

Influences of Culture Media, Temperature and Light/Dark Conditions on Growth and Antifungal Activity of *Streptomyces* spp. Against *Botrytis cinerea*, *in vitro* and on Tomato Leaf

Sawai Boukaew (✉ sawai.bo@skru.ac.th)

Songkhla Rajabhat University <https://orcid.org/0000-0001-8438-7393>

Siriporn Yossan

Sisaket Rajabhat University

Benjamas Cheirsilp

Prince of Songkla University - Hat Yai Campus: Prince of Songkla University

Wanida Petlamul

Songkhla Rajabhat University

Poonsuk Prasertsan

Prince of Songkla University - Hat Yai Campus: Prince of Songkla University

Research Article

Keywords: Antifungal compound, *Botrytis cinerea*, Culture media, Light/dark condition, Temperature, *Streptomyces* spp.

Posted Date: April 28th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-385032/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The influences of culture media, temperature, and light/dark conditions on growth and antifungal activity of the three strains *Streptomyces* spp. against *Botrytis cinerea* was studied. Results of in vitro study indicated that the GYM agar and incubated at 28°C exhibited good mycelial growth and a spore mass production of the three strains of *Streptomyces* spp. On the other hand, the PDA and incubation at 21°C were suitable for the mycelial growth of *B. cinerea*. Moreover, light/dark conditions had an effect on the growth of the two strains of *S. philanthi*. The strains RL-1-178 and RM-1-138 of *S. philanthi* grown in all media and incubation temperatures tested possessed antifungal activity against *B. cinerea* (100% inhibition) while *S. mycarofaciens* showed different results on PDA (83% inhibition) and GYM (88% inhibition) with the optimum incubation temperature at 21°C. Then, the antifungal compounds in culture filtrates produced by the three antagonistic strains against *B. cinerea* were tested on tomato leaf. They showed a significantly higher inhibitory effect on the symptoms of blight disease on tomato leaf compared with the control. The better protection efficacy against *B. cinerea* on tomato leaf was observed with the culture filtrates of *S. philanthi* RM-1-138 (82.89% and 0.33 cm² lesion areas symptoms). Moreover, the antifungal compounds in the culture filtrate of *S. philanthi* RM-1-138, identified by GC-MS, were greatly altered relative to concentration components under different temperatures and light/dark conditions tested. Our results clearly demonstrated that the environmental factors have an influence on antifungal activity of the three strains of *Streptomyces* spp.

Introduction

The necrotrophic fungi *Botrytis cinerea* Pers. [teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel] is among the world's most dangerous fungal pathogens that caused economically important diseases in numerous fruits and vegetables (Kamensky et al. 2003; Elad et al. 2015). Biocontrol is an environmentally friendly and efficient alternative to chemical fungicide management of these pathogens (Chen et al. 2016; Kamil et al. 2018). Species of *Streptomyces* are potential biocontrol agents since they are ubiquitous in the environment and many of them produce secondary metabolites such as enzyme inhibitors and antibiotics with diverse biological activities, including the ability to inhibit plant pathogenic fungi (VazJauri et al. 2016). Several species of *Streptomyces* have been isolated and used to control plant pathogens in various crops, such as *B. cinerea* (Ge et al. 2015; Kim et al. 2015; Bi and Yu 2016; Boukaew et al. 2017; Vijayabharathi et al. 2018).

The influence of culture media and temperatures on the growth of antagonistic *Streptomyces* sp. and microbial plant pathogens are important parameters. The temperature is known to have profound influences on the growth and production of bioactive compounds in *Streptomyces* species (Srinivasan et al. 1991) and disease development of pathogen *B. cinerea*. Several studies have shown that the growth of *Streptomyces* species is dependent on the culture medium and temperature (Kontro et al. 2005; Rashada et al. 2015). The medium containing 3 % starch, 0.75% peptone, 0.025% yeast extract, 1% (w/v) soybean meal and minor/trace elements, initial pH of 6.5, and incubation at 28°C was the best condition for growth and antifungal activity of *S. platensis* 3-10 against *Plasmodiophora brassicae* (Shakeel et al.

2015). GYM, containing 0.4% glucose, 0.4% yeast extract, and 1.0% malt extract, with an initial pH of 7.5 and a temperature of 30°C were found to be optimum for both cell growth and antifungal activity of *S. philanthi* RM-1-138 against *Rhizoctonia solani* (Boukaew and Prasertsan 2014). The medium consists of 3.0% glucose, 3.5% corn starch, 2.5% soybean meal, 1.2 mM MgCl₂, and 5.9 mM glutamate, and a temperature of 28°C was found to be the best condition for growth and antifungal activity of *S. rimosus* AG-P1441 against *Phytophthora capsici* (Ju et al. 2015). Soyabean meal medium has influenced the production of antibacterial compounds of *Streptomyces* sp. (Khattab et al. 2016).

B. cinerea requires several specific growth conditions for growth and reproduction. It is well known that growth and sporulation are important phases during the life of fungi, which is considerably influenced by external growth factors (Mishra and Tripathi 2015). Among the external growth factors, nutrition was one of the determinants that were previously proved by several workers in many fungal pathogens using different culture media sources (Kasar et al. 2004; Rani and Murthy 2004; Younis et al. 2004; Kim et al. 2005; Zhao et al. 2010; Lazarotto et al. 2013). Potato dextrose agar and incubation, ranging from 18 to 22°C were the best conditions for the growth of *B. cinerea* (Whipps 1987; Aqueveque et al. 2017; Boukaew et al. 2017; Meng et al. 2020).

Light conditions play a significant role in the growth of *Streptomyces* spp. and pigment production (Koyama et al. 1976; Takano et al. 2015; Khattab et al. 2016) and during disease development and pathogenicity of *B. cinerea* (Fillinger and Elad 2016; Zhang et al. 2016; Cheng et al. 2019; Meng et al. 2020). In *Streptomyces*, carotenoid production is a widespread metabolic activity, which occurs in a constitutive, light-dependent, or cryptic manner (Koyama et al. 1976). Koyama et al. (1975) found that light could enhance pigment production in the growing mycelia of *S. phaeopurpureus* 5125, *S. salmonicida* 5472, and *S. fulvissimus* more than cultivated under dark conditions. The pigmented antibiotics of *S. coelicolor* A3 are produced under light induction (Takano et al. 2005). Light is known to affect the population of several fungi and the circadian clock of one fungal phytopathogen has been linked to the pathogen's virulence programme (Hevia et al. 2015). *B. cinerea* is a light-responsive strain that actively senses light conditions to fine-tune its development and pathogenicity (Zhang et al. 2016). Light is an essential developmental signal for the *B. cinerea* as it triggers the exclusive formation of conidia, whereas constant darkness initiates the sole formation of sclerotia (Fillinger and Elad 2016). Zhang et al. (2016) reported that *B. cinerea* could produce conidia production in dark conditions more than in light conditions.

Under these circumstances, the present study aimed to study the growth conditions of antagonistic bacteria and pathogenic fungi in different growing conditions. The objectives of this study are (i) to study the influences of cultures media, temperature, and light/dark conditions on the growth of the three strains of antagonistic bacteria *Streptomyces* spp. and pathogenic fungi of *B. cinerea*, (ii) to evaluate the inhibitory ability of the three strains of *Streptomyces* spp. on the growth of *B. cinerea in vitro* and in tomato leaf and (iii) to identify the chemical composition of the antifungal compounds of the selected strain of *Streptomyces* spp. grown under different temperatures and light/dark conditions by GC-MS.

Materials And Methods

Microorganisms

The antagonistic strain SS-2-243 of *Streptomyces mycarofaciens* and the strains RM-1-138 and RL-1-178 of *S. philanthi* were isolated from the rhizosphere of chili peppers in southern Thailand (Boukaew et al. 2011) and kept on 20% glycerol at -20°C as a stock culture. They were sub-cultured freshly on glucose yeast-malt agar (GYM) and incubated at 28°C for 10 days before use in this study. Spore suspensions of each strain of *Streptomyces* spp. were prepared by removing the spores from a 10-day-old culture and suspending them in sterile distilled water, then adjusting the spore concentration with sterile distilled water to 10^7 spores per milliliter using a hemocytometer.

The pathogen strain *Botrytis cinerea* was isolated from infected tomatoes, showing typical gray mold symptoms. The fungal strains were grown on potato dextrose agar (PDA) slants and kept at 4°C . The fungal pathogen was sub-cultured freshly on PDA and incubated at 21°C for 3 days before use in this study.

Influence of culture media, temperature, and light/dark conditions on the growth of *Streptomyces* spp., and *B. cinerea*

In a previous study, Boukaew et al. (2011) have shown that GYM medium and incubation at 28°C were the best conditions for the growth of the three strains of *Streptomyces* spp. However, the growth conditions on PDA medium and 21°C are not yet known. A 10^7 spores mL^{-1} of each strain of *Streptomyces* spp. were streaked on GYM agar and PDA and incubated at 21 and 28°C . To evaluate their ability to grow at different mediums and temperatures, the growth and pigment of spore mass were qualitatively determined after 14 days of incubation. Each treatment had three replicates.

The influence of light conditions on the growth of *Streptomyces* spp. was also studied. A 10^7 spores mL^{-1} of each strain of *Streptomyces* spp. were streaked on the selected medium and incubated in the light (photo period of light-dark (L:D) at 14:10 hours) and dark (24 hours of dark) growth chamber at 21°C for 14 days. Samples were taken at a time interval to evaluate the ability of *Streptomyces* spp. to grow in the light/dark condition.

Most researchers have shown that the PDA and incubation, ranging from 18 to 22°C were the best conditions for the growth of *B. cinerea* (Whipps 1987; Aqueveque et al. 2017; Meng et al. 2020). However, the growth conditions on GYM medium and 28°C are not yet known. A 5-mm-diameter mycelial plug, excised from a three-day-old *B. cinerea* colony, was transferred to the center of GYM and PDA plate media and incubated at 21 and 28°C . The ability of pathogenic fungi to grow at different mediums and temperatures was determined after three days of incubation. Growth was measured based on the radius of mycelium produced (in cm). Each treatment had three replicates.

Influences of culture media and temperature on antifungal activity of *Streptomyces* spp. against *B. cinerea*

The two strains of *S. philanthi* RM-1-138 and RL-1-178 and *S. mycarofaciens* SS-2-243 were evaluated for their antagonistic properties against *B. cinerea* using a dual culture technique (Boukaew et al. 2011). For each strain of *Streptomyces* spp., a streak of spore suspension at 10^7 spores mL⁻¹ was deposited on one side of a GYM or PDA media in Petri dishes. Plates were then incubated in a growth chamber for seven days at 28°C.

A 5-mm-diameter mycelial plug, excised from a three-day-old sample of the pathogenic fungi colony, was transferred to the center of each plate. As a control, a mycelial plug of the pathogenic fungi was placed on a GYM and PDA plate without any *Streptomyces* strain. The dual culture plates were incubated in a growth chamber at 21°C and 28°C for three days, after which the radial mycelial growth of the pathogenic fungi was measured and compared to that of the control. Three replicates were conducted for each *Streptomyces*– the pathogenic fungal strain combination. The colony size in each treatment was recorded and the percentage inhibition of hyphal growth was calculated by the following formula: Protection percentage = [(Control-Treatment)/Control] × 100.

***In vivo* antagonistic activity of culture filtrates produced by *Streptomyces* spp. against *B. cinerea* on tomato leaf**

The culture filtrates of *S. mycarofaciens* SS-2-243 and the strains RL-1-178 and RM-1-138 of *S. philanthi* were prepared in a 250 mL flask containing 100 mL GYM medium (pH adjusted at 7.0 before autoclaving) and incubated in the light (photoperiod L:D 14:10 hours) growth chamber at 28°C. After three days of incubation, 5 mL aliquots of this culture was transferred into 100 mL fresh GYM medium and incubated for ten days under the same condition. The culture broth was centrifuged (8880 ×g for 20 min), then filtered through a 0.45 mm Millipore membrane to recover the culture filtrate. These culture filtrates were sprayed on tomato leaf *Streptomyces* spp. (10 mL/leaf). When the leaves were dried (1 h), they were removed, and three leaflets were placed in a transparent plastic box with humid absorbent paper to maintain high relative humidity (close to 100 %). Mycelial plugs (5-mm in diameter) of *B. cinerea* excised from the growing margin of three-day-old PDA cultures were deposited onto the center of the leaflets of tomato. Leaves were treated with GYM medium as a control. After 2 days of growth with 12 h of light and 12 h of darkness alternately at 28 °C, the lesion areas were measured (Zhang et al. 2020). Each treatment included three replicates and each replicate consisted of three leaflets (three leaflets/plastic boxes). The strain of *Streptomyces* spp. possessing the highest *in vivo* antagonistic activity was selected for the next study.

Identification of antifungal compounds produced by the selected strain of *Streptomyces* spp. grown in different light/dark conditions and temperatures by GC–MS

A 5 mL aliquots of the selected strain of *Streptomyces* spp. was transferred into a 200 mL GYM medium, incubated in the light (photoperiod L:D 14:10 hours) and dark (24 hours) growth chamber at 21°C and

28°C. After 10 days of incubation, the culture broth was centrifuged (8880 ×g for 20 min), then filtered through a 0.45 mm Millipore membrane to recover the culture filtrate. The chemical composition of the antifungal compounds in all culture filtrates was determined. Each culture filtrate was acetylated (Sangmanee and Hongpattarakere 2014), then analyzed by a gas chromatograph-mass spectrometer (GC-MS) (7890 B GC-7000 MS, Agilent, USA) equipped with a VF-WAXms column (30 m, film thickness 0.25 µm, I.D. 0.25 mm). Mass spectra were obtained using the scan modus (total ion count, 35–500 m/z). The retention time and fragmentation pattern in the mass spectra were used to identify each compound by comparing them to those available standards from the Library of the National Institute of Standards and Technology (NIST).

Statistical analysis

The data were submitted to analyses of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) version 26 (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). A *P* value < 0.05 was considered significant.

Results

Influences of culture media, temperature, and light conditions on the growth of *Streptomyces* spp.

Table 1A represents the growth level of *S. mycarofaciens* SS-2-243 and the two strains of *S. philanthi* (RM-1-138 and RL-1-178) under different media and temperatures. The growth of these three strains depended on the nutrient composition of the media and incubation temperature. *Streptomyces* spp. had an excellent spore mass production depending on the temperature and medium composition. The two strains of *S. philanthi* showed higher growth on GYM medium and incubation at 28°C than on PDA medium incubated at 21°C. GYM media contained higher nutrients (glucose and yeast-malt extract) for microbial growth than PDA (potato and dextrose) 21°C. Unlike *S. philanthi*, culture media had no influence on the growth of *S. mycarofaciens* but the strain was more affected by incubation temperature. The strain grew better at 28°C than at 21°C. Therefore, the optimum temperature for growth of all three strains of *Streptomyces* spp. was 28°C. While the two strains of *S. philanthi* preferred GYM to PDA, the medium composition did not affect *S. mycarofaciens*.

Light/dark conditions influenced the growth of *S. mycarofaciens* SS-2-243 but did not affect the two strains of *S. philanthi* (RM-1-138 and RL-1-178) (Table 1B). *S. mycarofaciens* SS-2-243 grew better under dark conditions than the light conditions

In the case of *B. cinerea*, the culture media (PDA and GYM) and temperatures (21 and 28°C) had a profound effect on the mycelial growth (colony diameter) after three days of incubation (Fig. 1). The PDA medium and temperature at 21°C gave significantly higher mycelial growth of *B. cinerea* with an average colony diameter of 8.41 cm compared with the GYM (6.86 cm).

Influence of culture media and temperature on antifungal activity of *Streptomyces* spp. against *B. cinerea*

Influence of culture media and temperature on antifungal activity of *S. mycarofaciens* SS-2-243 and the two strains of *S. philanthi* against *B. cinerea* were investigated (Fig. 2). Figure 2A shows that the three strains of *Streptomyces* spp. exhibited 83-100% inhibition on the mycelial growth of the pathogenic fungi on PDA or GYM medium after three days incubation at 21 or 28°C, compared with the control treatment without *Streptomyces* spp. Significant differences in antifungal activity of *S. mycarofaciens* SS-2-243 were observed between medium and temperature (the same as the result of growth). Notably, the strains RM-1-138 and RL-1-178 of *S. philanthi* showed the strongest antagonistic activity (100% inhibition) against the mycelial growth of *B. cinerea* tested on both PDA and GYM medium after incubation at 21 or 28°C. The growth appearance of each *Streptomyces* against the pathogenic fungi on PDA and GYM plates after incubation at 21°C and 28°C is illustrated (Fig. 2B and C).

***In vivo* antagonistic activity of culture filtrate produced by *Streptomyces* spp. against *B. cinerea* on tomato leaf**

Because control efficacy *in vitro* is not always consistent with the results *in vivo*, the disease controls of the efficacy of culture filtrate produced by three strains of *Streptomyces* sp. against gray mold was evaluated on tomato leaf as a screening procedure for predicting the actual control efficacy. The development and expansion of disease symptoms induced by *B. cinerea* were inhibited effectively by culture filtrate produced by the strain SS-2-243 of *S. mycarofaciens* and the strains RM-1-138 and RL-1-178 of *S. philanthi* and in *in vivo* leaf test. As shown in Figure 3A, the lesion areas on tomato leaves inoculated by *B. cinerea* were significantly reduced ($P < 0.05$) after being treated with culture filtrate produced by *Streptomyces* spp. using detached leaf assay. For leaves in the control group with no treatment of culture filtrate, the lesion areas extended to 3.12 cm² after 2 days incubation at 28°C, whereas for the leaves treated with the culture filtrate produced by *S. mycarofaciens* SS-2-243 and the strains RL-1-178 and RM-1-138 of *S. philanthi*, the lesion areas were limited to 1.78, 0.98 and 0.33 cm², respectively, (Fig. 3B). The cultural filtrates of *S. philanthi* RM-1-138 showed a strong efficacy protection on tomato leaf caused by *B. cinerea* (82.89%) (Fig. 3C). Therefore, the culture filtrates of this strain RM-1-138 were selected to identify the chemical composition.

Identification of antifungal compounds produced by *S. philanthi* RM-1-138 grown at different light/dark condition and temperature by GC-MS

The metabolic compounds produced by *S. philanthi* RM-1-138 greatly varied under the light (L:D of 14:10 h) and dark (24 h) conditions at two temperatures (Table 2). The chemical compounds mainly fell into several categories including acids, ketones, alcohol, amines, amides, and others. Among them, there were only three components: acetic acid, 2-propanone, and 2-furanmethanol that were detected in all conditions tested. In the presence of the light condition, 11 and 19 compounds were identified after incubation at 21 and 28°C, respectively. At 21°C, the dominant component was acetic acid (67.41%) (Fig. 4A) while at 28°C the dominant component was benzeneacetamide (43.58%) (Fig. 4B). Under dark conditions, *S. philanthi* RM-1-138 produced 11 and 32 antifungal compounds after cultivation at 21 and

28 °C, respectively. Surprisingly, acetic acid (68.77%) (Fig. 4C) was produced abundantly when incubated at 21°C while at 28°C the dominant components were propanamide (20.68%) (Fig. 4D).

Discussion

This paper provides useful information related to the influence of culture media, temperatures, and light conditions on growth and antifungal activity by the antagonistic strains of *Streptomyces mycarofaciens* SS-2-243 and *S. philanthi* RL-1-178 and RM-1-138 against *Botrytis cinerea* *in vitro* and tomato leaf. Besides, the antifungal compounds produced by the promising strain grew at different lights, and temperatures were identified by GC–MS. The results revealed that culture media, temperatures, and light conditions had a profound effect on the growth of the three antagonistic strains and fungal pathogenic strains. Moreover, temperatures and light conditions influenced metabolic compounds produced by the selected strain RM-1-138 of *S. philanthi*.

The culture media and environmental factors were found to be the most influential factors related to the growth and production of secondary metabolites of microorganisms (Saxena et al. 2001; Kim et al. 2005; Saha et al. 2008). Kathiresan et al. (2005) reported that the ability of bacteria to produce antimicrobial agents can be increased or lost under different culture conditions. In our study, the growth medium, temperature, and light/dark conditions had a significant effect on the three strains of *Streptomyces* spp. The strains RL-1-178 and RM-1-138 of *S. philanthi* were grown on GYM medium and incubated at 28°C and grew and produced a spore mass higher than on PDA medium incubated at 21°C. GYM medium containing glucose and yeast-malt extract was more enriched with nutrients for microbial growth than PDA containing only potato and dextrose. Nitrogen and carbon sources were reported to have a profound influence on the growth and production of secondary metabolites by *Streptomyces* species (Narayana and Vijayalakshmi 2008). The study indicated that the nutrient source has an important on bacterial and fungal growth.

The light/dark cycle influenced the growth of the three antagonistic strains of *Streptomyces* spp. There were no significant differences in the growth of *S. philanthi* RL-1-178 and RM-1-138 between light and dark conditions. On the other hand, *S. mycarofaciens* SS-2-243 had a significantly higher growth rate under dark conditions than light conditions. Different previous studies have shown that *S. phaeopurpureus* 5125, *S. salmonicida* 5472, and *S. fulvissimus* could grow well in light conditions (Koyama et al. 1975). Some strains of *Streptomyces* species such as *S. viridosporus*, *S. lividans* and *S. coelicolor* were reported to be sensitive to light, while others remained unaffected (*S. ambofaciens* and *S. pilosus*) (Imbert and Blondeau 1999). It has been demonstrated that light conditions influenced the growth of *Streptomyces* sp.

B. cinerea is able to deploy a wide range of strategies to complete its infection cycle. The induction of these strategies must be mainly in response to environmental conditions. Culture media (PDA and GYM) and temperature (21 and 28°C) influenced the mycelial growth of *B. cinerea*. The PDA medium and temperature at 21°C gave a rapid mycelial growth of *B. cinerea* while GYM medium was not significantly

different after incubation at 21 or 28 °C. This finding agreed with that of Ciliberti et al. (2016) who reported that the highest mycelial growth and conidia production of *B. cinerea* was observed on PDA and temperature at 20°C. Several other researches also stated that PDA was the best media for mycelial growth (Xu et al. 1984; Maheshwari et al. 1999).

The PDA medium, with a temperature of 21°C not only had a profound positive effect on growth but also on the antifungal activity of *S. mycarofaciens* SS-2-243 against gray mold *B. cinerea*. The two strains of *S. philanthi* (RM-1-138 and RL-1-178) showed the strongest antagonistic activity (100% inhibition) against the mycelial growth of *B. cinerea* tested on both media at an incubation temperature of 21 and 28°C *in vitro* test. The three antagonistic strains of *Streptomyces* spp. produced antifungal substances and secreted them into the agar medium in dual culture plates.

In addition to the *in vitro* trials, the three antagonistic strains of *Streptomyces* spp. also exhibited positive results against *B. cinerea* during the *in vivo* trials on tomato leaves with remarkable protection efficiency after 2 days of cultivation at 28°C. Blight symptoms and lesion diameter caused by the fungal pathogen in tomato leaves were all significantly reduced by the strains of *S. mycarofaciens* SS-2-243 as well as *S. philanthi* RM-1-138 and RL-1-178 when compared with the control. Among the three strains tested, the antifungal compounds in the culture filtrates produced by *S. philanthi* RM-1-138 (82.89%) showed the highest inhibitory effect on the protection of tomato leaf caused by *B. cinerea* when compared with *S. philanthi* RL-1-178 (68.45%) and *S. mycarofaciens* SS-2-243 (42.78%), respectively. These results confirmed the potential of an antifungal compound in the secondary metabolites produced by antagonistic microorganisms to control gray mold *B. cinerea* in this study or other plant pathogenic fungi such as *Streptomyces* sp. (Li et al. 2011; Boukaew and Prasertsan 2014; Lyu et al. 2017; Brzezinska et al. 2019; Kim et al. 2020), *Bacillus* sp. (Kurniawan et al. 2018; Masmoudi et al. 2017; Shafi et al. 2017; Toral et al. 2018), and *Pseudomonas* sp. (Simionato et al. 2017; Strano et al. 2017; Kurniawan et al. 2018; Dutta et al. 2020; Köhl et al. 2020).

The antimicrobial substances produced by *Streptomyces* were variable and diversified depending on multiple factors. Therefore, the influence of temperatures (21 and 28°C) and light/dark conditions on antifungal production of the selected strain *S. philanthi* RM-1-138 was studied. Interestingly, the metabolic compounds produced by this particular strain were greatly altered by relative concentration components under the conditions tested. In light/dark conditions tested, the anti- *B. cinerea* of *S. philanthi* RM-1-138 was established at a higher level in several metabolic compounds in the dark condition (11 and 32 antifungal compounds after incubation at 21 and 28°C, respectively) than in the light condition (11 and 19 antifungal compounds after incubation at 21 and 28°C, respectively). Moreover, we found that the dominant antifungal compounds showed significant differences between dark and light conditions. Temperature conditions tested showed that incubation temperature plays a significant role in the number of antifungal compounds and dominance compound of *S. philanthi* RM-1-138. Incubation was at 21°C and, the dominant component was acetic acid (68.77%, dark condition and 67.41%, light condition) while at 28°C the dominant components were propanamide (20.68 %, dark condition) and benzeneacetamide (43.58%, light condition). Besides, we found that acetic acid, 2-propanone, and 2-furanmethanol were

detected in all conditions tested. Moreover, some of the antifungal compounds such as lactic anhydride, 2,5-pyrrolidinedione, 1-methyl, propanamide, benzeneacetamide, butyl aldoxime, 2-methyl-, syn- were found only under incubation at 28°C. According to this study, different environmental factors may release similar or different types of antifungal substances, with a variety of effects. All researchers proposed that *Streptomyces* spp. was commonly reported for its broad spectrum of antimicrobial activity (Ouhdouch et al. 2001; Prabavathy et al. 2006; Prapagdee et al. 2008). Our results clearly demonstrated that the light/dark condition have an influence on a rich source of secondary metabolites of *S. philanthi* RM-138.

Declarations

Acknowledgments

The first and second authors were supported by the Thailand Research Fund (RTA6280014).

Compliance with ethical standards

Competing interests

The authors declare that no competing interest exists.

Human and animals rights

No human and/or animal participants were involved in this research.

References

- Aqueveque P, Cespedes CL, Becerra J, Aranda M, Sterner O (2017) Antifungal activities of secondary metabolites isolated from liquid fermentations of *Stereum hirsutum* (Sh134-11) against *Botrytis cinerea* (grey mould agent). *Food Chem Toxicol* 109:1048–1054
- Awla HK, Kadir J, Othman R, Rashid TS, Hamid S, Wong MY (2017) Plant growth-promoting abilities and biocontrol efficacy of *Streptomyces* sp. UPMRS4 against *Pyriculariaoryzae*. *Biol Control* 112:55–63
- Bi Y, Yu Z (2016) Diterpenoids from *Streptomyces* sp. SN194 and their antifungal activity against *Botrytis cinerea*. *J Agric Food Chem* 64:8525–8529
- Bonaldi M, Chen X, Andrea Kunova A, Pizzatti C, Saracchi M, Cortesi P (2015) Colonization of lettuce rhizosphere and roots by tagged *Streptomyces*. *Front Microbiol* 6:25
- Boukaew S, Chuenchit S, Petcharat V (2011) Evaluation of *Streptomyces* spp. for biological control of *Sclerotium* root and stem rot and *Ralstonia* wilt of chili pepper. *BioControl* 56:365–374
- Boukaew S, Prasertsan P, Troulet C, Bardin M (2017) Biological control of tomato gray mold caused by *Botrytis cinerea* by using *Streptomyces* spp. *BioControl* 62:793–803.

- Chen X, Pizzatti C, Bonaldi M, Saracchi M, Erlacher A, Kunova A, Berg G, Cortesi P (2016) Biological control of lettuce drop and host plant colonization by rhizospheric and endophytic *Streptomyces*. *Front Microbiol* 7:714
- Chen YY, Chen PC, Tsay TT (2016) The biocontrol efficacy and antibiotic activity of *Streptomyces plicatus* on the oomycete *Phytophthora capsici*. *Biol Control* 98:34–42
- Elad Y, Pertot I, Cotes Prado AM, Stewart A (2015) Plant hosts of *Botrytis* spp. In: Fillinger, S., Elad, Y. (eds) *Botrytis—the fungus, the pathogen and its management in agricultural systems*. Springer, Berlin, pp 413–486
- Fillinger S, Elad Y (2016) *Botrytis—the fungus, the pathogen and its management in agricultural systems*. Pub. Springer, New York
- Ge BB, Cheng Y, Liu Y, Liu BH, Zhang KC (2015) Biological control of *Botrytis cinerea* on tomato plants using *Streptomyces ahngroscopicus* strain CK-15. *Lett Appl Microbiol* 61:596–602
- Hockberger EP (2000) The discovery of the damaging effect of sunlight on bacteria. *J Photochem Photobiol B Biol* 58:185–191
- Imbert M, Blondeau R (1999) Effect of light on germinating spores of *Streptomyces viridosporus*. *FEMS Microbiol Lett* 181:159–163
- Jung SJ, Kim NK, Lee DH, Hong SI, Lee JK (2018) Screening and evaluation of *Streptomyces* Species as a potential biocontrol agent against a wood decay fungus, *Gloeophyllum trabeum*. *Mycobiology* 46 138–146
- Kamil FH, Saeed EE, El-Tarabily KA, AbuQamar SF (2018) Biological control of mango dieback disease caused by *Lasiodiplodia theobromae* using Streptomyces and Non-streptomyces actinobacteria in the united arab emirates. *Front Microbiol* 9:829
- Kamensky M, Ovadis M, Chet I, Chernin L (2003) Soil-borne strain IC14 of *Serratia plymuthica* with multiple mechanisms of antifungal activity provides biocontrol of *Botrytis cinerea* and *Sclerotinia sclerotiorum* diseases. *Soil Biol Biochem* 35:323–331
- Kathiresan K, Balagurunathan K, Selvam MM (2005) Fungicidal activity of marine actinomycetes against phytopathogenic fungi. *Indian J Biotechnol* 4 271–276
- Khattab AI, Babiker EH, Saeed HA (2016) *Streptomyces*: isolation, optimization of culture conditions and extraction of secondary metabolites. *Int Curr Pharm J* 5:27–32
- Kim YS, Lee IK, Yun BS (2015) Antagonistic effect of *Streptomyces* sp. BS062 against *Botrytis* diseases. *Mycobiology* 43:339–342

- Kontro M, Lignell U, Hirvonen MR, Nevalainen A (2005) pH effects on 10 *Streptomyces* spp. growth and sporulation depend on nutrients. *Lett Appl Microbiol* 41:32–38
- Koyama Y, Kato F, Oshibi S, Takamatsu T, Yamagishi S (1975) Effect of light on nonphotosynthetic microorganisms II. photochromogenicity in genus *Streptomyces*. *Japan J Microbiol* 19:387–393
- Koyama, Y., Kato, F., & Yazawa, Y. (1976). Effect of light on the pigmentation of bacteria in Actinomycetales. p. 65–85. In T. Arai (ed.), Actinomycetales, the boundary microorganisms. Toppan, Tokyo, Japan.
- Kumara P, Dubey KK (2017) Mycelium transformation of *Streptomyces toxytricini* into pellet: role of culture conditions and kinetics. *Bioresour Technol* 228:339–347
- Narayana KJP, Vijayalakshmi M (2008) Optimization of antimicrobial metabolites production by *Streptomyces albidoflavus*. *Res J Pharm* 2:4–7
- Meng L, Mestdagh H, Ameye M, Audenaert K, Höfte M, Van Labeke MC (2020) Phenotypic variation of *Botrytis cinerea* isolates is influenced by spectral light quality. *Front Plant Sci* 11:1233
- Rashada FM, Fathy MH, El-Zayat AS, Elghonaimy AM (2015) Isolation and characterization of multifunctional *Streptomyces* species with antimicrobial, nematocidal and phytohormone activities from marine environments in Egypt. *Microbiol Res* 175:34–47
- Srivastava S, Patel JS, Singh HB, Sinha A, Sarma BK (2015) *Streptomyces rochei* SM3 induces stress tolerance in chickpea against *Sclerotinia sclerotiorum* and NaCl. *J Phytopathol* 163:583–592
- Sunpapaoa A, Chairina T, Ito SI (2018) The biocontrol by *Streptomyces* and *Trichoderma* of leaf spot disease caused by *Curvularia oryzae* in oil palm seedlings. *Biol Control* 123: 36–42
- Takano H, Obitsu S, Beppu T, Ueda K (2005) Light-induced carotenogenesis in *Streptomyces coelicolor* A3 (2): identification of an extracytoplasmic function sigma factor that directs photodependent transcription of the carotenoid biosynthesis gene cluster. *J Bacteriol* 187:1825–1832
- Vijayabharathi R, Gopalakrishnan S, Sathya A, Kumar MV, Srinivas V, Mamta S (2018) *Streptomyces* sp. as plant growth-promoters and host-plant resistance inducers against *Botrytis cinerea* in chickpea. *Biocontrol Sci Technol* 28 1140–1163
- Vijayabharathi R, Gopalakrishnan S, Sathya A, Srinivas V, Sharma M (2018) Deciphering the tri-dimensional effect of endophytic *Streptomyces* sp. on chickpea for plant growth promotion, helper effect with *Mesorhizobium ciceri* and host-plant resistance induction against *Botrytis cinerea*. *Microb Pathog* 12:298–107
- Zhang ZQ, Li H, Qin GZ, He C, Li BQ, Tian SP (2016) The MADS-box transcription factor Bcmads1 is required for growth, sclerotia production and pathogenicity of *Botrytis cinerea*. *Sci Rep* 6:33901

Tables

Table 1 Influences of culture media and temperature **(A)** and light conditions **(B)** on growth of the strain SS-2-243 of *S. mycarofaciens* and the strains RM-1-138 and RL-1-178 of *S. philanthi* after 14 days incubation at 21 or 28°C.

(A)

<i>Streptomyces</i> species	21°C		28°C	
	PDA	GYM	PDA	GYM
<i>S. mycarofaciens</i> SS-2-243	++	++	+++	+++
<i>S. philanthi</i> RM-1-138	+	+	++	+++
<i>S. philanthi</i> RL-1-178	+	+	++	+++

(B)

<i>Streptomyces</i> species	Light	Dark
<i>S. mycarofaciens</i> SS-2-243	++	+++
<i>S. philanthi</i> RM-1-138	+	+
<i>S. philanthi</i> RL-1-178	+	+

+++ = Good growth and produced black spore

++ = Moderate growth and produced white spore

+ = Weak growth and no produced spore

PDA = Potato dextrose medium

GYM = Glucose yeast-malt extract medium

Table 2 Comparative chemical composition analyzed by GC-MS of the culture filtrate from the strain RM-1-138 of *S. philanthi* grown in a GYM medium under the light and dark condition, and incubated at 21 and 28°C for 10 days

Chemical compositions	% relative concentration			
	Light condition		Dark condition	
	21°C	28°C	21°C	28°C
Acid				
Acetic acid (C ₂ H ₄ O ₂)	67.41	4.91	68.77	4.27
Propanoic acid (C ₃ H ₆ O ₂)	2.66	-	3.08	-
Propanoic acid, 2-methyl- (C ₄ H ₈ O ₂)	1.22	-	2.00	-
Butanoic acid, 2-methyl- (C ₅ H ₁₀ O ₂)	1.85	-	2.24	-
Lactic Anhydride (C ₆ H ₁₀ O ₅)	-	1.49	-	3.09
2-Propenoic acid, 2-hydroxyethyl ester (C ₅ H ₈ O ₃)	-	3.13	-	-
Pentanoic acid, 2-hydroxy-, ethyl ester (C ₇ H ₁₄ O ₃)	-	-	-	7.61
Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester) (C ₉ H ₁₁ NO ₄)	-	-	-	0.24
Ketones				
2-Propanone, 1-hydroxy- (C ₃ H ₆ O ₂)	1.68	3.55	2.15	7.15
2(3H)-Furanone, dihydro-4-hydroxy- (C ₄ H ₆ O ₃)	1.03	1.96	0.79	-
2(3H)-Furanone, dihydro- (C ₄ H ₆ O ₂)	-	-	-	0.39
2,5-Pyrrolidinedione, 1-methyl- (C ₅ H ₇ NO ₂)	-	0.70	-	0.34
2-Butanone, 3-hydroxy- (C ₄ H ₈ O ₂)	-	-	-	0.52
2(5H)-furanone (C ₄ H ₄ O ₂)	-	-	-	1.19
Alcohol				
2-Furanmethanol (C ₅ H ₆ O ₂)	0.75	1.49	0.74	3.15
3,4-Furandimethanol (C ₆ H ₈ O ₃)	-	2.46	1.69	-
1,2-Propanediol (C ₃ H ₈ O ₂)	-	1.30	-	-

Table 2 (continued).

Chemical compositions	% relative concentration			
	Light condition		Dark condition	
	21°C	28°C	21°C	28°C
Benzenemethanol, .alpha.-(1-aminoethyl)-, [S-(R*, R*)]- (C ₉ H ₁₃ NO)	-	-	-	5.01
1,3-Butanediol, (S)- (C ₄ H ₁₀ O ₂)	-	-	-	1.31
Cyclobutanol-DO (C ₄ H ₇ DO)	-	-	-	1.28
Carveol (C ₁₀ H ₁₆ O)	-	-	-	0.95
9-Hydroxy-linalool (C ₁₀ H ₁₈ O ₂)	-	-	-	0.22
p-Mentha-1,8-dien-7-ol (C ₁₀ H ₁₆ O)	-	-	-	1.79
3,4-Furandimethanol (C ₆ H ₈ O ₃)	-	-	-	5.09
(2-[1-(3,3-Dimethoxy-propyl)-vinyl]-5-methylcyclopentyl)-methanol (C ₁₄ H ₂₆ O ₃)	-	-	-	0.23
Amines				
Benzeneethanamine, 2,5-difluoro-.beta.,3,4-trihydroxy-N-methyl- (C ₉ H ₁₁ F ₂ NO ₃)	-	2.44	1.54	-
Amides				
Propanamide (C ₄ H ₉ NO)	-	20.39	-	20.68
Heptanamide (C ₇ H ₁₅ NO)	-	0.85	-	-
Butanamide, 3-methyl- (C ₅ H ₁₁ NO)	-	2.15	-	-
Benzeneacetamide (C ₈ H ₉ NO)	-	43.58	-	10.34
Hexanamide (C ₆ H ₁₃ NO)	-	-	-	0.36
Others				
Benzeneacetaldehyde (C ₈ H ₈ O)	10.01	-	-	-
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (C ₆ H ₈ O ₄)	6.80	-	8.52	-

Table 2 (continued).

Chemical compositions	% relative concentration			
	Light condition		Dark condition	
	21°C	28°C	21°C	28°C
1,4:3,6-Dianhydro-.alpha.-d-glucopyranose (C ₆ H ₈ O ₄)	4.46	-	-	-
2-Furancarboxaldehyde, 5-methyl- (C ₆ H ₆ O ₂)	2.14	-	-	-
Butyl aldoxime, 2-methyl-, syn- (C ₅ H ₁₁ NO)	-	1.34	-	3.76
Diethyl .alpha.-acetylglutarate (C ₁₁ H ₁₈ O ₅)	-	0.58	-	-
Nonanal (C ₉ H ₁₈ O)	-	1.41	-	-
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)- (C ₁₀ H ₁₆ O)	-	2.14	-	-
1,4:3,6-Dianhydro-.alpha.-d-glucopyranose (C ₆ H ₈ O ₄)	-	2.47	2.97	-
3-Carene (C ₁₀ H ₁₆)	-	-	-	0.31
4,4-Ethylenedioxy-pentanenitrile (C ₇ H ₁₁ NO ₂)	-	-	-	0.24
(Z)-4-(Methylamino)-3-penten-2-one (C ₆ H ₁₁ NO)	-	-	-	0.25
5-Methyl-2-hexanone oxime (C ₇ H ₁₅ NO)	-	-	-	0.42
1,3,5-Triazine-2,4,6-triamine, 1,3,5-trioxide	-	-	-	0.43
Arginine (C ₆ H ₁₄ N ₄ O ₂)	-	-	-	1.51
2,7-Anhydro-l-galacto-heptulofuranose	-	-	-	0.23
2-N-propyl-5-oxohexanal	-	-	-	0.37
1,4:3,6-Dianhydro-.alpha.-d-glucopyranose (C ₆ H ₈ O ₄)	-	-	-	9.15
2 - hydroxy - butanedial (C ₄ H ₆ O ₃)	-	-	-	5.36

Figures

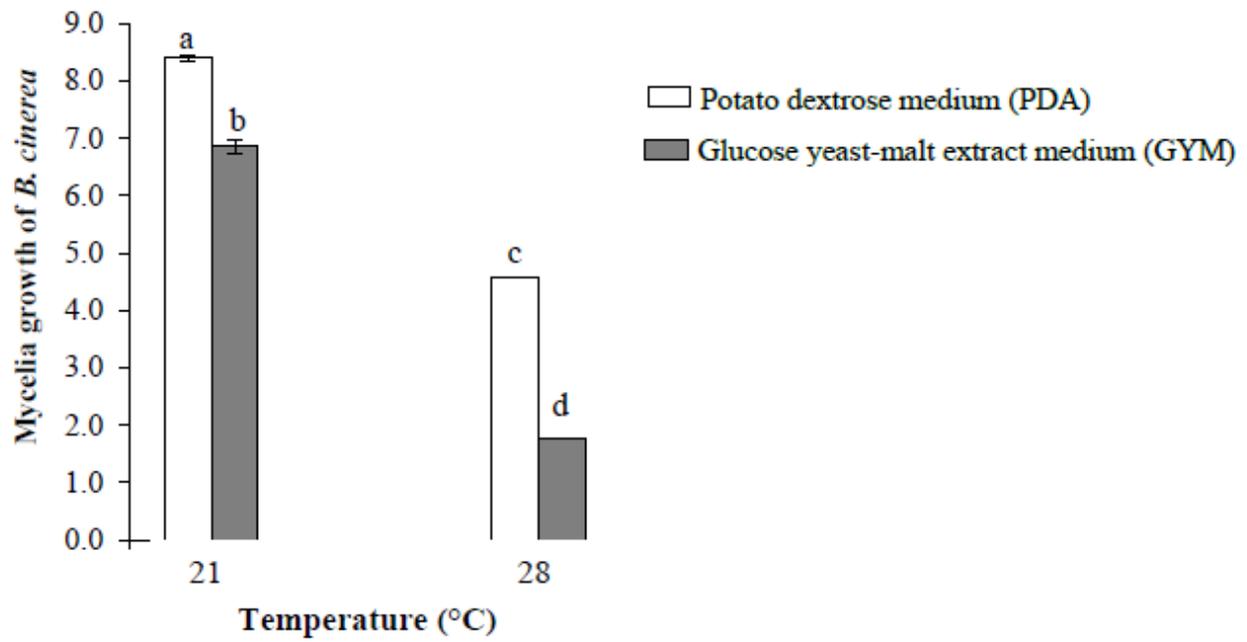


Figure 1

Influences of culture media (potato dextrose agar; PDA and glucose yeast-malt agar; GYM) and temperature (21 and 28°C) on mycelial growth of *B. cinerea* were determined after three days incubation. Values are the meaning of three replications (\pm SD).

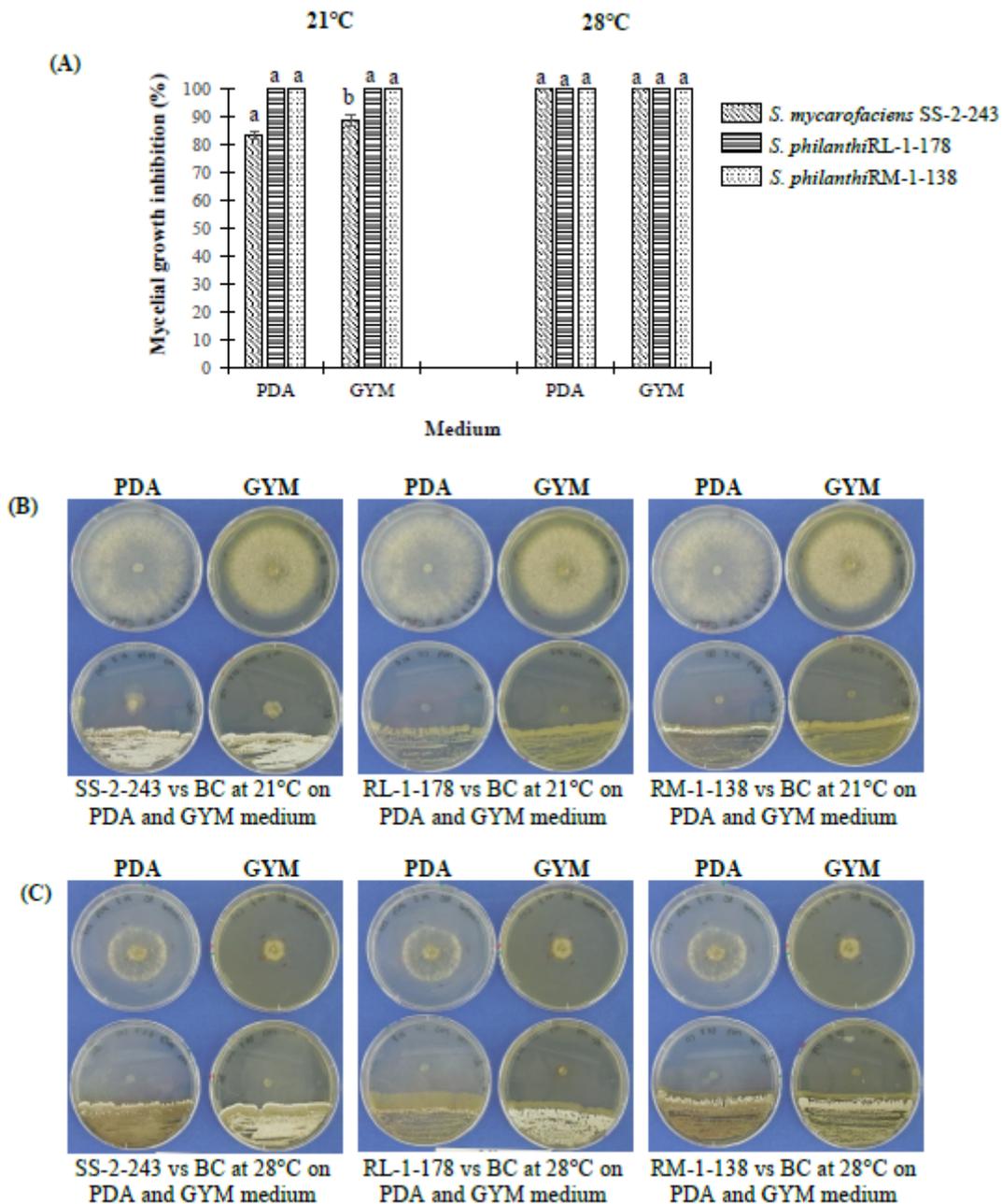


Figure 2

Mycelial growth inhibition of *B. cinerea* (BC) caused by the strain SS-2-243 of *S. mycarofaciens* and the strains RM-1-138 and RL-1-178 of *S. philanthi* using dual culture technique on potato dextrose gar (PDA) or glucose yeast-malt extract agar (GYM) after incubated at 21°C or 28°C (A). The growth appearance of each *Streptomyces* – the pathogenic fungal strain combination on PDA or GYM plates after incubated at 21 °C (B) or 28 °C (C). Top of figures *B. cinerea* only (control) and below the *Streptomyces* spp. vs *B. cinerea* (dual culture tested).

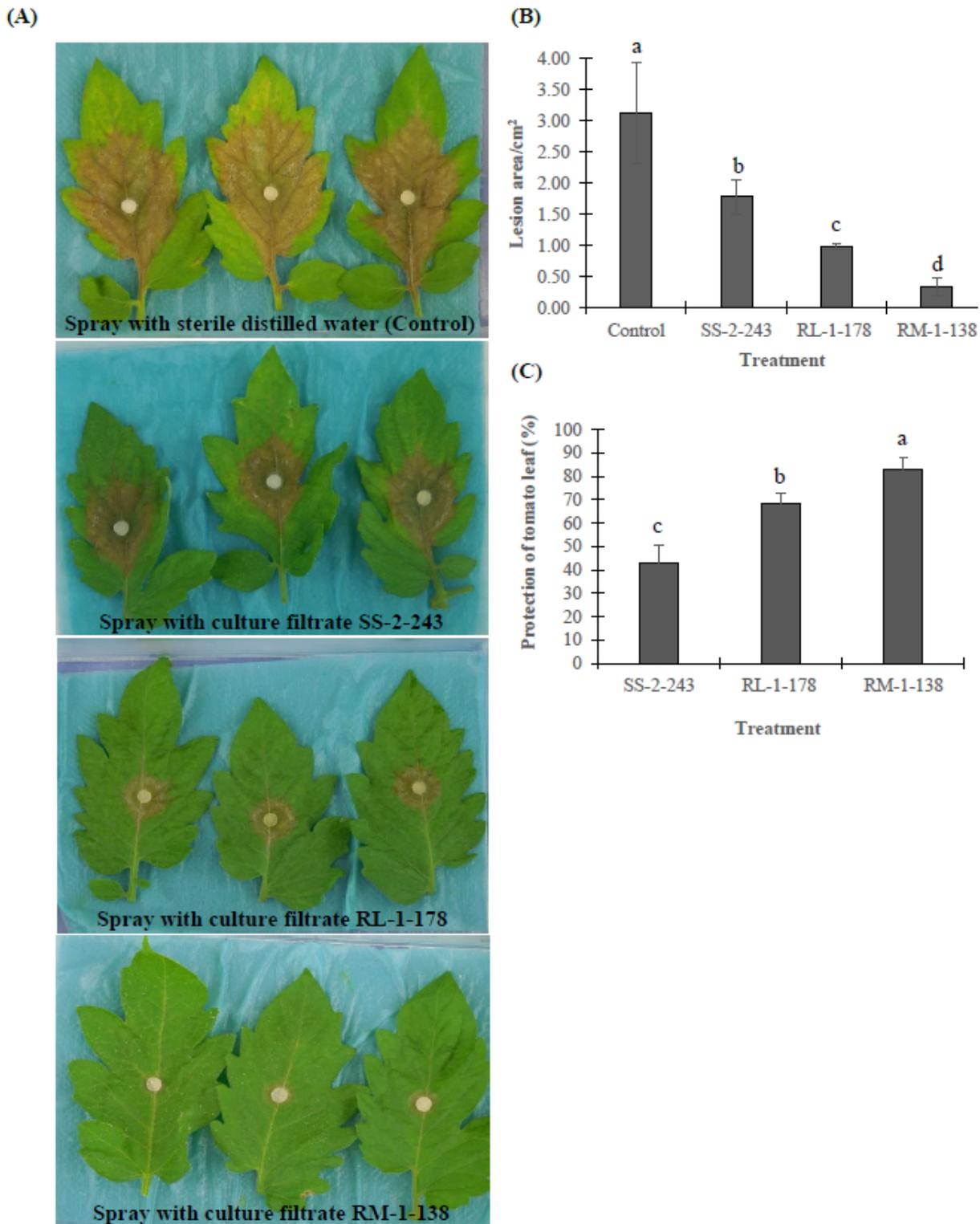


Figure 3

Antagonistic effects of culture filtrate produced by the strain SS-2-243 of *S. mycarofacien* and the strains RM-1-138 and RL-1-178 of *S. philanthi* on the development of blight symptoms in tomato leaf (A), lesion areas of tomato leaves with or without treatment by the culture filtrates (B), and protection of tomato leaf (in percentage) against *B. cinerea* with the culture filtrate produced by the three strains of *Streptomyces*

spp. (C). The data presented are the mean \pm SD ($n = 6$). The same letter on the bars for each column indicates no significant difference according to a Tukey test at $P < 0.05$.

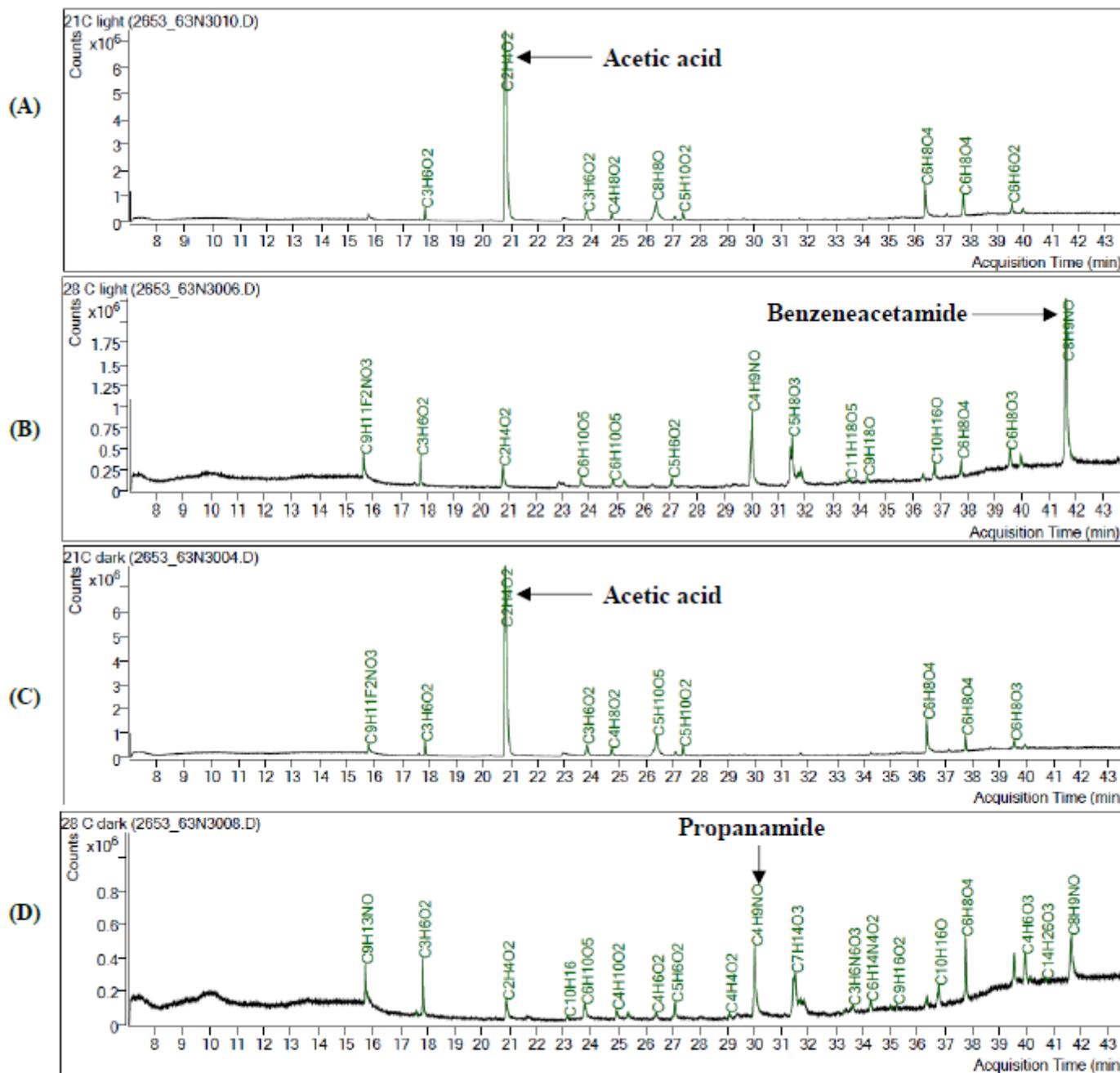


Figure 4

GC chromatograms of the culture filtrate from the strain RM-1-138 of *S. philanthi* grew in a GYM medium under the light and dark condition, and incubated at 21 and 28°C for 10 days: light condition and incubated at 21 °C (A) and 28°C (B) and dark condition and incubated at 21 °C (C) and 28°C (D), highlighting one major compound of each condition.