused 80.88% mycelia growth inhibition, 78.26% spore germination inhibition and significant morphological alterations in *M. oryzae*. Meanwhile, strain Ahn75 also exhibited several growth-promoting properties, including phosphate and potassium solubilization, siderophore production, and nitrogen fixation. Genome sequencing results showed 40 gene clusters of secondary metabolites, including lactones, non-ribosomal peptide synthetases (NRPS), siderophores, polyketide antibiotics, terpenes, and peptides, that may synthesize active compounds against *M. oryzae*, and several genes related to plant growth promotion were exhibited in the genome of Ahn75. In greenhouse test, Ahn75 dramatically reduced rice leaf blast incidence by 59.76% and increased grain yield by 55.57% and 59.22% in wet and dry weight, respectively. These findings suggest that strain Ahn75 could be a potential biocontrol and growth-promoting agent for rice.

Introduction

Rice blast disease caused by *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*) is one of the most serious diseases in cultivated rice (*Oryza sativa* L.), which is the staple food resource for more than half of the world's population (Amruta et al. 2018). At present, the management of rice blast mainly relies on chemical fungicides and resistant rice varieties. However, chemical fungicides not only bring serious pollution to ecology and the environment, but also have deleterious implications for human health. Meanwhile, some resistant varieties became susceptible after a few years of cultivation due to blast fungus evolution and adaptation (Miah et al. 2013). Therefore, microbiological control has been considered as one of the most promising alternatives for the management of rice blast. Previous studies showed that rhizospheric *Bacillus amyloliquefaciens* UASBR9 (Amruta et al. 2018), *Pseudomonas* sp. EA105 (Spence et al. 2014), *Trichoderma sp*. (Prabhakaran et al. 2015), and *Streptomyces* sp. (Law et al. 2017) could inhibit the growth of the rice blast fungus. Among the microbial candidates for biocontrol, actinomycetes, especially endophytic actinomycetes, are of the utmost value because of their high antibiotic productivity, filamentous and sporulating properties, and excellent colonization aptitudes (Vurukonda et al. 2018; Xu et al. 2019).

Endophytic actinomycetes, which live in the inner parts of rice and do not cause any damage or disease to the host plants, can kill or inhibit pathogenic microorganisms, induce systemic resistance, or promote growth in host plant through the secretion of abundant active compounds (Vurukonda et al. 2018). The unique plant inner space also creates more probability of novel endophytic actinomycetes and active compounds and favors endophytes to exert beneficial effects without being influenced by field operations, climate change, and other factors for their growth (Eljounaidi et al. 2016; Kandel et al. 2017).

For example, endophyte *Streptomyces hygroscopicus* OsiSh-2 has been found to inhibit the growth of *M. oryzae* and reduce rice blast disease severity by 59.64%, which may be due to the antagonistic activity by antibiotic nigericin (Xu et al. 2017), iron competition strategy by siderophores (Zeng et al. 2018), and damage to the cell wall and membrane in pathogens by lytic enzymes (Xu et al. 2019). Endophytic *Streptomyces* sp. SS1, SS5, and SS8 colonizing rice tissues not only inhibit *M. oryzae* by the production of active metabolites and upregulation of rice defense response genes but also significantly enhance rice growth, with an increased wet shoot weight of 55–518% (Patel et al. 2018). The endophytic actinomycetes resources used as biopesticides and biofertilizers agents are still insufficient to meet the needs of rice cultivation, although some of them have showed good biocontrol and plant growth-promotion properties.

To explore novel high-efficiency biocontrol resources against rice blast, this study isolated and characterized rice endophytic actinomycetes against *M. oryzae*. An endophyte *Streptomyces* isolate with good antagonistic effects against *M. oryzae* was obtained from the stem of rice in Hainan, China. This work analyzed the antagonistic activity of this strain and evaluated its biocontrol and plant growth-promotion potential in vitro and in vivo.

Material And Methods

Isolation of endophytic actinomycetes

Endophytic actinomycetes were isolated from rice tissues collected from rice variety ‘Xiangliangyou 900’ in Sanya, Hainan, China. The rice plants were washed thoroughly, dried overnight, divided into root, stem, sheath and leaf tissues, cut into pieces (5 cm in length), and then subjected to a surface sterilization procedure as described by Xiong et al. (2014).

After surface sterilization, the dried healthy and diseased plant segments were cut into pieces (1 cm in length) and transferred onto five different isolation media: humic acid vitamin B agar (HV), mannitol soybean agar (MS), tap water yeast extract agar (TWYE), water agar (WA) (Garbeva et al., 2014; Le et al., 2016; Xu et al., 2017) and nutrient agar (NA). Each medium was pre-supplemented with 20 $\mu g \cdot mL^{-1}$ nalidixic acid and 50 $\mu g \cdot mL^{-1}$ benomyl as antibacterial and antifungal agents respectively. Isolation plates were incubated at 27°C and 37°C and checked every 2 days. The emerged actinomycetes colonies were transferred on half-strength potato dextrose agar (half-PDA) for purification.

Screening of antagonistic actinomycetes against *M. oryzae*

Rice blast pathogenic fungi *M. oryzae* TJ40-2-1 were kindly provided by Zhou Hu of Hunan Agricultural University (Changsha, China) (Zhou et al. 2021). The antagonistic activity of actinomycetes against *M. oryzae* was analyzed using a dual culture assay. *M. oryzae* was cultured on International *Streptomycetes* Project (ISP)-2 agar medium for 5–7 days; then, a new 8-mm-diameter mycelia plug was cut and inoculated onto the center of a new ISP2 plate, and three actinomycetes were inoculated onto the same plate, 30 mm from the center, by sterile

toothpick. There were three replicates for each strain. After incubation at 28°C for 7 days, the radius of the *M. oryzae* colony was measured. The inhibition rate of mycelia growth was determined by the following equation:

Inhibition rate = (Radius of control *M. oryzae* colony – Radius of treated *M. oryzae* colony) / (Radius of control *M. oryzae* colony – Radius of the original block).

Identification of endophytic actinomycete Ahn75

The cultural characteristics of Ahn75 were determined on PDA and ISP2 medium. The mycelia and spores of Ahn75 were examined by phase microscopy (Zeiss A1, Oberkochen, Germany). Molecular characterization was performed by taxonomical identification based on the sequence of the 16S rRNA gene and multilocus sequence analysis (MLSA) based on the sequences of five housekeeping genes (*gyr*B, *atp*D, *rec*A, *rpo*B, and *trp*B), which was proved to provide better resolution and stability for species delineation within *Streptomyces*, than MLSA using six housekeeping genes (16S rRNA, *gyr*B, *atp*D, *rec*A, *rpo*B, and *trp*B) and alone 16S rRNA gene scheme (Rong and Huang, 2010). Genomic DNA was extracted using a commercial DNA extraction kit (Ezup Column Bacteria Genomic DNA Purification Kit, Sangon Biotech, Shanghai, China) following the manufacturer's protocol. The 16S rRNA gene was amplified by PCR with universal primers 27F/765R and 705F/1492R (Coombs and Franco 2003), and other five housekeeping genes, *atp*D, *gyr*B, *rec*A, *rpo*B, and *trp*B, were PCR amplified and sequenced using primers described previously (Guo et al. 2008; Suárez-Moreno et al. 2019).

The sequences of 16S rRNA genes were used to construct the neighbor-joining phylogenetic tree by Clustal W2 and MEGA7.0. The sequences for five loci, namely, *atp*D, *gyr*B, *rec*A, *rpo*B, and *trp*B genes from Ahn75, were concatenated head to tail in frame and exported in FASTA format (Guo et al. 2008; Jolley and Maiden 2010; Rong and Huang 2010). The sequences of representative *Streptomyces* strains were downloaded from the *Streptomyces* PubMLST database (http://pubmlst.org/streptomyces/). All sequences were aligned using Clustal W2 and MEGA7.0, and a neighbor-joining phylogenetic tree was constructed from the concatenated sequences of all five loci.

Effect of Ahn75 on the mycelial growth and spore germination of *M. oryzae*

Strain Ahn75 was inoculated into ISP2 liquid medium and incubated at 28°C and 180 rpm for 7 days, and the cell-free culture was obtained by centrifugation at 12,000 g for 10 min at 4°C, then filtered through a 0.22 μm membrane. The culture filtrate was diluted with sterile water; then, 1 mL stock or diluted liquid was added to 9 mL of ISP2 liquid medium at 50°C, mixed rapidly, and poured into Petri dishes (90 mm in diameter). The control comprised 1 mL sterile water in place of the culture filtrate. After the agar cooled down, a new 8-mm-diameter mycelia plug of *M. oryzae* was inoculated onto the center of the ISP2 medium containing 0, 1%, 2%, 5%, and 10% culture filtrate of Ahn75. Each experiment was performed three times. After incubation at 28°C for 7 days, the diameter of the *M. oryzae* colony was measured, and the inhibition rate and IC_{50} was measured by Excel 2010 and SPSS v19.0. The hyphal morphology of *M.*

oryzae grown on coverslips, which was inserted obliquely in the ISP2 agar medium containing culture filtrate of Ahn75, was observed using a phase contrast microscope (Zeiss A1, Oberkochen, Germany).

For spore germination inhibition tests, spores of *M. oryzae* were harvested in sterile distilled water by flooding the oatmeal–tomato agar plates (30 g·L^{-1} oatmeal, 150 mL·L^{-1} tomato juice, and 20 g·L^{-1} agar) of 10-day-old cultures. Then, the spore suspension was filtered through a muslin cloth to remove the mycelia, adjusted to approximately 5×10^5 spores·mL^{-1}, and used in the following bioassays. The culture filtrate of Ahn75 was added to 100 µL ISP2 liquid medium containing the spore suspension (1×10^5 spores·mL^{-1}) of *M. oryzae* to yield a final concentration of 5, 10, 20, and 50% culture filtrate (v/v). The ISP2 liquid medium only containing the spore suspension (1×10^5 spores·mL^{-1}) was used as the control. After incubation at 28°C for 16 h, the germinated and non-germinated spores in each culture were visualized and counted using phase microscopy (Zeiss A1, Oberkochen, Germany). Six images were visualized on each treatment, which was repeated three times. Percentage germination was calculated by the number of germinated spores and the total number of spores in the images (Spence et al. 2014). Spore germination inhibition rate = (Control spore germination rate – Treated spore germination rate) / (Control spore germination rate)

Plant growth-promoting (PGP) related traits

The PGP traits of the antagonists, including phosphate and potassium solubilization, siderophore production, and nitrogen fixation, were qualitatively determined by following standard procedures. Strain Ahn75 was grown on ISP2 agar medium for 3–5 d, and spores were harvested by flooding the plates with sterile distilled water containing 0.5% Tween 80. The spore suspension was adjusted to approximately 1×10^8 spores·mL^{-1}. The spore suspension (10 µL) was spotted on different agar medium plates. Pikovskaya's agar containing tricalcium phosphate (Verma et al. 2018), Aleksandrov medium with potassium feldspar powder as the only source of K (Zhou et al. 2021), chrome azurol-s (CAS) agar (Jasim et al. 2014), and nitrogen-free (NFM) agar plates (Ben Abdallah et al. 2018) were used for the evaluation of phosphate and potassium solubilization, siderophore production, and nitrogen fixation, respectively.

Genome sequencing, assembly, and annotation

Genomic DNA was extracted and used to construct an Illumina PE library, which was sequenced on an Illumina HiSeq 2500 using the PacBio RS II sequencer. After quality filtering, qualified reads were assembled into a single contig by SOAPdenovo v2.04; and the local cavity was filled and the base was corrected by GapCloser v1.12. Gene predictions were performed by Glimmer 3.02 (http://www.cbcb.umd.edu/software/glimmer/). rRNA and tRNA genes were predicted by Barrnap 0.4.2 and tRNAscan-SE v1.3.1, respectively. Functional annotations of predicted genes were based on BlastP similarity searches (E-value < 10^{-5}) against different databases, including the NCBI Non-Redundant Protein database (Nr, http://www.ncbi.nlm.nih.gov/), the STRING database (http://string-db.org/), Clusters of Orthologous Groups database (COG, http://www.ncbi.nlm.nih.gov/COG/), Kyoto Encyclopedia of

Genes and Genomes database (KEGG, http://www.kegg.jp/kegg/), and the Gene Ontology database (GO, http://www.geneontology.org/). Secondary metabolite gene clusters were predicted by antiSMASH v5.0.0 (http://antismash.secondarymetabolites.org/).

Greenhouse pot experiment

The biocontrol and plant growth-promoting effects of strain Ahn75 were investigated on 'Xiangliangyou 900' in the greenhouse. For the pot experiment, rice seeds were soaked in sterile distilled water for 30 min, and healthy seeds were selected, surface sterilized with 75% ethanol for 5 min, washed five times with sterile distilled water, and transferred to a double layer drain basket. Seeds were germinated in an artificial climate incubator at 25°C for 2 weeks. After root-soaking in an Ahn75 spore suspension (containing 10^8 spores·mL^{-1} in 0.05% v/v Tween 80) for 1 h, the seedlings were transplanted to plastic pots (30 cm*18 cm*20 cm) filled with 4.0 kg field soil and 0.5 kg vermiculite, with 3 seedlings per pot. Rice plants were routinely grown in the greenhouse with natural sunlight, and inoculated with an Ahn75 spore suspension again, using foliar spray technique at the tillering stage. A control assay was conducted simultaneously, using sterile water in place of Ahn75 spore suspension. Then, all treated plants were sprayed with *M. oryzae* conidial suspension (10^4 spores·mL^{-1}) 5 days after the second Ahn75 spray application. The disease lesions on the leaf surface were counted 10 days after pathogen infection, and the leaf blast incidence was calculated by dividing the number of diseased plants by the total plants in each pot. The experiment was repeated three times. The grains were harvested at maturity. Then, the fresh weight and dry weight of grains from each pot were obtained to determine the yield parameters.

Statistical analysis

Means and standard deviations were calculated and statistically analyzed by an analysis of variance (ANOVA) at the 5% level using SPSS v19.0.

Results

Isolation and screening of antifungal endophytic actinomycetes

In this study, 122 endophytic actinomycete strains were isolated from healthy and diseased rice segments. Among them, 65.57% strains showed antagonistic activity against *M. oryzae*, and the frequency of antagonistic endophytes was associated with the tissue source, culture medium, and temperature by statistical analysis (Table 1). More antagonistic actinomycetes appeared from healthy rice stems, and on the TWYE and MS media at 37°C. One isolate, Ahn75 (CCTCC No. M 2019890), which was obtained from a healthy stem on TWYE medium at 37°C, showed an inhibition rate of 53.49% ± 3.38% against *M. oryzae* by dual culture assay.

Identification of strain Ahn75

Strain Ahn75 grew on different agar media. However, it grew better on ISP2 medium than PDA medium. When it was grown on ISP2 agar medium, the colonies were round with white aerial mycelium and gray-green spores after 2–3 d. The substrate mycelium was white at early cultivation and then turned brown gradually (Fig. 1a).

From 16S rRNA gene sequence analysis using BLAST at NCBI, the strain was classified as belonging to the genus *Streptomyces*. The phylogenetic tree based on the neighbor-joining method showed that strain Ahn75 had the closest sequence similarity with type strain *S. griseobrunneus* ATCC 4.1838 (99.48%) (Fig. 1b). To improve taxonomic identification, MLSA of five housekeeping genes (*atp*D, *gyr*B, *rec*A, *rpo*B, and *trp*B) was used to analyze strain Ahn75. A neighbor-joining tree based on five housekeeping genes suggested that strain Ahn75 was most similar to *S. griseobrunneus* ATCC 4.1838 and *S. bacillaris* CGMCC 4.1584 (Fig. 1c), which are known as heterotypic synonyms of the same species according to Rong and Huang (2010). All loci obtained from strain Ahn75 showed 98.93% and 99.05% sequence similarity to *S. griseobrunneus* ATCC 4.1838 and *S. bacillaris* CGMCC 4.1584, respectively, and the evolutionary distance calculated with Kimura 2 parameters was below the species definitive MLSA distance of 0.007 , which suggested that Ahn75 is the same species as *S. griseobrunneus* or *S. bacillaris*.

Activity of strain Ahn75 against *M. oryzae*

The mycelial growth of *M. oryzae* on ISP2 agar medium containing culture filtrate of Ahn75 was inhibited, and the inhibition rate was dependent on the concentration of the culture filtrate (Fig. 2, Fig. 3a). When the concentration of the culture filtrate ranged from 1% to 10%, the inhibition rate of mycelial growth gradually increased from 25.05% to 80.88% with an IC_{50} value of 2.21% (95% confidence interval, 0.522% to 4.645%).

Meanwhile, the spore germination of *M. oryzae* was also be inhibited by the culture filtrate of Ahn75 (Fig. 3b). When 50% culture filtrate by volume was used, the inhibition rate of spore germination reached 79.15% ± 7.18%.

Using a phase-contrast microscope, it was observed that the mycelia of *M. oryzae* became shorter when treated with 2% and 5% culture filtrate of Ahn75, as did the single cell, and part of the cells appeared swollen, distorted, or formed vesicle structures (Fig. 4).

These results indicated that the culture filtrate of Ahn75 can significantly inhibit mycelial growth and spore germination of *M. oryzae* with concentration dependence, and cause mycelia to be shorter, swollen, or distorted.

Plant growth-promoting features of strain Ahn75

Strain Ahn75 developed small hydrolysis zones around the colonies grown on Pikovskaya's and Aleksandrov medium plates, indicating phosphate and potassium solubilizing ability. Ahn75 also showed

a positive result in nitrogen fixation by growing well on nitrogen-free medium for more than 5 generations. Ahn75 decolored the blue-colored ferric CAS complex to orange with a clear zone surrounding the colony on the CAS plate, which indicated the presence of siderophores (data not shown). These results suggests that Ahn75 can decomposite mineral phosphate and potassium, fix atmospheric nitrogen, and produce siderophores.

Genomic features of strain Ahn75

The draft genome of strain Ahn75, which was deposited at GenBank under accession number JAJQWZ000000000, had a consensus length of 7,550,402 bp assembled by 115 scaffolds. Gene predictions resulted in 6980 open reading frames (ORFs). The function of 6435 ORFs was annotated and 3556 ORFs were classified into 21 COG categories by their function. Among the 21 COG categories, the cluster for “amino acid transport and metabolism” represented the largest group (368, 9.97%), followed by “transcription” (351, 9.51%) and “general function prediction” (314, 8.51%) (Fig. 5).

Through genome mining by the antiSMASH tool, 40 candidate secondary metabolite clusters were predicted in the Ahn75 genome, including three lactones, seven non-ribosomal peptide synthetases (NRPS) (valinomycin, salinomycin, griseoviridin, malacidin, phosphonoglycans and daptomycin), two Nrps-like, three Nrps-pks (diisonitrile antibiotic SF2768, cosmomycin D, and SGR PTMs.), four siderophores (desferrioxamine B, griseobactin, coelichelin, and ficellomycin), eight polyketide antibiotics (bafilomycins, salinomycin, brasilinolide, nonactin, auricin, alkylresorcinol, and herboxidiene), five terpenes (geosmin, hopene, isorenieratene, and stambomycin), six peptides, and two others (Table S1). The total lengths of these gene clusters were estimated to be about 1196 kb, which suggested that 15.84% of the genome may be occupied by genes concerned with the biosynthesis of secondary metabolites, a far higher proportion than that found in other sequenced genomes. Among these secondary metabolites, valinomycin inhibited the mycelia growth of *M. oryzae* with an inhibition rate of 52.27% ± 4.55% at a concentration of 15 $\mu g \cdot mL^{-1}$ in our inhibition assay. Siderophore and bafilomycin have been reported to be active against *M. oryzae* (Zeng et al. 2018; Zhang et al. 2011), and lactones, Nrps, and polyketide antibiotics were reported to possess excellent antibacterial and antifungal activities (Katz and Baltz 2016; Pimentel-Elardo et al. 2010; Sansinenea and Ortiz 2011). The exhibition of multiple antimicrobial metabolite gene clusters provide a good genetic basis for Ahn75 as a biocontrol resource.

Furthermore, the Ahn75 genome also contains a series of genes/gene clusters associated with plant growth promotion, including siderophore biosynthesis, nitrogen fixation, phosphate and potassium transport, growth-promoting hormones (indole acetic acid (IAA), phytase, trehalose), and spermidine (Table S2). Siderophore can not only inhibit plant pathogens but also promote plant growth by providing iron to the plant. In addition, five nitrogen utilization related genes, *nifU* coding for the nitrogen fixation protein, *glnB* coding for nitrogen regulatory protein II, *moeA* and *moaD* coding for molybdenum cofactor biosynthesis protein, *nir* coding for nitrite reductase, four phosphate transporter genes *pst*A, *pst*B, *pst*C, and *pst*S, and three potassium transporter genes *trk*A, *ktr*B, and *kdp*FABC were found in the Ahn75 genome. IAA synthesis related genes ysnE coding for N-acetyltransferase, dhaS

coding for indol 3-acet-aldehyde dehydrogenase, trpC coding for indole-3-glycerol-phosphate synthase, yhcX coding for nitrilase, the phytase synthesis gene *phy*, trehalose synthesis-related genes, *tps*A coding for alpha, alpha-trehalose-phosphate synthase, *tre*PP coding for trehalose 6-phosphate phosphorylase, and *tre*Z coding for malto-oligosyltrehalose trehalohydrolase were also identified in the Ahn75 genome. The genome also contains spermidine synthase gene *spe*E, and agmatinase gene *spe*B, which are also involved in plant growth and shoot differentiation (Chen et al. 2019).

This genetic basis suggests that strain Ahn75 has great potential as a biocontrol and growth-promotion agent in rice cultivation.

Biocontrol and growth-promoting efficacy of strain Ahn75

The biocontrol and growth-promoting efficacy of strain Ahn75 was assessed in pot experiments. The leaf blast disease symptoms detected at the tillering stage showed that leaves treated with Ahn75 developed significantly less lesions than the control leaves treated with water. The majority (64.17%) of plants developed disease lesions after pathogen infection, but only small parts (26.04%) of plants treated with Ahn75 before pathogen infection developed disease lesions, which suggests that Ahn75 can protect rice plants against leaf blast by reducing disease incidence by 59.76% (Table 2). Meanwhile, rice plants treated with Ahn75 before pathogen infection showed the increase in wet and dry weight of grains by 56.33% and 50.65%, respectively, compared to the control plants only infected with fungal pathogens (Table 2).

Discussion

Plant endophyte actinomycetes were confirmed as new important resources for plant biological control and growth promotion. In this study, 80 antifungal strains were isolated from healthy and diseased rice tissues using five media with different nutrient levels, which were incubated at 27°C and 37°C. It showed that a higher isolation frequency of antifungal actinomycetes were obtained from healthy tissues with few tiny lesions than diseased tissues with severe lesions. Few infected pathogenic fungi can stimulate the colonization and growth of antifungal microorganisms in the plant and improve their predominance in the endophyte microbial community. Moderate nutrient media, such as TWYE and MS, and a higher culture temperature, can also improve the isolation frequency of antifungal actinomycetes.

Then, strain Ahn75, which is highly active against *M. oryzae*, was obtained and identified as *S. griseobrunneus* or *S. bacillaris* by 16S rRNA and MLSA. Based on the results of MLSA, DNA-DNA hybridization, and cultural and morphological characteristics, *S. griseobrunneus* was considered as the heterotypic synonyms of *S. bacillaris* (Rong and Huang 2010). Although all the 6 housekeeping genes from Ahn75 have more than 97% sequence similarity to that of *S. griseobrunneus* 4.1838 and *S. bacillaris* 4.1584, the allele analysis of the six genes still showed two loci from Ahn75, *gyr*B and *apt*D, have the different allelic profiles to that of *S. griseobrunneus*, and three loci from Ahn75, 16S rRNA, *rec*A, and *trp*B, have the different allelic profiles to that of S. *bacillaris* (Table S3), which concluded Ahn75 is a new isolate of *S. griseobrunneus* or *S. bacillaris.* To the best of our knowledge, *S. griseobrunneus* with

antimicroorganism activity has not been reported, but some strains of *S. bacillaris* showed inhibition against Gram-positive bacteria including *Staphylococcus aureus, Enterococcus faecium*, *E. faecalis*, and *Salmonella enterica* (Chung et al. 2021), autophagy (Hu and MacMillan 2012), and protozoal (Pagmadulam et al. 2020). However, antifungal activity against *M. oryzae* is the first to be reported from *S. griseobrunneus* and *S. bacillaris*.

Ahn75 and its culture filtrate strongly inhibited the mycelia growth and spore germination of *M. oryzae* in vitro. The in vivo greenhouse pot assay also demonstrated that Ahn75 can protect rice against leaf blast by reducing the area of the lesion and disease incidence. The high-efficiency antifungal activity may be due to the abundant secondary metabolites produced by Ahn75. Siderophore is one of the most vital antifungal metabolites from Ahn75 and was identified by the CAS assay and the search for gene clusters on the genome sequence. Valinomycin is another active compound that was found in the culture filtrate from Ahn75 by LC-HRMS/MS and genome sequence analysis. Zeng (2018) reported that siderophores can inhibit the growth of *M. oryzae* by competing for iron, and valinomycin is a macrolactone antibiotic with a broad range of bioactivities, including antifungal, antibacterial, antiviral, insecticidal-nematocidal, and cytotoxic/anticancer activities (Sharma et al. 2017). In this paper, we determined that valinomycin can also significantly inhibit the mycelia growth of *M. oryzae*. In addition, the genome of Ahn75 was predicted to contain 35 other gene clusters for the biosynthesis of antimicrobial compounds, such as bacteriocins, lactones, Nrps, and polyketide antibiotics (Katz and Baltz 2016; Pimentel-Elardo et al. 2010; Sansinenea and Ortiz 2011). More importantly, bafilomycin A, K, and D were shown to have antifungal activity against *P. oryzae* (*M. oryzae*) (Zhang et al. 2011). In the genome of strain Ahn75, two gene clusters for the biosynthesis of bafilomycin were also predicted and showed 83% and 38% sequence similarity with that of bafilomycin in the MIBiG BGC database (BGC0000028). Using fluorescence microscopy, our previous study showed that Ahn75 can colonize the stems, roots and leaves of rice seedlings. The re-isolation assay of endophyte Ahn75 also showed that the stem has the highest colonization with more than 10^3 CFU·g^{-1} tissue at 27 days after spraying on the surface of rice seedlings, followed by roots and leaves (Hu et al. 2019). The good colonization capacity may be another important way in which Ahn75 protects rice against leaf blast.

Nitrogen-fixing endophyte bacteria, such as *Azoarcus*, *Burkholderia*, *Azospirillum*, *Burkholderia*, *Klebsiella*, and *Frankia*, have been found in many different plants, including rice, maize, wheat, potato, and tomato, and facilitate the growth of the host plant in nutrient-poor conditions (Kandel et al. 2017; Vurukonda et al. 2018). However, few nitrogen-fixing *Streptomyces* have been identified in rice. *Streptomyces* Ahn75 can grow well on nitrogen-free media, which suggests that Ahn75 can convert atmospheric nitrogen into usable N, which is required for its growth. The nitrogenase synthetic-related genes found in the Ahn75 genome provide the basis for its nitrogen fixation capacity. Strain Ahn75 can reside in internal rice tissues, which may favor a microaerobic environment for nitrogenase activity (Santoyo et al. 2016), as well as provide a continuous supply of nitrogen for host rice and improve the rice growth. Furthermore, Ahn75 can also supply P, K and Fe for rice plants by degrading insoluble phosphate, potassium and chelating iron from the rice rhizosphere environment. The nutrient supply from Ahn75 may directly

promote the growth of rice plants, significantly increasing the grain yield of rice treated with Ahn75 spore suspensions, compared with non-inoculated rice. The antifungal activity of Ahn75 may indirectly promote rice growth by reducing rice blast incidence. In addition, some plant growth-promoting hormones secreted by Ahn75, such as IAA, phytase, and trehalose, may also stimulate rice growth.

In conclusion, rice endophyte *S. griseobrunneus* Ahn75 can significantly inhibit the growth of the rice blast pathogen *M. oryzae* and promote rice growth in vitro and in vivo, demonstrating that Ahn75 has great potential in the biocontrol of rice blast and improving yield in cultivated rice. However, the effects of Ahn75 as a biocontrol against rice blast and growth promotor in the field deserve deeper investigation because the effects are correlated with the colonization level of Ahn75, which was influenced by the forms and quantity of beneficial microorganisms, weather conditions, and inoculated rice growth period.

Declarations

Author contribution

All authors contributed to the study conception and design. Performed the experiments: Strain isolation and identification-RX, RL, Genome analysis-HY, Secondary metabolites-ZH, Pot assay: WC, SW, SS, Analyzed the data: ZF, ZG, PL, Wrote the manuscript-ZF. All authors read and approved the final manuscript.

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Data availability

The genome sequence of strain Ahn75 was submitted in NCBI GenBank. Data and material for this article are available upon request from the authors.

Compliance with ethical standards

Conflict of interest

The authors declare that there is no conflict of interest.

Supplementary Information

The online version of this article contains supplementary material, which is available to authorized users.

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Tables

Tables 1 and 2 are available in the Supplementary Files section

Figures

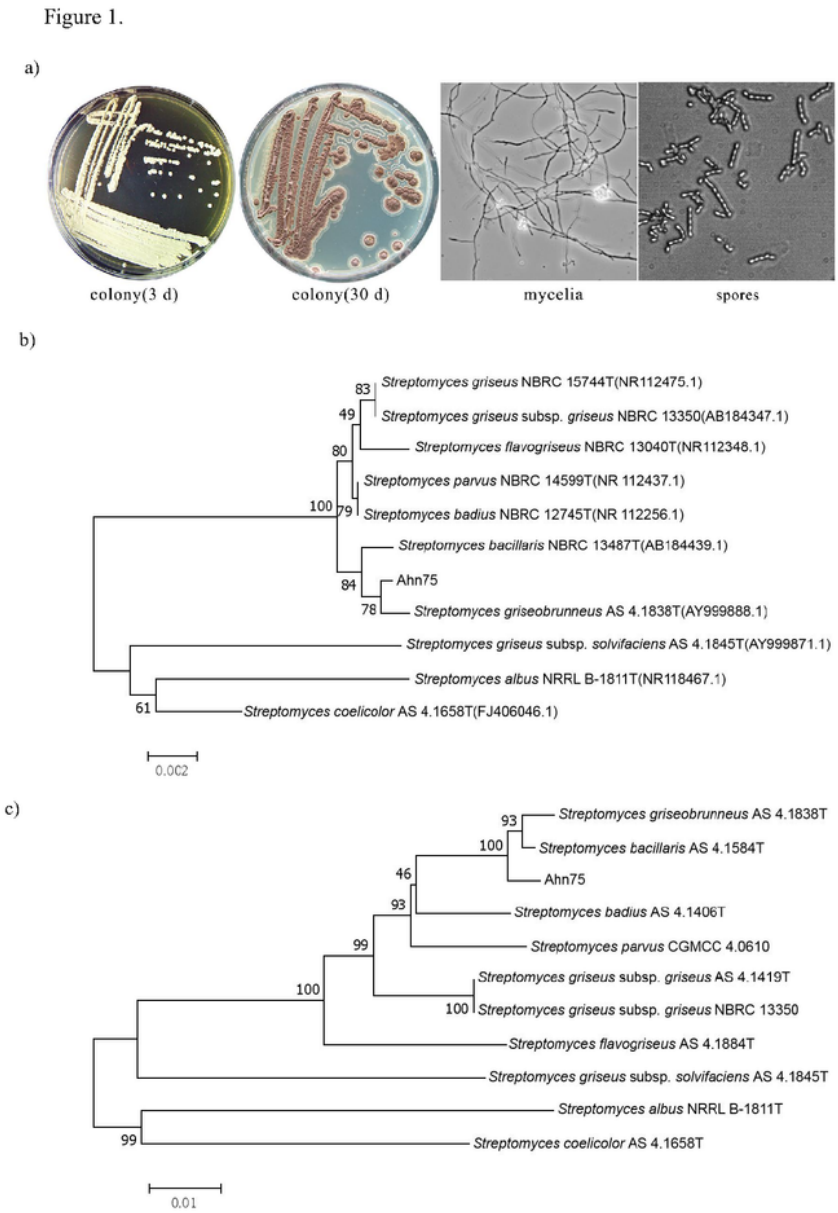

Figure 1

Colony and cell morphology of strain Ahn75 cultured in ISP2 media (a), neighbor-joining trees based on 16S rRNA sequences (b) and MLSA from five loci (*atp*D, *gyr*B, *rec*A, *rpo*B, and *trp*B) (c) of strain Ahn75. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. Nucleotide

sequences of 10 reference strains in Fig. 2a and Fig. 2b came from the GenBank database and the *Streptomyces* PubMLST database, respectively. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7.0.

Figure 2.

Figure 2

Magnaporthe oryzae growing on ISP2 plates mixed with 0–10% culture filtrate of Ahn75.

Figure 3

Inhibition of the culture filtrate of Ahn75 on the mycelial growth and spore germination of *Magnaporthe oryzae*. a) Inhibition rate on the mycelial growth of *M. oryzae*; mean ± SD, n = 3. b) Inhibition rate on the spore germination of *M. oryzae*; mean ± SD, n = 5.

Figure 4.

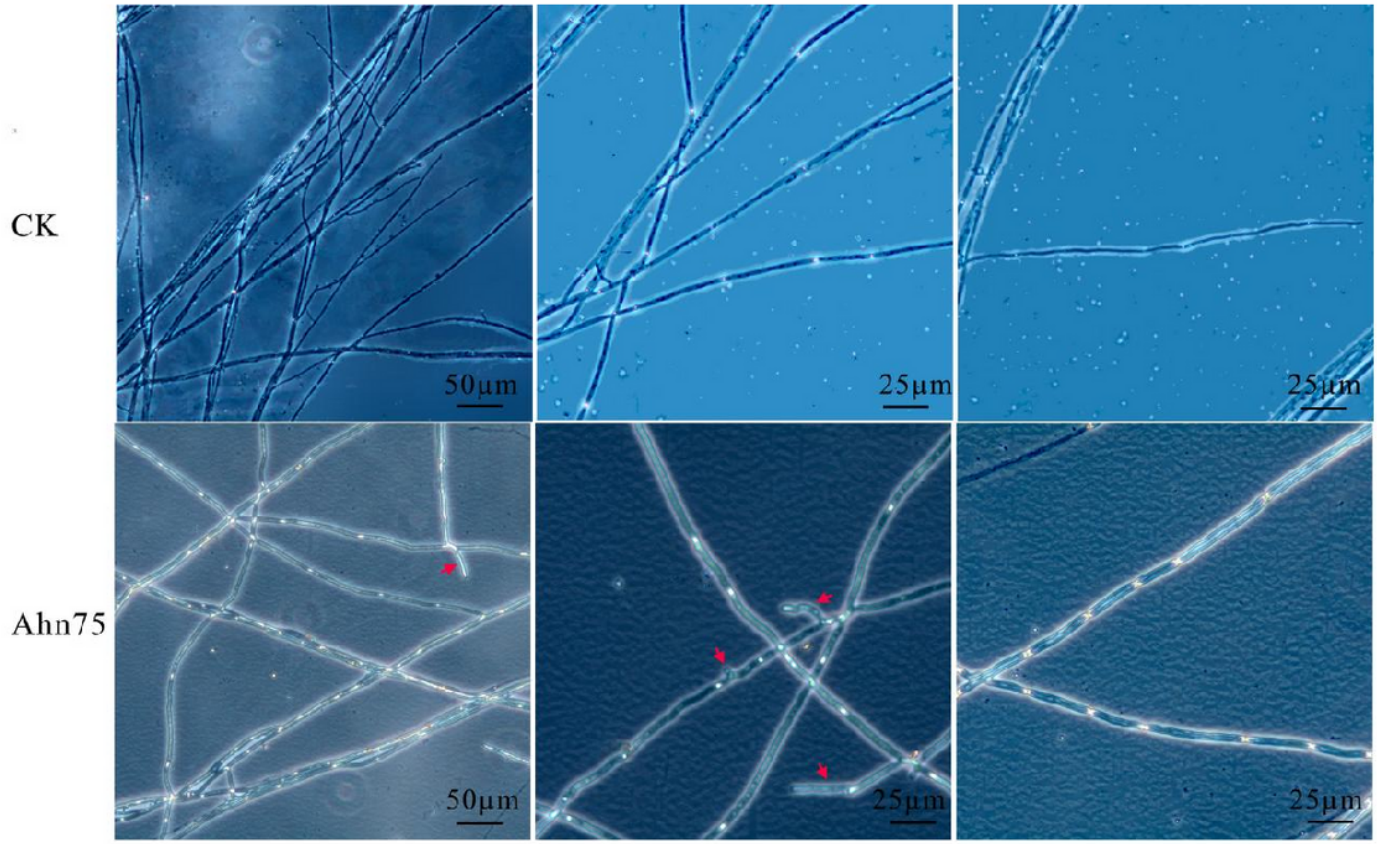

Figure 4

Mycelial morphology of *Magnaporthe oryzae* treated with a Ahn75 culture filtrate. CK: *Magnaporthe oryzae* grown on ISP2 medium; Ahn75: *Magnaporthe oryzae* grown on ISP2 medium with 5% Ahn75 cultural filtrate. The mycelial was observed in phase microscopy, and the the arrow indicates the deformed mycelial.

Figure 5.

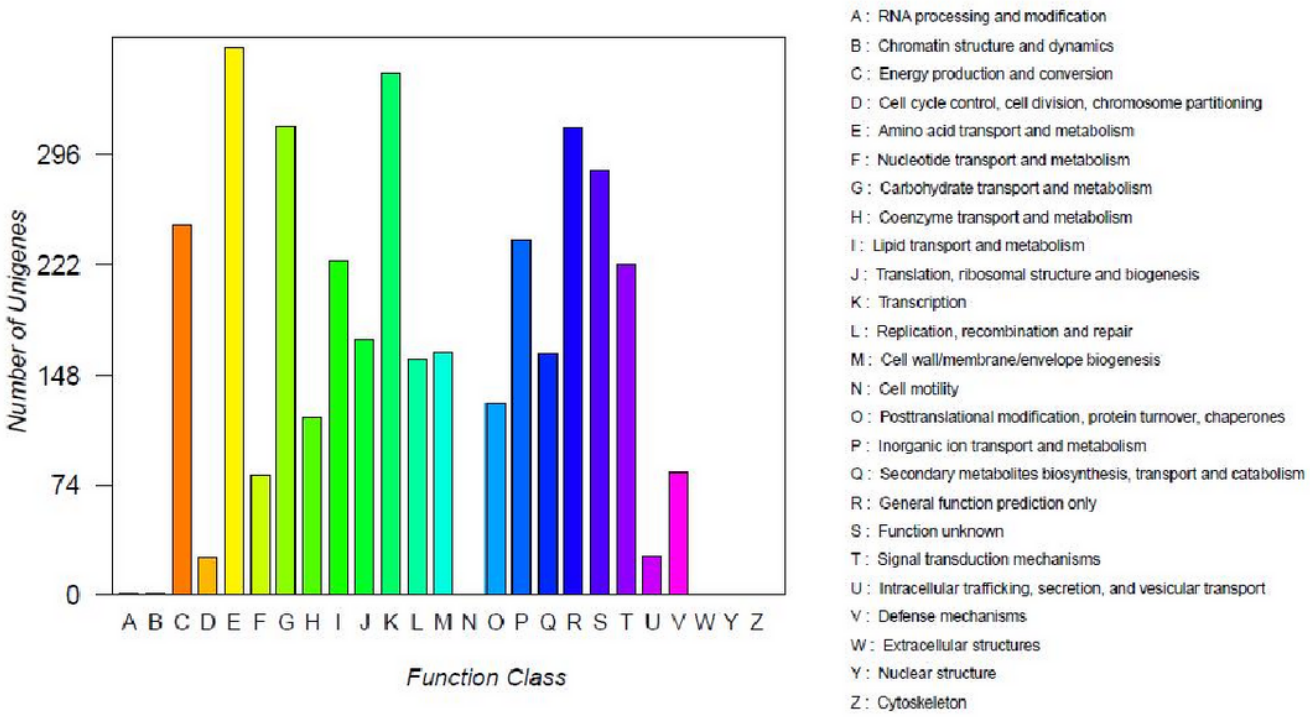

Figure 5

Function class of strain Ahn75 by Clusters of Orthologous Groups database (COG) function classification

Supplementary Files

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