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FUNGAL DISEASES

Control of tomato southern blight caused by *Athelia rolfsii* (syn. *Sclerotium rolfsii*) using the soil isolate *Streptomyces sasae* strain GT4041

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Abstract
Damping off of tomato caused by *Athelia rolfsii* causes serious losses in the Benin Republic despite the use of chemical fungicides. To reduce pesticide dependency and provide better control, we evaluated the activity of soil isolate GT4041 against *A. rolfsii* in Japan for use in Benin. Isolate GT4041, applied in its PC1 culture broth, strongly inhibited *A. rolfsii* mycelial growth in dual culture and completely inhibited sclerotial germination. In growth chamber tests, sclerotia were pretreated with GT4041 or GT4041 was preincubated in soil before application or applied directly on sclerotia on the soil; GT4041was always used in its culture broth. Its direct application on the soil reduced disease incidence the most in the growth chamber and in the greenhouse. GT4041 inhibited mycelial growth of five other tomato fungal pathogens and also increased tomato root and shoot dry mass. GT4041 produced proteases and indole acetic and it grew optimally at 28 °C. Scanning electron microscopy of GT4041 showed cylindrical, smooth spores in flexuous chains. This is the first report of *S. sasae* as a biocontrol agent for tomato damping off caused by *A. rolfsii*.

Keywords *Athelia rolfsii* · Biocontrol · Disease incidence · GT4041 · Stem rot · *Streptomyces sasae* · Tomato plant

Introduction

Tomato is among the most appreciated vegetable fruits grown in the Benin Republic (James et al. 2010), where production nearly doubled from approximately 2.4 million tons in 2011 to approximately 5 million tons in 2020 (FAO 2020). In 2020, worldwide tomato production was estimated to be 186 million tons. Pests, especially fungal pathogens such as *Athelia rolfsii*, however, are a serious threat to tomato cultivation. First reported by Rolfs on tomato in 1892, *A. rolfsii* is a telluric, omnipathogenic, and facultative, soil-borne parasite of many crops and causes damping off and stem rot, and an economically important tomato pathogen in the Benin Republic (Aycock 1966; James et al. 2010). Sikirou et al. (2015) studied the geographical distribution and prevalence of the main tomato fungal pathogens in the Benin Republic and concluded that *A. rolfsii* is the most disseminated and devastating. *A. rolfsii* also infects many other agricultural crops such as peanut and cowpea and more than 500 species of horticultural crops in warm tropical and subtropical areas (Muthukumar and Venkatesh 2014; Punja 1985), where it thrives in rainy and humid environments (Adandonon et al. 2004; Bhuiyan et al. 2012; Mukherjee and Raghu 1997; Punja 1985). Fungicides and tillage, the main control strategies, have limited efficacy because the fungus has a wide host range, grows rapidly and produces a high number of sclerotia that remain viable in the soil for many years (Adandonon et al. 2004; Sennoi et al. 2013). In addition, the numerous fungicides that have been developed against *A. rolfsii* must be applied in large quantities (Punja 1985). Although chemical fungicides such as flutolanil, pentachloronitrobenzene (PCNB) prothioconazole, pyraclostrobin and tebuconazole have been effective in the control of *A. rolfsii*

The nucleotide sequence presented in this paper is available in the DDBJ/EMBL/GenBank database under accession number LC720967.

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(Khatri et al. 2017; Scinos 1989; You et al. 2021), the fungus has developed resistance to PCNB and tebuconazole (Franke et al. 1998; Shim et al. 1998), and its sensitivity decreases to fungicides after frequent use (Franke et al. 1998; Le et al. 2012). Fungicides also have undesireable side effects; they can inhibit soil enzymatic activity, decrease microbial populations and change soil biological activity (Baćmaga et al. 2016, 2019; Ratna Kumar et al. 2015; Roman et al. 2021). As an alternative to fungicides, biological control agents (BCAs) such as *Bacillus amyloliquefaciens*, *Bacillus halotolerans, Bacillus safensis*, *Bacillus subtilis*, *Bacillus velezensis*, *Ganoderma* spp., *Pseudomonas fluorescens*, *Trichoderma harzianum*, and *Trichoderma* sp. inhibited the growth of *A. rolfsii* (Adandonon et al. 2006; Chen et al. 2019; Dutta et al. 2022; Farhaoui et al. 2022; Mukherjee and Raghu 1997; Osemwegie et al. 2010). However, *Streptomyces* species have not been tested (Sahu et al. 2019).

Streptomyces species are gram-positive, spore-producing actinobacteria and main bioactive compound producers among prokaryotic species, producing 39% of all microbial metabolites and 80% of the bioactive metabolites known to be synthesized by species belonging to the order *Streptomycetales* (Bérdy 2012). *Streptomyces* species also produce numerous antifungal compounds that suppress the growth of diverse fungal pathogens (Taechowisan et al. 2003). For instance, antibiotics and antimicrobial enzymes produced by cells of *S. griseus* or present in cell-free extracts of *S. plumbeus* suppress the growth of *Fusarium oxysporum* f. sp. *cubense* (Abdullah et al. 2021; Zacky and Ting 2013). *S. blastmyceticus* also inhibits powdery mildew caused by *Podosphaera xanthii* (Ganphung et al. 2019). In the Benin Republic, banana column juice was found to inhibit *A. rolfsii* in vitro and in tomato plants, but biocontrol agents against *A. rolfsii* on tomato plant have not yet been reported (Sikirou et al. 2010). Toward eventual use against tomato damping-off in the Benin Republic, candidate BCAs were isolated from soils in Gotsu City (Japan), tested for inhibitory activity, and the best candidate was further evaluated in vitro and on tomato seedlings in the growth chamber and greenhouse.

Materials and methods

Plant and pathogen

Seedlings of tomato cv. Reika were used for growth chamber and greenhouse experiments. Seeds were sown in nursery plastic trays (80 × 70 mm) containing commercial soil (Kumiai Nippi Engeibaido No. 1; Nihon Hiryo, Fujioka, Japan). Seedlings were transplanted to 7-cm-diameter vinyl pots and grown using standard cultural practices in a growth chamber (26 ± 2 °C) with 12 h of fluorescent light and sunlight from the chamber window. Seedlings were used 21 days later in the same pot for growth chamber experiments. For greenhouse experiments, 21-day-old seedlings were transplanted to 10-l plastic pots containing 8 l of Sakata Super Mix A soil (Sakata Seed, Yokohama, Japan) and 40 g of Magamp K fertilizer (HYPONeX JAPAN, Osaka, Japan). For all plant experiments, plants were watered according to standard practices.

Two strains of *Athelia rolfsii* were used in this study: MAFF103050 from the Japan NARO Genebank and SHIMANE002 isolated from an experimental field of Shimane University. Each strain was preserved on potato sucrose agar (PSA; 200 g/l potatoes, 2.0% w/v sucrose, 2.0% w/v agar) slants for further use. A mycelial disc (6 mm diameter) or one sclerotium was placed on the center of potato dextrose agar (PDA; 200 g/l potatoes, 2.0% w/v dextrose, 2.0% w/v agar) plates and maintained at 26 ± 2 °C with 12 h light/12 h dark for 7 days to generate mycelia and for 14 days to produce sclerotia.

Antagonist GT4041 isolation and culture

In a study by the plant pathology laboratory of Shimane University, 166 soil microbial isolates were collected from Gotsu City (Kawahira), Shimane Prefecture, Japan, isolated as described by Lemtukei et al. (2016), then screened for inhibtion of mycelial growth of *A. rolfsii*. Among 25 isolates that inhibited the fungus, strain GT4041 was the most inhibitory and tested further in the present study. It was stored in 15–20% v/v glycerol solution at −80 °C until used. From this stock, isolate GT4041 was grown on Luria-Bertani (LB) agar (0.5% w/v yeast extract, 1% w/v bactotryptone, 1% w/v NaCl, 2% w/v agar) in an incubator for 3 days at 28 °C. Individual colonies were transferred to separate culture tubes containing 5 ml of PC1 broth (1% w/v each of starch, polypeptone, molasses, and beef extract in 1000 ml deionized water at pH 7.2). The cultures were incubated at 26 ± 2 °C for 3 days with constant shaking at 180 rpm. For all experiments, the cultures had an $OD_{600nm} > 2$ when used. Unless otherwise specified, these PC1 broth cultures were used in all experiments.

In vitro assays of antifungal activity of GT4041

GT4041 was tested for inhibition of mycelial growth and sclerotial germination of *Athelia rolfsii*. Each experiment was done three times, with five Petri plates per treatment. For tests of mycelial growth inhibition, a 6-mm-diameter mycelial plug of each strain of *A. rolfsii* was taken from the edge of 5-day-old PDA cultures and placed on soil agar (SA) (SA; 4.0% w/v soil, 2.0% w/v agar) at one edge of separate plates. A paper disc (8-mm diameter, Advantec, Tokyo, Japan) was placed 5 cm from the mycelial plug on the same plate, then 50 µl of GT4041 or 50 µl of PC1 broth

(control) was dropped onto the paper disc. Plates were then incubated at 26 ± 2 °C in the dark for 10 days, and mycelial area of inhibition (mm^2) was determined using LIA 32 software (http://www.agr.nagoya-u.ac.jp/~shinkan/LIA32/index-e.html). For tests of sclerotial inhibition, 10 sclerotia were incubated with 3 ml of GT4041 cultured in PC1 broth or in PC1 broth only (control) in tubes at 26 ± 2 °C for 24 h with constant shaking at 180 rpm. One sclerotium was then placed on the center of a PDA plate, then incubated and mycelial area measured as described for the mycelial assay.

Growth chamber and greenhouse tests of GT4041 disease suppression

Three types of applications of GT4041 were tested in growth chamber experiments; either sclerotia were preincubated with a GT4041 culture (treatment 1) or GT4041 cultures were mixed with soil (treatments 2 and 3). (1) Twenty sclerotia of each pathogen strain were incubated in GT4041 broth cultures for 24 h (10 sclerotia per 3 ml), then placed on the soil near the base of the stem of a 3-week-old tomato seedling and lightly covered with untreated soil. (2) GT4041 in its PC1 culture broth (5 ml) was mixed with 15 g of soil in small plastic pots, covered and incubated for 3 days in the dark before being applied over 20 sclerotia that had just been placed around the base of the tomato stem. (3) GT4041 in its PC1 culture broth (5 ml) was mixed with 15 g of soil in small plastic pots, then the mixture was immediately applied in the same manner as the second application. For each strain of *A. rolfsii*, 20 sclerotia were incubated in PC1 broth for 24 h, then placed on the soil near the basal stem of a 3-week-old tomato seedling and covered with previously mixed soil. As a positive control, incubated sclerotia were placed on the soil near the tomato stem and covered with untreated soil. Two weeks after inoculation with *A. rolfsii*, the percentage of plants that developed damping off was calculated as disease incidence (DI): (Number of diseased plants / Total number of Plants) × 100. Five plants were used per treatment per fungal strain, and the completely randomized experiment was done three times.

In experiments in a vinyl greenhouse (< 27 ± 5 °C) from June to September 2021 at Shimane University, 20 sclerotia were placed on soil near the plant stems as in the growth chamber experiments, 10 ml of GT4041 was either mixed with 10 g of soil or not and directly applied on the sclerotia. After application of GT4041-only, it was lightly covered by untreated soil. Five pots of plants were used per treatment, and the completely randomized experiment was done three times. After 9 weeks, DI was determined as described in the previous paragraph.

In vitro activity of GT4041 against other tomato fungal pathogens

GT4041 was also tested in dual culture, as described above, with a mycelial plug from a 14-day-old PDA culture of six other tomato fungal pathogens: *Alternaria alternata*, *Botrytis cinerea*, *Stemphylium lycopersici*, *Fusarium oxysporum* f. sp. *lycopersici*, and *Sclerotinia sclerotiorum*. The area of mycelial inhibition was determined after 3 weeks.

Growth chamber tests of tomato growth promotion by GT4041

GT4041 (3 ml; or PC1 broth as the control) was applied on the soil around the base of 3-week-old tomato seedlings. Five plants were used per treatment, and the experiment was done three times. After 3 weeks, the shoot and the roots of each plant were separated, oven-dried at 70 °C until constant mass, and weighed separately.

Identification of GT4041

DNA was extracted from a GT4041 colony as described by Suzuki et al. (2006), and the 16 S rDNA region was amplified by PCR using the primers described by Huong et al. (2007), Matsui et al. (2009) and Nakagawa et al. (2001) in Table S1. PCR amplification, DNA sequencing, and sequence homology search using BLAST were done as described by Abdullah et al. (2021). A phylogenetic tree was generated using CLUSTAL W for multiple sequence alignments (Thompson et al. 1994). Genetic distances between the aligned DNA were determined using Kimura's two-parameter model (Kimura 1980) and the neighbor-joining method (Saitou and Nei 1987) in MEGA X (https://www.megasoftware.net/). The pathogen *Actinoalloteichus cyanogriseus* NBRC14455 was used as the outgroup.

Scanning electron microscopy

GT4041 was grown on LB agar plates for 5 days, then 3–4 pieces (8 mm diameter) of the colony were cut from the colony and placed in a 2 ml microtube, 1 ml of 6.25% (v/v) glutaraldehyde in 0.2 M phosphate buffer was added, and the tube held at 4 °C for 24 h. The glutaraldehyde was then removed with a pipette, and samples were washed three times for 10 min each with 2 ml of 0.2 M phosphate buffer. Samples were then dehydrated with an ethanol series (2 times × 10 min for each step): 50%, 70%, 90%, 100%. The ethanol was then removed, 2 ml of *tert*-butyl-alcohol was added (2 times), and then the sample was freeze-dried in a *tert*-butyl-alcohol freeze dryer (VDF-21 S: Vacuum Device, Mito, Japan). The freeze-dried sample was coated with platinum using ion sputtering (E-101, Hitachi, Tokyo, Japan).

Mycelia and spores were observed for morphology using a Hitachi S-4800 field-emission SEM at 5 kV (Hitachi High-Tech, Tokyo, Japan).

Production of protease, indole-3-acetic acid (IAA), and optimal growth temperature for GT4041

Protease production was tested on skim milk agar (skim milk powder 5 g; agar 15 g; 1 l water); an 8-mm-diameter paper disc (Advantec, Tokyo, Japan) was placed in the center of the plate and inoculated with 50 µl of GT4041; for the control, 50 µl of PC1 broth was added to the disc. The clear area (mm^2) on the agar was measured after 7 days using LIA 32 software (http://www.agr.nagoya-u.ac.jp/~shinkan/LIA32/index-e.html). For the test for IAA production using colorimetry and Salkowski's reagent (Gordon and Weber 1951), GT4041 was grown in nutrient broth (peptone, 5 g; yeast extract, 1.5 g; beef extract, 1.5 g; and NaCl, 5 g; per liter water) with 0.1% l-tryptophan (w/v), which is a known precursor for IAA synthesis by *Streptomyces* sp (Datta and Basu 2000). The tubes were maintained at 26 ± 2 °C for 4 days with constant shaking at 180 rpm. One milliliter of supernatant was added to 2 ml of Salkowski's reagent and incubated for 30 min at ambient temperature. Absorption was measured at 530 nm using a spectrometer (UV mini-1240: Photometric Mode, Shimandzu co., Kyoto, Japan). A 3-indoleacetic acid standard (Wako Chemical Pure Industries Ltd, Osaka, Japan) was used at 5, 10, 15, 20, 25, and 30 µg/ml to calculate the amount of IAA produced by GT4041 using a regression line (Sarker and Al-Rashid 2013). GT4041 was grown at 4, 20, 28, and 38 °C to test for optimal growth using the method of Haidary et al. (2018), then the colonies on each plate were counted after 7 days.

Statistical analyses

Significant differences ($P < 0.05$) among means for treatment groups in each set of experiment were calculated using Tukey-Kramer's test or a *t* test in SPSS ver. 22.0 for Windows (IBM, Armonk, NY, USA).

Results

In vitro activity of GT4041 against *A. rolfsii*

In dual culture, GT4041 significantly inhibited mycelial growth of *A. rolfsii* with an inhibition zone of 2162.6 ± 622.6 mm^2 (mean ± SD) for strain MAFF103055 and 2910.2 ± 352.4 mm^2 for strain SHIMANE002 compared to 0 mm^2 for the control (Fig. 1). GT4041 also completely inhibited germination of the sclerotia of both strains compared to the control (Fig. 2).

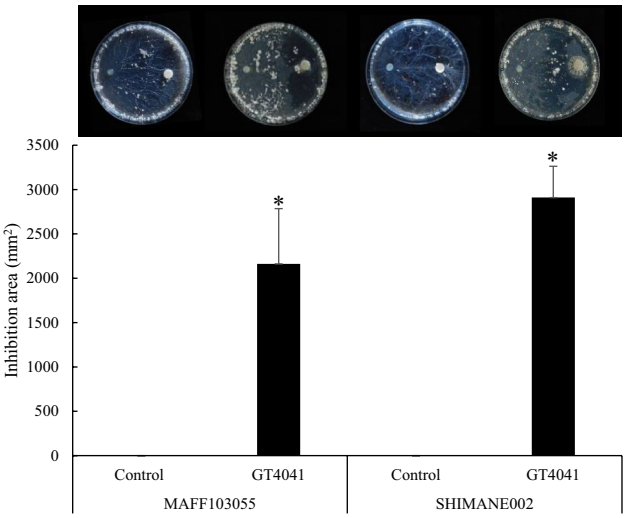

Fig. 1 Mean (± SD) inhibition of mycelial growth of *Athelia rolfsii* (strain MAFF103055 and SHIMANE002) by isolate GT4041 in dual culture on soil agar (SA). An asterisk indicates a significant difference compared to the control (Student's *t* test, $P < 0.05$)

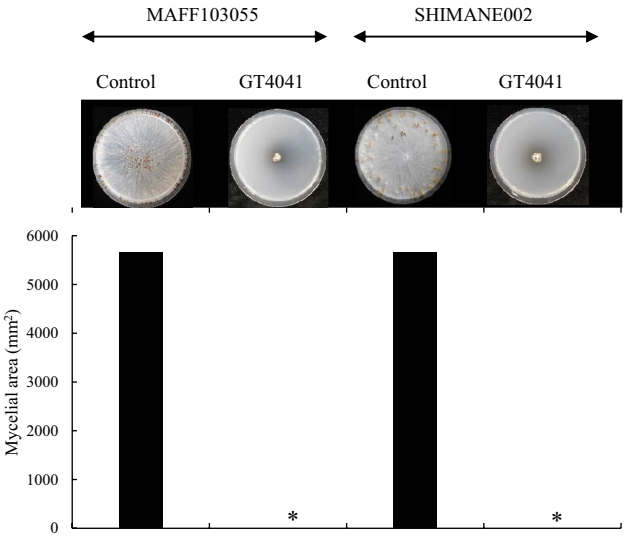

Fig. 2 Mean (± SD) inhibition of sclerotial germination and mycelial growth of *Athelia rolfsii* (strain MAFF103055 and SHIMANE002) by isolate GT4041 on potato dextrose agar (PDA). An asterisk indicates a significant difference compared to the control (Student's *t* test, $P < 0.05$)

GT4041 disease suppression in growth chamber and greenhouse

In the growth chamber tests of the first application type, sclerotia were incubated with GT4041 in its PC1 culture broth before being placed on soil near the plant stems. The mean disease incidence (DI) ± SD caused by strain MAFF103055 was 0% and 23.3 ± 25.2% for SHIMANE002 compared to

100% for the broth-treated controls (Fig. 3A). The pretreatment of the sclerotia thus inhibited disease by pathogenic strain MAFF103055 but not for strain SHIMANE002. In the second application type, GT4041 in its culture broth was mixed with soil, held in the dark for 3 days, then applied on the sclerotia near plant stems. In this case, control of the two strains was not as effective as the pretreatment with the antagonist; DI was 46.7 ± 11.5% MAFF103055 and 53.3 ± 11.5% for SHIMANE002 compared to 100% for the controls (Fig. 3B). In the third application, soil mixed with GT4041 in its culture broth was placed on sclerotia near the stems. DI was 13.3 ± 11.5% with strain MAFF103055 and 26.6 ± 11.5% with SHIMANE002 compared to 100% for the controls (Fig. 3C). The third treatment, in which the soil was mixed with GT4041 in its culture broth and immediately applied, DI was significantly reduced more than in the second treatment, which was applied after a 3-day incubation of the antagonist in the soil.

In the greenhouse experiment, GT4041 in its culture broth was directly applied to sclerotia on soil near plant stems, DI was 6.7 ± 11.5% with MAFF103055 and 0% with SHIMANE002 compared to 100% for the controls (Fig. 4). However, when GT4041 in its culture broth was first mixed with soil, then applied on the sclerotia, DI was 60.0 ± 20.0% with MAFF103055 and 6.7 ± 11.5% with SHIMANE002 compared to 100% for the controls (Fig. 4). Thus, direct application of GT4041 in its culture broth provided better control than when mixed with soil.

In vitro inhibition of other tomato fungal pathogens by GT4041

In dual culture tests, GT4041 inhibited mycelial growth of five of the six tomato pathogens: *Alternaria alternata*, *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *lycopersici*, *Stemphylium lycopersici* and *Sclerotinia sclerotiorum*. The respective mean (± SD) inhibition areas were 438.6 ± 242.7 mm^2, 1368.4 ± 333.3 mm^2, 165.1 ± 216.1 mm^2, 578.4 ± 351.6 mm^2, and 1685.7 ± 596.9 mm^2 (Fig. 5).

Tomato growth promotion by GT4041 in a growth chamber

By 3 weeks after GT4041 was applied to the soil, the mean (± SD) dry mass of tomato shoots and roots was 0.32 ± 0.06 g and 0.11 ± 0.05 g, respectively, compared to the control mass of 0.12 ± 0.04 g for shoots and 0.03 ± 0.04 g for roots (Fig. 6).

Identification of GT4041

Phylogenetic analysis of the 16 S rDNA indicated 99.0% similarity between isolate GT4041 and *Streptomyces sasae* NBRC109809 (Fig. 7).

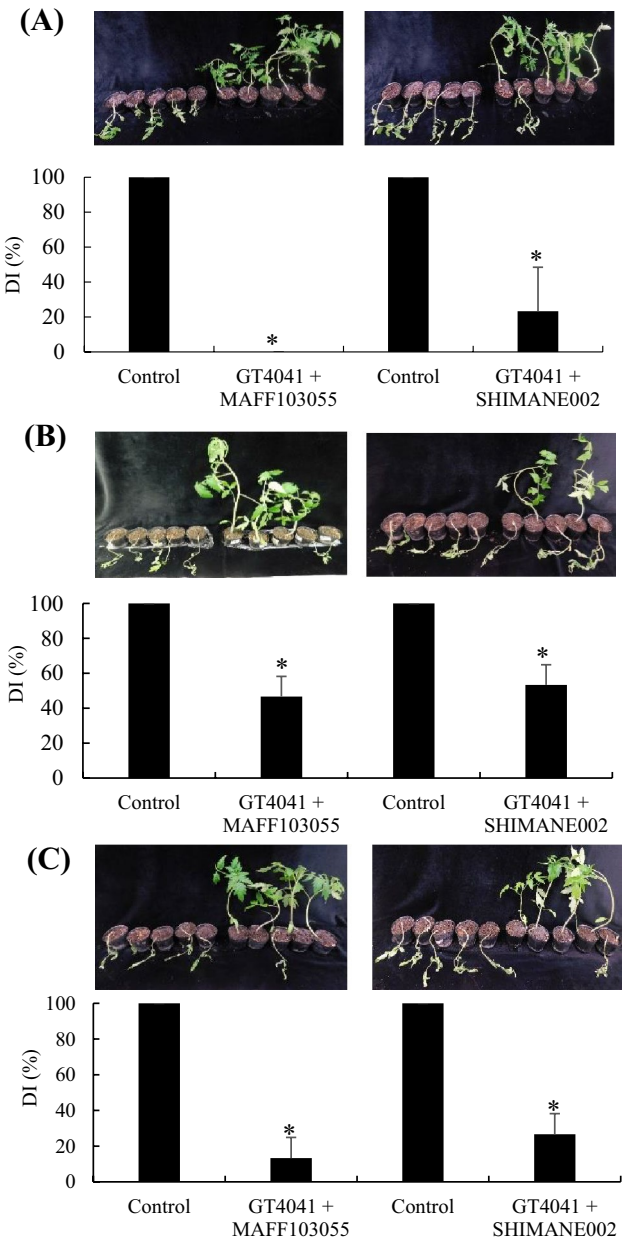

Fig. 3 Disease incidence (DI, % of diseased plants) caused by *Athelia rolfsii* (strain MAFF103055 and SHIMANE002) on tomato seedlings (cv. Reika) after different types of GT4041 application and 2 weeks of incubation in a growth chamber. In **A**, 20 sclerotia for each strain of *A. rolfsii* was incubated with GT4041 in PC1 broth for 24 h with constant shaking at 180 rpm, then placed on the soil near the base of 3-week-old seedlings and lightly covered with untreated soil. In **B**, 5 ml of GT4041 in its PC1 culture broth was mixed with 15 g of soil, held in the dark for 3 days, then applied directly on 20 sclerotia on soil near the stem. In **C**, 5 ml of GT4041 in its PC1 culture broth was mixed with 15 g of soil in small plastic pots, then immediately applied on 20 sclerotia on soil near of the stem. Means and standard deviations are shown; an asterisk indicates a significant difference compared to the control (Student's *t* test, $P < 0.05$)

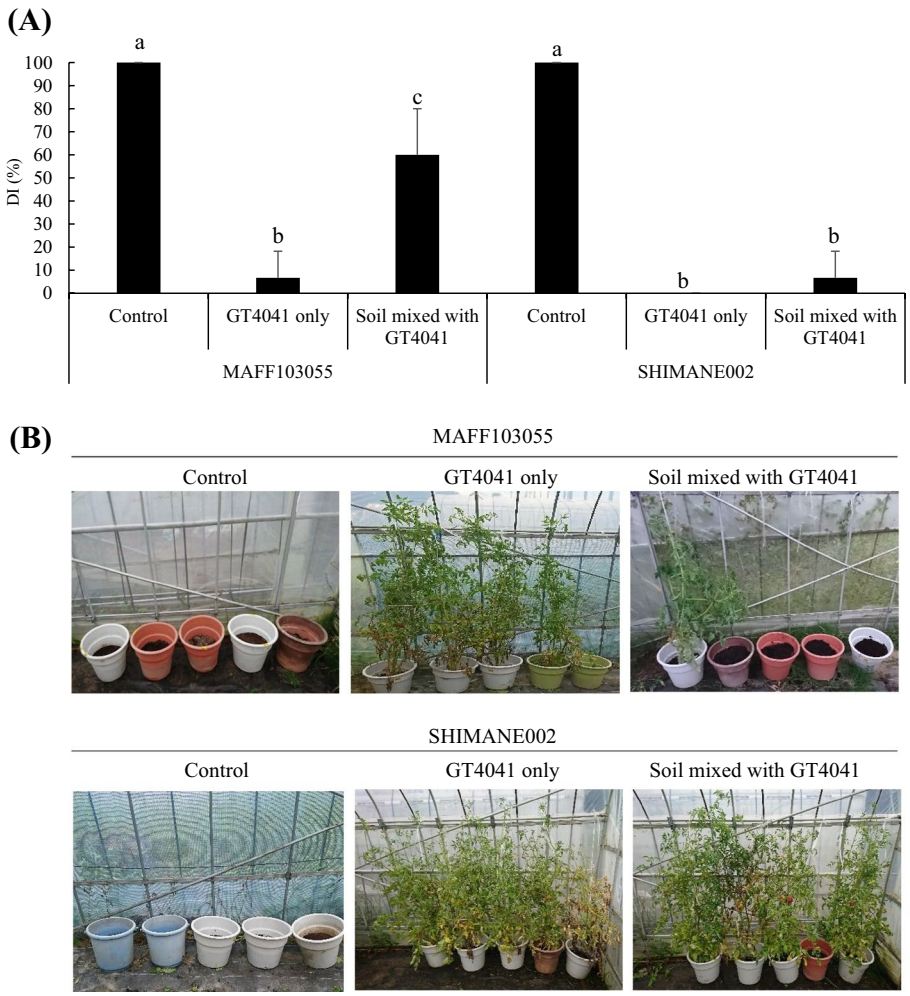

Fig. 4 Disease incidence (DI, % of diseased plants) caused by *Athelia rolfsii* (strain MAFF103055 and SHIMANE002) on tomato seedlings (cv. Reika) after different types of GT4041 application and 2 weeks of incubation in a greenhouse. GT4041 in its PC1 culture broth was mixed with soil (10 ml of GT4041 in 10 g of soil) or not (10 ml of "GT4041 only"), then directly applied on sclerotia on soil near of the stems. "GT4041 only" application was lightly covered with soil. Means and standard deviations are shown. Different letters above the means for a strain of *A. rolfsii* indicates a significant difference compared to the control (Tukey-Kramer's test, $P < 0.05$). The plants in **(B)** are results from the experiment in **(A)**

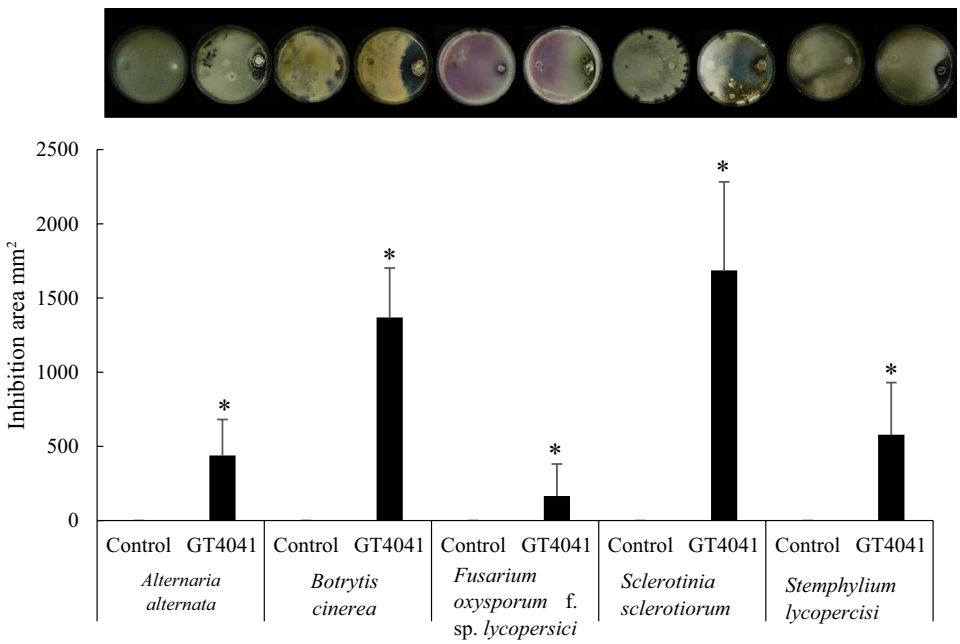

Fig. 5 Mycelial inhibition of six tomato fungal pathogens by isolate GT4041 in dual culture on potato dextrose agar (PDA). Means ± standard deviation are shown; an asterisk indicates a significant difference compared to the control (Student's *t* test, $P < 0.05$)

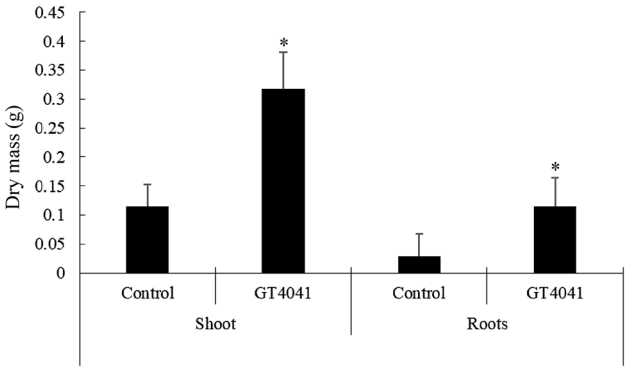

Fig. 6 Tomato (cv. Reika) growth promotion after soil application of isolate GT4041 in a growth chamber. Means ± standard deviations are shown; an asterisk indicates a significant difference compared to the control (Student's *t* test, $P < 0.05$)

Scanning electron microscopy of GT4041

GT4041 had flexuous chains of cylindrical, smooth spores (Fig. 8).

GT4041 production of protease and IAA, optimal growth temperature

GT4041 was positive for protease and IAA production. The clear zone area indicating the presence of protease was 1226.6 ± 153.7 mm^2 (mean ± SD) compared to 0 mm^2 for the control (Fig. 9), and 42.3 ± 4.1 µg/ml of IAA (mean ± SD) was measured compared to 0 µg/ml for the control (data not shown). The mean (± SD) number of colonies at 20 °C, 28 °C, and 38 °C differed significantly (268.2 ± 41.3, 297.2 ± 73.2, and 244.1 ± 64.7, respectively) compared to 4 °C (Fig. 10). Growth was optimal at 28 °C.

Discussion

Isolate GT4041 was identified as *S. sasae* (Fig. 7), which was first reported 7 years ago in South Korea, and its smooth, cylindrical spores in flexuous chains (Fig. 8) and optimal growth temperature agree with previous studies (Lee and Whang 2015; Srivastava et al. 2021). GT4041 can thus be more effective in warm ecosystems. GT4041 in its PC1 culture broth inhibited in vitro mycelial growth of both *A. rolfsii* strains by (Fig. 1), indicating that it produced antifungal compounds. These results are similar with those of other studies in which *A. rolfsii* mycelia were inhibited by *Pseudomonas* cf. *monteilii* strain 9 and *Bacillus subtilis* strain MZ488846 (Farhaoui et al. 2022; Rakh et al. 2011), and the inhibition of the mycelium on soil agar in our dual culture assay confirmed the efficacy of GT4041 on soil. Additionally, the total inhibition of sclerotial germination after the 24-h incubation with GT4041 in its culture broth (Fig. 2), confirmed the strong antifungal properties of GT4041. GT4041 also produced protease, which can degrade cell walls to contribute to its antifungal activity. These results are similar to those for the biological control properties of *Trichoderma harzianum*, which produces protease and inhibits hydrolytic enzymes of the pathogen

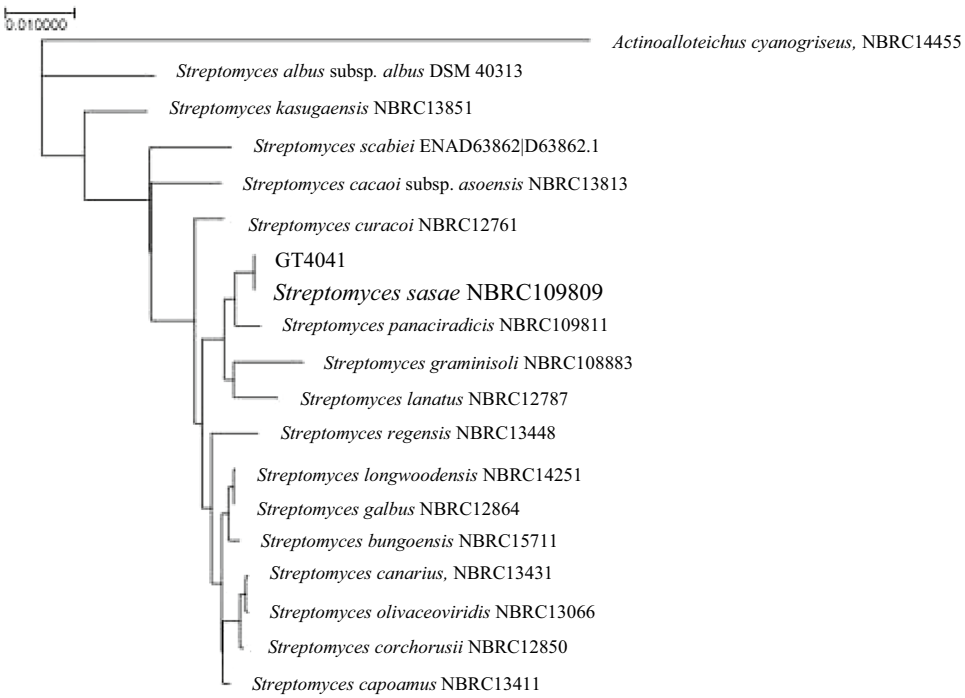

Fig. 7 Phylogenetic tree based on 16 S rDNA from *Streptomyces* species and isolate GT4041. *Actinoalloteichus cyanogriseus* NBRC14455 was used as the outgroup, and the scale bar represent 1% sequence dissimilarity

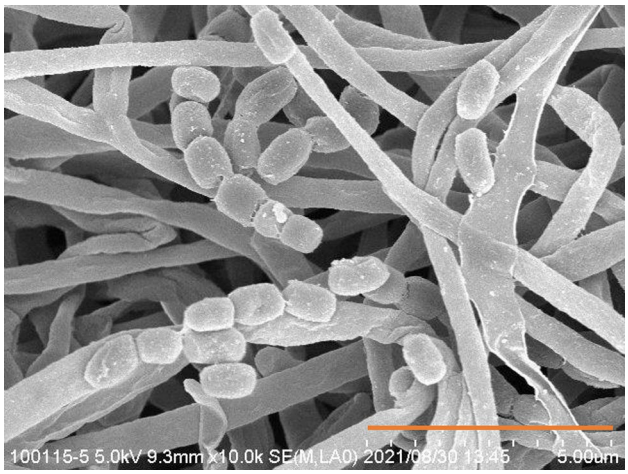

Fig. 8 Scanning electron micrograph of cells of strain isolate GT4041 on Luria-Bertani agar

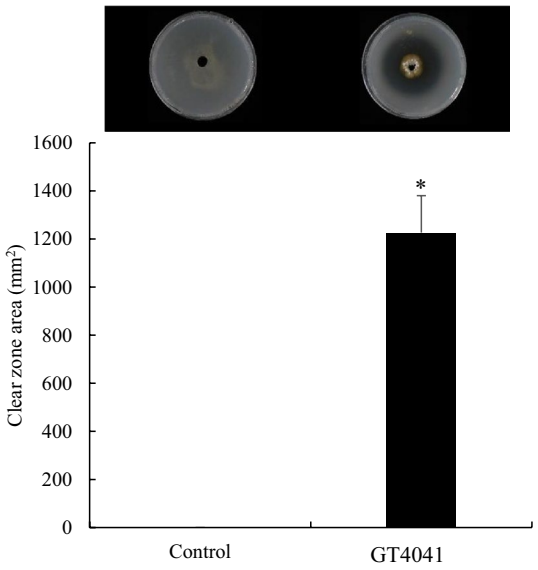

Fig. 9 Mean (±SD) area of clear zone on skim milk agar indicating protease production by GT4041. An asterisk indicates a significant difference compared to the control (Student's *t* test, $P < 0.05$)

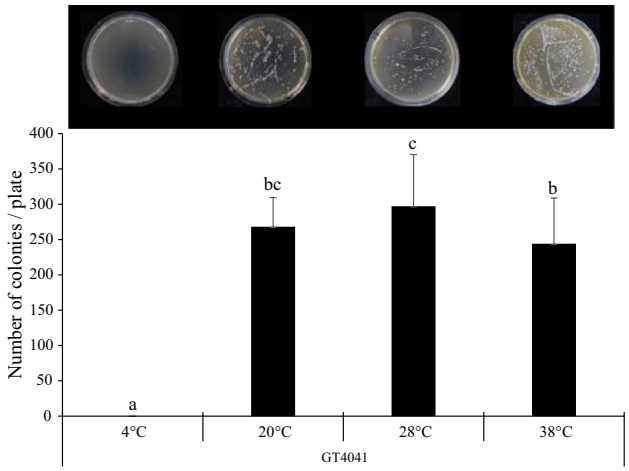

Fig. 10 Mean (±SD) number of colonies of isolate GT4041 after 1 week at 4, 20, 28, and 38 °C. The bar at the top represents the standard deviation of the mean. Different letters above means indicates a significant difference compared to the control (Tukey-Kramer's test, $P < 0.05$)

Botrytis cinerea (Elad and Kapat 1999). In our growth chamber experiment, in contrast to the total inhibition of sclerotial germination in vitro, strain SHIMANE002 infected tomato in the first treatment (Fig. 3A), which could be related to soil factors such as moisture and temperature, which might enhance sclerotial germination despite the antagonistic effect of GT4041, similar to the combined effect of moisture and temperature on *Sclerotium cepivorum* sclerotia found by Crowe and Hall (1980). Immediate application of soil mixed with GT4041 (Fig. 3C) was more efficient than applying the same mixture after 3 days (Fig. 3B). In addition, in the greenhouse, application of GT4041 in its culture broth provided better disease control efficient than mixing GT4041 with soil (Fig. 4). These results could indicate that the concentration of antifungal compounds was higher when GT4041 was applied directly in its culture broth. Applying GT4041 before and after soils are inoculated with sclerotia should help us understand whether the biocontrol mechanism involves priming of resistance-related genes. Based on the experiments with tomato plants, we recommend direct application of the GT4041 culture on plant stems for the best efficacy.

Additionally, GT4041 also inhibited the mycelial growth of five other fungal pathogens (Fig. 5), including soil- and airborne fungi. These results are consistent with those of other studies using *S. sasae*. For example, *S. sasae* strain St-3 strongly inhibits mycelial growth of *Fusarium oxysporum* f. sp. *lactucae* (KACC 42795) and *Pythium ultimum* (KACC 40705) on plates and on radish and pepper plants. (Santhanarajan et al. 2021) and *S. sasae* strain TG01 inhibits growth of *Fusarium solani* (InaCCF76) and *F. oxysporium* (InaCCF78) (Sudiana et al. 2020). An analysis of the secondary metabolites produced by *S. sasae* TG01 revealed the presence of a bioactive compound, 2-methyl-1,3-dioxolane (Sudiana et al. 2020). Perhaps this compound is also responsible for the antifungal property of GT4041 because it also inhibits the growth of *Candida albicans* ATCC 10231 a fungi (yeast) and gram-positive and gram-negative bacteria such as *Enterobacter faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Staphylococcus*

epidermidis ATCC 12228 (Küçük et al. 2011). The acetyl acetate extract of the culture of another *Streptomyces* sp. UK-201, which shares 96.88% similarity with *S. sasae* (JR-39, Hq267987), inhibited diverse microorganisms such as *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus epidermis*, *Brevibacterium linens*, *Escherichia coli, Pseudomonas fluorescens* and *Saccharomyces cerevisiae*. GC-MS analysis of the secondary metabolites produced by *Streptomyces* sp. UK-201 showed a diverse range of compounds such as undecane, 2-propyl-1-pentanol, *tetra*-butylbenzene, 1,2,4,5-tetramethyl benzene, (*E*)-3-tetradecane, 2-methyl-1,3-dioxolane (Srivastava et al. 2021). We will also analyze the secondary metabolites produced by GT4041 to determine whether they contribute to fungal inhibition and disease control.

The growth promotion of tomato by isolate GT4041 (Fig. 6) can be explained by its production of IAA. In addition to synthesizing IAA, *Streptomyces* sp. can also synthesize gibberellic acid and zeatin (Solans et al. 2011); however, IAA production by *S. sasae* has not yet been reported. IAA is known to stimulate plant growth through the production of long roots with more root hairs and lateral roots, which enhances nutrient uptake (Datta and Basu 2000). IAA can be synthesized through the precursor tryptophan (Trp) via the Trp-dependent IAA biosynthetic pathway (Mohite 2013; Lee and Whang 2016). In the presence of Trp, *S. fradiae* NKZ-259 produces a maximum IAA yield of up to 82.363 µg/ml (Myo et al. 2019), which is two times higher than our result (42.3 ± 4.1 µg/ml). Here, we only confirmed that IAA was produced on medium amended with Trp, but for a better understanding of requirements for IAA production by *S. sasae*, further studies with and without Trp are needed.

In conclusion, the selected soil isolate *S. sasae* strain GT4041, which is not pathogenic to tomato plants, was inhibitory to *A. rolfsii* (in vitro and in planta) and stem rot and damping of tomato while also promoting tomato growth promotion. It thus has promise as a good biological control agent and source for the development of a new microbial fungicide.

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Declarations

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

Ethical approval Not applicable.

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