

Article

Assessment of the Role of Local Strawberry Rhizosphere—Associated Streptomycetes on the Bacterially—Induced Growth and *Botrytis cinerea* Infection Resistance of the Fruit

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Received: 22 November 2010 / Accepted: 10 December 2010 / Published: 22 December 2010

Abstract: The future need for sustainable agriculture will be met in part by wider use of biological control of plant pathogens over conventional fungicides hazardous to the environment and to public health. Control strategies involving both (i) direct use of microorganisms antagonistic to the phytopathogen, and (ii) use of bioactive compounds (secondary metabolites/antibiotic compounds) from microorganisms on the phytopathogen were both adapted in order to investigate the ability of streptomycetes isolated from the rhizosphere of strawberry plants to promote the growth of the fruit and suppress *Botrytis cinerea* causing strawberry rot on the Sunshine Coast, Queensland, Australia. *In vitro* studies showed that 25/39 streptomycetes isolated from strawberry field soils inhibited *B. cinerea* growth by antifungal activity, ranging from antibiosis to volatile compound production. However, when non-volatile antifungal compounds were extracted and applied aerially to the actively growing strawberry fruits infected with *B. cinerea*, a significant disease reduction was not recorded. On the other hand, plant and fruit growth was promoted by the presence of actively growing streptomycetes in container media. Findings might indicate that live streptomycete inoculum can be used as growth promoting agent in container media for this economically important crop.

Keywords: strawberry; *Botrytis cinerea*; streptomycetes; biological control; growth promotion

1. Introduction

Currently, the strawberry industry in Australia has an economic value of \$250 million with a farm gate value of \$100 million supporting 700 producers, employing 3,000 full-time and 15,000 casual workers. This makes it important for the horticultural industry, with approximately 30% of current production taking place in Queensland during the winter period and 70% of production in Victoria, Tasmania, New South Wales and Western Australia during the spring/autumn period [1]. *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Phytophthora cactorum* are the three major strawberry pathogens and *B. cinerea* has proved extremely difficult to control, due to its complex epidemiology and various pathways of infection. It has the ability to rapidly develop fungicide resistance, with resistant strains reported in Victoria, Australia [2].

Under cultivation, *B. cinerea* of strawberries is generally managed with an integrated program of synthetic chemical fungicides and cultural practices, such as removing infected fruit and leaf material from the plants [3]. Currently, the strains of *B. cinerea* resistant to two fungicide groups, benzimidazoles and dicarboximide are of particular concern as these chemicals are recommended and widely utilized for controlling disease on strawberries in Australia [4,5]. Biological control might be an alternative route to inhibit this pathogen by reducing the use of some chemical fungicides, which may in turn help to combat resistance problems often experienced with chemical control [6,7].

Extensive research into fungal and bacterial antagonists as potential biocontrol agents of *B. cinerea* has been carried out over the past 50 years in various countries throughout the world, including Australia. However, very few commercially viable biocontrol products have been released on the agronomic market, especially when compared to the number of synthetic based fungicides available for pathogen control [8-10]. Investigations of fungal and bacterial antagonists of *B. cinerea* have concentrated mainly on *Trichoderma*, *Gliocladium*, *Pseudomonas*, and *Bacillus* species [11]. Continued research on these antagonists as well as definition of the activities of new ones will aid in the formulation of commercially viable control products to reduce fungicide applications to crops.

The investigation described below targeted streptomycete species from strawberry field soils to determine whether these isolates would produce diffusible and/or volatile antifungal compounds inhibitory towards *B. cinerea* *in vitro*. The study further investigated whether live streptomycete inoculum incorporated into container media used to grow the fruit or the chemical extracts of the streptomycete fermentation products would reduce disease incidences caused by *B. cinerea* and induce growth promotion to the fruit.

2. Experimental Section

2.1. Isolation of the Pathogen

Commercial strawberry farmers commonly report *B. cinerea* infections in the Sunshine Coast Area. Isolations of the pathogen were therefore conducted from four cultivars (cultivars *Camarosa*, *Selva*, *Parker* and *Kabala*) collected from a commercial strawberry farm in the region. Infected fruit portions (~5 mm²) were placed centrally onto malt yeast extract agar (MYA) [12] containing streptomycin (50 µg/mL) to prevent bacterial growth, and incubated at 22 °C ± 2 °C for four days. The resulting 18 fungal isolates were purified onto the MYA and were identified and deposited at the Herbarium (BRIP),

Queensland Department of Primary Industries, Indooroopilly, Australia. Strain BRIP 28032-14 which was the most aggressive strain colonizing the fruit in laboratory trials, was selected as the test pathogen for this study to determine the effects of selected streptomycete antagonists on *B. cinerea*.

2.2. Isolation and Antifungal Screening of Streptomycetes against the Pathogen

Soil samples aseptically collected to a depth of 10 cm within the rhizosphere of each strawberry cultivar were used for streptomycete isolations using Starch-casein agar [13] supplemented with antifungal antibiotics (Nystatin and Cycloheximide, 50 ug/mL, Sigma) and incubated at 28 ± 2 °C for seven days. Selected streptomycete isolates were purified on Oatmeal agar [14] and incubated at 28 ± 2 °C for 10 days. Tentatively identified streptomycete isolates [15,16] were then stored in 20% glycerol at -20 ± 2 °C [17].

Streptomycete isolates that showed antagonistic properties towards *B. cinerea* from the dual culture assay [18] described above were selected for further study (Table 1). A dialysis membrane overlay technique was also used [19] to confirm the production of diffusible antifungal metabolites from the streptomycete isolates inhibitory towards *B. cinerea*. Potato Dextrose Agar (Oxoid) (PDA) plates untreated with the streptomycete antagonists and inoculated with *B. cinerea* agar plugs served as the control. The production of volatile inhibitory compounds against *B. cinerea* by the streptomycete isolates was studied using the volatile compound production assay [20].

Table 1. Details of streptomycetes isolated from the strawberry rhizosphere soil.

Cultivar	Total number of isolates	Isolate codes
Selva	8	S1#, S2, S5#, S6, S7, S8, S9, S10
Kabala	9	K1#, K2#, K3#, K4#, K5#, K6#, K7, K8#
Camarosa	16	C1, C2#, C3#, C4#, C6#, C7#, C8, C9, C10# CN1#, CN2#, CN3#, CN4#, CN5#, CN6#, CN7#
Parker	7	P1, P2#, P3, P4#, P5, P6, P7#
USC isolates*		S4#, S21#

*Antifungal streptomycetes selected from the USC Microbial Library and were previously isolated from container media soil used to grow cut flowers in the region, #Bioactive isolates.

In both tests, the colony diameters (mm) of *B. cinerea* were measured on the plates treated with and without the streptomycetes following incubation. A one-way analysis of variance (ANOVA) (performed on SPSS version 10.1 for Windows) was performed to test for significant differences between the colony diameters of *B. cinerea* from treated and control plates. If a significant ANOVA result was calculated, significant differences between means were determined by Duncan's multiple-range test (DMRT) at $P = 0.05$.

2.3. Antifungal Compound Extractions and Purifications

Four selected streptomycete strains (S21, S4, C3 and K5), which displayed the greatest inhibitory effect on the above-mentioned assays, were prepared by evenly streaking spores from 7–10 day old cultures over the 12 replicated oatmeal agar plates, and incubating at 28 ± 2 °C for 14 days. Following

the incubation, diffused compounds were extracted by using two different solvents from equally divided plate contents [21]. Dried and concentrated extracts were transferred to small glass vials and stored at $-20\text{ }^{\circ}\text{C}$ until required. The concentrated extracts were then assayed for antifungal activity utilizing a cup-plate method [18]. Extracts were also tested to determine their inhibitory activity against the spore germination of *B. cinerea* [22].

Fermentation metabolites of streptomycetes were studied using the methods described by Kurtboke *et al.* [23]. The methanol extract obtained from isolate S4, (S4-MeOH), proved to have a strong inhibitory action against *B. cinerea*. The crude extract was fractionated on compound polarity by passing through a C_{18} solid phase extraction (SPE) tube (Supelco LC-18 6 mL). The SPE tube was conditioned by running 3 mL of MeOH through the tube, followed by 3 mL of distilled water. The S4-MeOH extract, 4 mL, (turbid, deep red) was run into tube, followed by three washes; 3 mL with H_2O producing a light cream slightly turbid fraction (S4-A), 3 mL with 1/3 MeOH/ H_2O producing a light brown fraction (S4-B) and 3 mL with MeOH, producing a bright red fraction (S4-C). All three fractions were vacuum evaporated and assayed for activity utilising the methods described above. The most active fraction, S4-C, was further purified by preparative high performance liquid chromatography (HPLC) utilising a Perkin Elmer series 200 pump equipped with a diode array detector, separating on a $100\text{ mm} \times 10\text{ mm}$ Omnifit RP C_{18} column, with detector parameters set at 205 nm (Channel A) and 260 nm (Channel B). The mobile phase solvents were prepared from HPLC grade acetonitrile (ACN) and Milli-Q water (H_2O). Solvent A consisted of 25/75 ACN/ H_2O and solvent B consisted of 75/25 ACN/ H_2O . Total flow was maintained at 0.2 mL per minute, while maintaining 100% solvent A for 3 minutes. The flow rate was then increased to 1.0 mL per minute, while grading elution to 30/70 solvent A/B over 27 minutes, maintaining this for 4 minutes, before grading back to 100% solvent A over 6 minutes. The fraction visibly eluting as pink was collected and re-analysed as one peak by HPLC. The collected fraction was evaporated to 0.25 mL under reduced pressure on a Büchi Rotavapor, then transferred to a 2 mL vial and frozen at $-20\text{ }^{\circ}\text{C}$. The sample was subsequently freeze-dried under vacuum in a dessicator which contained phosphorus pentoxide and anhydrous sodium carbonate in separate beakers. The dry extract was analysed in deuterio (d_4)-methanol by ^1H NMR (nuclear magnetic resonance) on a Varian UNITY 400 MHz spectrometer, and by VG platform II electrospray LC-MS located at Griffith University, Queensland, Australia.

2.4. Pot Trial

A rye grass (cultivar *Tetila*) seed-based inoculum was prepared for selected streptomycete isolates for the pot trial conducted to grow strawberry plants in the presence and absence of the *B. cinerea* pathogen and the streptomycete antagonists [24]. Pasteurised soil (0.35 m^3) for use in the pot trial was supplied by the Department of Primary Industries Maroochy Research Station located at Nambour, Queensland. The soil consisted of a mixture of 60% sand and 40% peat and was heat pasteurised with steam at $60\text{ }^{\circ}\text{C}$ for 45 min. Certified strawberry runners (cultivar *Camarosa*), grown in fumigated soil in Victoria, under the Victorian Strawberry Industry Certification Authority (VSICA), were obtained for pot trials. Prior to planting, strawberry runners were thoroughly cleaned in water to remove all dirt particles. A total of six treatments were prepared for the pot trial (Table 2). Treatments were arranged in a random block design, with plots consisting of the five treatments replicated 10 times.

Table 2. Treatments used in the pot trial.

Pot treatment	Description
Treatment-1	Control
Treatment-2	Disease control
Treatment-3	C3-LP-EtOAc extract spray solution on aerial plant surfaces
Treatment-4	Soil inoculated with non-inhibitory streptomycete isolates C1, C8, P6, S2
Treatment-5	Soil inoculated with inhibitory streptomycete isolates K3, K5, S4, S21
Treatment-6	Rye grass seed inoculum control (Soil inoculated only with sterilised rye grass seeds)

Disease index used to assess the effectivity of the above treatments: 1: No visible disease on fruit, 2: No greater than $\frac{1}{4}$ of fruit infected, 3: No greater than $\frac{1}{2}$ of fruit infected, 4: No greater than $\frac{3}{4}$ of fruit infected, 5: Whole fruit surface infected.

Control and aerial spray treatments were prepared by filling 10, 200 mm black plastic pots for each treatment with pasteurised soil to approx 1.5 cm from the pot rim. Osmocote® controlled release fertiliser was added to the soil surface as per manufacturer's specifications, prior to covering the pots with silver/black plastic (silver side up) and securing to the rim. A cross was cut into the plastic to expose the soil, with the strawberry runners planted using a metal planter to push the root system deep into the soil, leaving the leafless crown above soil level. For other treatments, inocula were introduced with a ratio of 0.84% (w/w) into the potting soil. Plants were placed on plant stands in full sunlight and enclosed in wire netting to prevent fruit damage by wild animals and were irrigated by hosing every second day or when required. Treatments were arranged in a random block design, with plots consisting of the five treatments replicated 10 times [25].

2.5. Pathogen Inoculations

B. cinerea spores were harvested from MYA plates and the resulting spore suspension diluted into Tween 80 (0.01%), with a final spore concentration of 1×10^5 spores mL^{-1} . The spore suspension was sprayed onto the plants until runoff, concentrating on fruit, flowers and buds [18]. Treatment-1 (control) was sprayed only with sterilised Tween 80 (0.01%) solution. Strawberry plants were returned to the enclosure and covered with clear plastic for 24hrs to promote humid conditions required for *B. cinerea* infection [26].

B. cinerea spores were inoculated onto the aerial surfaces of strawberry plants for Treatments 2, 3, 4, 5, and 6 utilising a handheld trigger action atomiser spray unit. The first inoculation event occurred approximately seven weeks after planting runners, when all plants were at the flowering and fruiting stage. Due to difficulties in establishing grey mould disease on strawberry plants, two additional inoculation events were performed at intervals of two weeks, following the initial inoculation event.

2.6. Application of Antifungal Extract

C3 was selected to produce large volumes of antifungal metabolite to be further tested to spray the plant aerially. A total of 20 mL of active extract was produced (following evaporation) using the

solvent ethyl acetate, which was utilised for the preparation of an aerial spray treatment for the subsequent pot trial involving the pathogen.

The extract C3-LP-EtOAc was formulated into a solution by diluting the extract (3.5 mL) into a sterile Tween 80 (0.1%) solution (200 mL). The extract solution was transferred into a handheld atomiser spray unit (as utilised for spore suspension inoculations) and applied to plants until runoff.

2.7. Harvesting of Strawberry Fruit

Ripe and/or severely diseased strawberry fruits were harvested from strawberry plants every four days. Each individual fruit harvested was weighed (g), assessed for presence of disease, and any lesion/s of *B. cinerea* present measured in millimetres. Disease severity of strawberry fruits was rated with a disease index based on a scale from 1–5, adapted from the index described by Archbold *et al.* [27] (Table 2). Samples were taken from diseased fruit not showing classic symptoms and plated onto the MYA (incubated at room temperature approx. 22–24 °C for five days) to determine that *B. cinerea* was the actual causative agent. In addition, the wet and dry weights (drying at 60 °C for four days) of each strawberry plant were obtained for root and shoot portions [28].

A two-way ANOVA, performed on SPSS version 10.1 for Windows, was used to determine (i) differences in the harvested fruit weights between each treatment (g) and (ii) differences in the shoot, root, and combined shoot/root weights (g) of the strawberry plants between each treatment. If a significant ANOVA result was calculated, significant differences between means were determined by DMRT at $P = 0.05$.

3. Results and Discussion

3.1. Detection of Antifungal Activity from Streptomyces Isolates

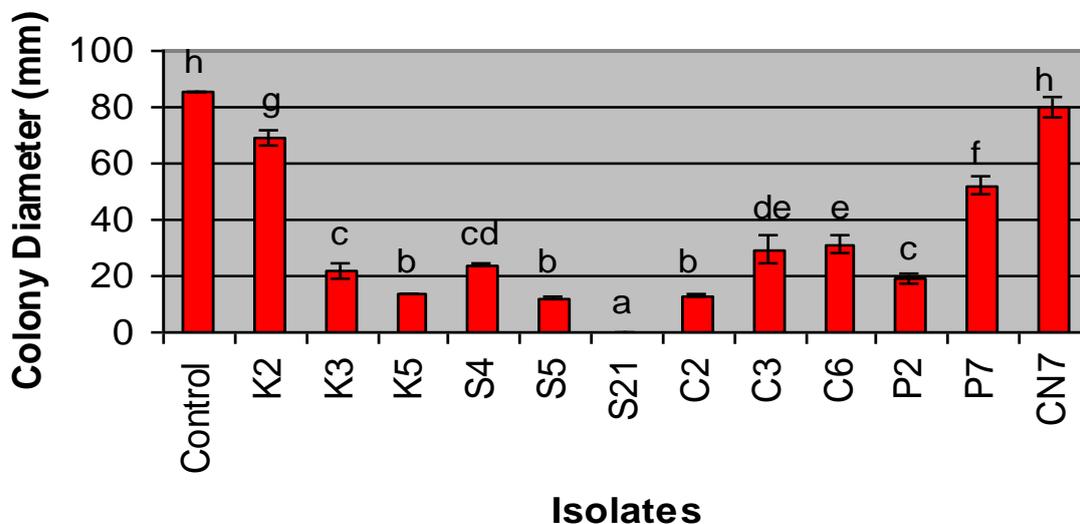
In dual culture assays, most of the streptomyces isolates were observed to produce coloured extracellular compounds that diffused into the agar surrounding the inoculation site. These compounds were evident following the incubation of inoculated PDA plates before the addition of mycelium plugs of *Botrytis cinerea*. The observed inhibitory activity towards *B. cinerea* was not always associated with the production of these pigmented compounds by the isolates, however, 25 of the 39 streptomyces isolates were found to be capable of inhibiting *B. cinerea* growth (Table 2). Streptomyces isolates exhibiting various degrees of antagonistic activity towards the pathogen were selected for further screening of their activity *in vitro* and *in vivo*.

All streptomyces isolates were observed to produce pigmented metabolites, which diffused through the dialysis membrane into PDA during incubation. *B. cinerea* radial growth was significantly ($P < 0.05$) inhibited by the diffused metabolites produced by 11 of the 12 streptomyces isolates when statistically compared to the control, as indicated by one-way ANOVA and DMRT results (Figure 1a).

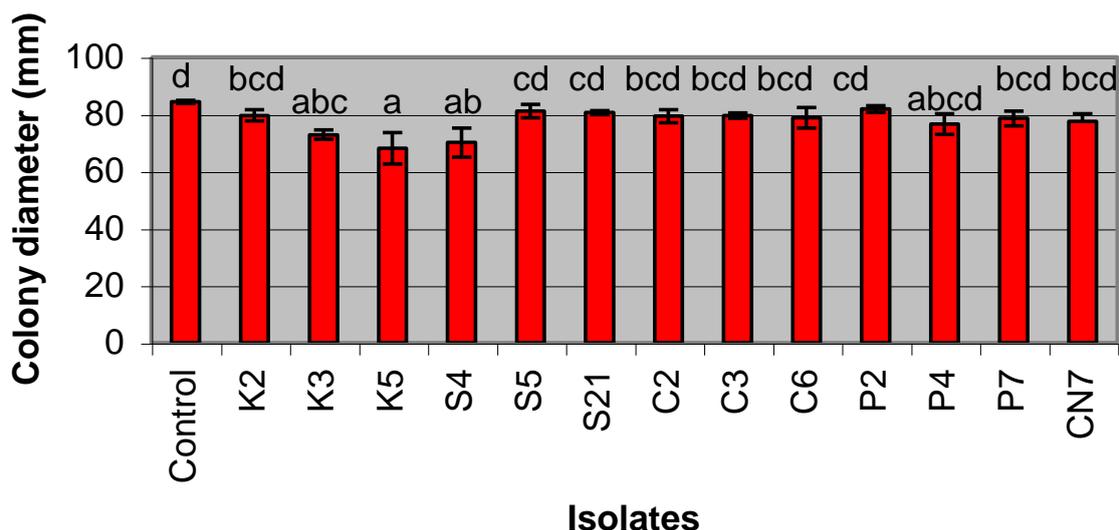
Comparison of *B. cinerea* colony diameter measurements, obtained from the volatile compound production assay, proved that certain streptomyces isolates had a volatile inhibitory effect with a statistically significant ($P < 0.05$) result obtained when analysed by one-way ANOVA. Streptomyces isolates K3, K5, and S4 were found to be significantly different from the control and other isolates

using DMRT ($P = 0.05$) (Figure 1b), indicating that these isolates were able to produce inhibitory volatile metabolites towards *B. cinerea*.

Figure 1. *B. cinerea* colony diameter in (a) dialysis membrane assay (b) volatile compound production assay.



Bars represent the mean colony diameter of 4 replicate plates (\pm SE). Measurements were square root transformed before analysis. Bars annotated with the same letter are not significantly ($P > 0.05$) different according to DMRT.



Bars represent the mean colony diameter of 4 replicate plates (\pm SE). Bars annotated with the same letter are not significantly ($P > 0.05$) different according to DMRT.

3.2. Extract Assays

The assays for inhibitory activity against *B. cinerea* indicated that the extracts prepared using the solvent ethyl acetate retained the greatest activity, with S21-EtOAc and S4-EtOAc producing the greatest inhibition of *B. cinerea* (Table 3). The activity of extracts prepared with methanol were observed to have less inhibitory activity than the ethyl acetate extracts, with C3-MeOH and K5-MeOH losing all inhibitory action. The extracts prepared with methanol were also observed to possess a

stronger colour than those prepared with ethyl acetate, particularly S4-MeOH and S21-EtOAc, which were deep turbid red and dark turbid brown, respectively.

Table 3. Inhibition of *B. cinerea* against antifungal metabolite extracts.

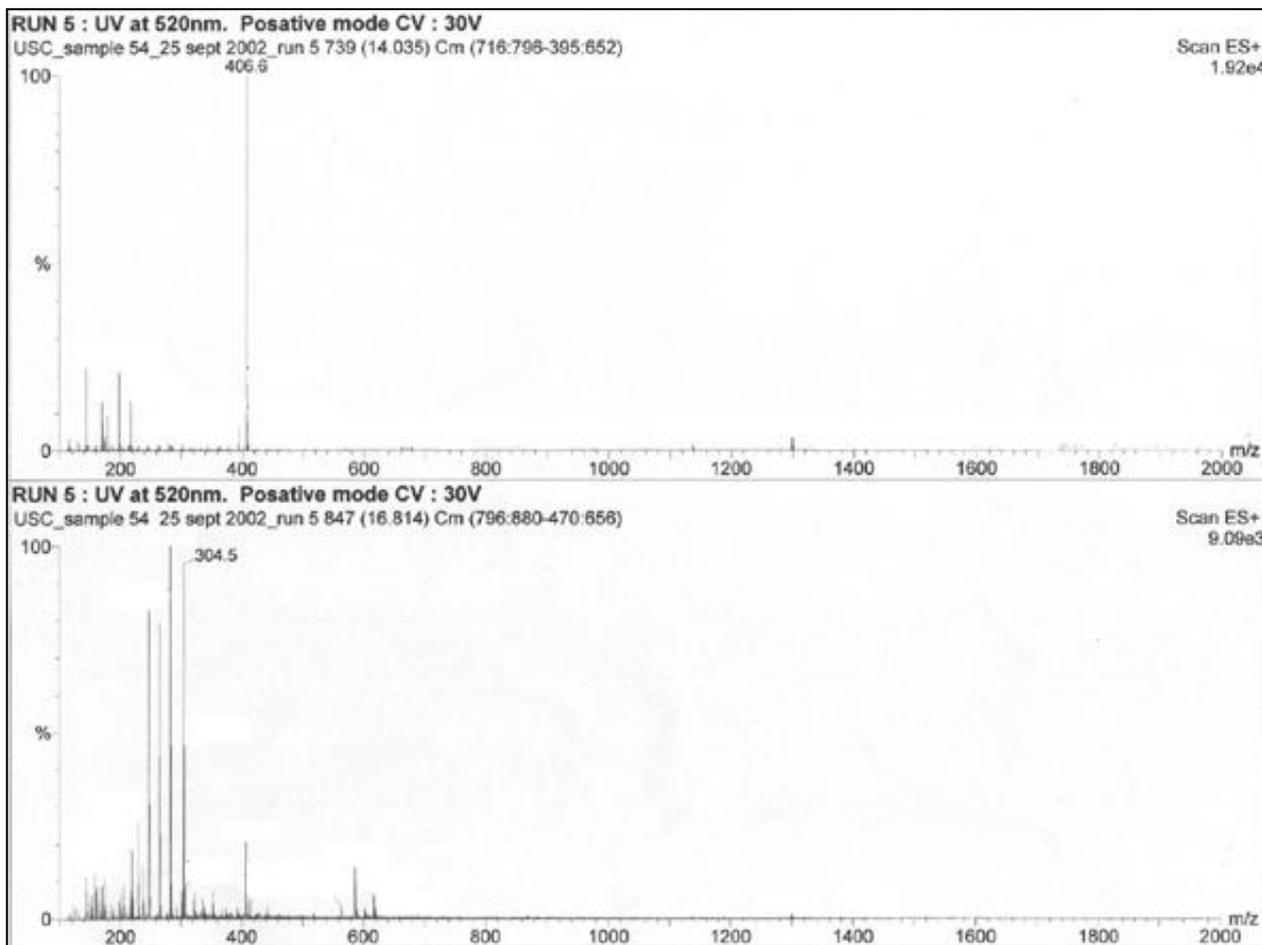
Extract	Inhibition Zone (mm)
S21-MeOH	8.3
S21-EtOAc	11.0
S4-MeOH	9.0
S4-EtOAc	13.0
C3-EtOAc	7.0
C3-MeOH	0.0
C3-LP-EtOAc	6.6
K5-MeOH	0.0
K5-EtOAc	1.6

Ethyl acetate has a lower polarity index ($P^1 = 4.3$) than methanol ($P^1 = 6.6$) [29], therefore ethyl acetate is more likely to extract compounds with lower polarity, whereas methanol will extract compounds with a greater polarity. Based on the polarity indexes of the solvents, isolates S21 and S4 were shown to produce both non-polar and polar bioactive compounds as the extracts prepared with both solvents were capable of inhibiting mycelial growth from *B. cinerea* cultures. Only ethyl acetate was able to extract bioactive compounds from isolates C3 and K5 with methanol extracts possessing no inhibitory activity, suggesting the bioactive metabolites produced by these streptomycete isolates were non-polar.

Following the 16 h incubation period, all extracts utilised in the spore germination assay were found to inhibit germination of *B. cinerea* spores when compared to the control. The results from the Kruskal-Wallis test indicated that there was a significant difference between the percentages of germinated spores recorded from the treatments, with a χ^2 value of 18.615 at 6 degrees of freedom (df) and a significance level of 0.005 (<set alpha level of 0.05). As the Kruskal-Wallis test proved significant, the highest and lowest *R* values were then compared. The control had the highest *R* value (26.50), with treatments S4-MeOH, S4-EtOAc, and S21-EtOAc having the lowest *R* value (10.50), indicating that these extract treatments had significantly lower spore germination percentages than the control.

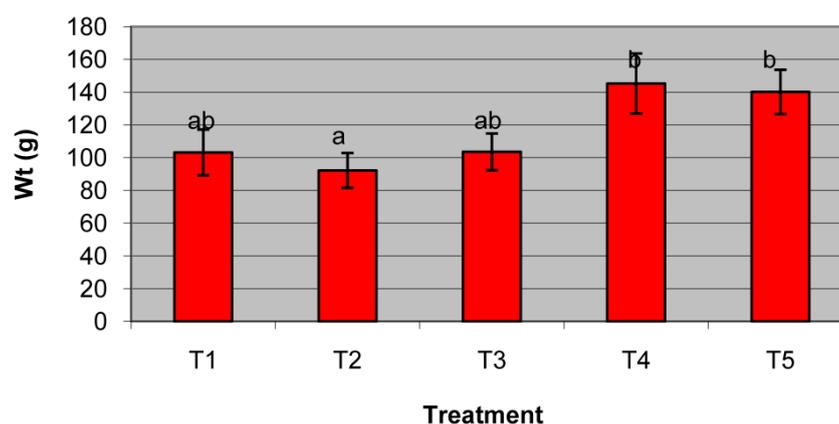
The final purification of the active fraction from S4-MeOH, S4-C fraction, by HPLC resulted in the collection of a pink fraction, observed as a single chromatographic peak, which was dried and further analysed. Partial decomposition of the S4-C fraction on storage was apparent by the shift in colour from pink to orange and the appearance of two peaks in the LC-MS spectrum. Compound identification was not possible but the ^1H NMR was consistent with the presence of a monosaccharide, flavin like compound and valine fragments and the LC-MS provided molecular weights of 406 and 304 amu for the two compounds (Figure 2).

Figure 2. LC-MS spectrum showing molecular weights of 406 and 304 amu for the two compounds detected with ^1H NMR.



3.3. Strawberry Pot Trials

The fruit weights (g) recorded from 14 harvesting occasions were found to be significantly ($P < 0.05$) different between treatments, with no significant ($P > 0.05$) difference found between blocks for pot Treatments 1 to 5. DMRT ($P = 0.05$) determined that the mean fruit weight for the disease control, Treatment-2 (92.14 g) was significantly lower than the mean fruit weights obtained from the two treatments consisting of soil inoculated with streptomycete isolates, Treatment-4 (145.26 g) and Treatment-5 (140.16 g) (Figure 3). The mean weight of fruit harvested from the control, Treatment-1 (103.16 g) and the plants treated with the C3-LP-EtOAc crude extract, Treatment-3 (103.54 g) were not found to significantly differ from the other three pot treatments.

Figure 3. Mean fruit weights per treatment.

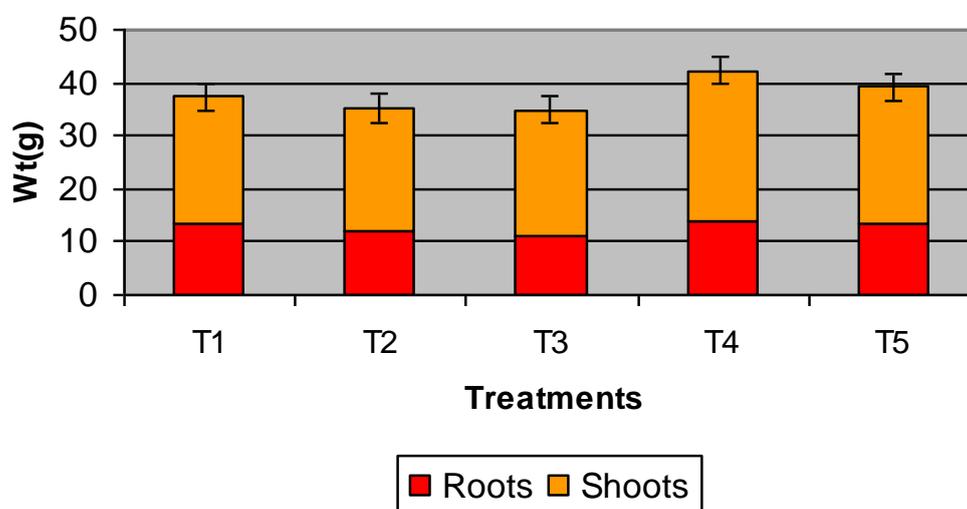
Bars represent the mean fruit weights for each treatment from 10 replicate plants (blocks) (\pm SE). Bars annotated with the same letter are not significantly ($P > 0.05$) different according to DMRT.

The incidence of each disease index (1 to 5), expressed as a percentage (%) of the total fruit harvested for Treatments 1 to 5, determined that 91% of fruit harvested from Treatment 1 showed no symptoms of *B. cinerea* incited disease (Table 4). Whereas, fruit harvested from the remaining treatments had a reduced proportion of fruit uninfected with *B. cinerea*, with 75% of fruit harvested from Treatments 2, 70% from Treatment-3, 62% from Treatment 4, and 70% from Treatment 5 showing no symptoms of *B. cinerea* disease. These results therefore indicate that the application of *B. cinerea* spore suspension to Treatments 2, 3, 4, and 5 plants was responsible for the increase in strawberry fruits showing symptoms of *B. cinerea* infection. Comparing the incidence of diseased fruit (represented by Indexes 2 to 5) between Treatments 2, 3, 4, and 5 showed that 25% of fruit from Treatment 2 was recorded as showing symptoms consistent with *B. cinerea* infection whereas Treatment 3 recorded 30%, Treatment 4 recorded 38% and Treatment 5 recorded 30% (Table 4). These results suggest that the three treatments (3, 4, and 5), consisting of crude C3-LP extract or soil inoculated with streptomycete isolates, were unsuccessful in reducing the *B. cinerea* fruit infection. Comparing the percentages of severely infected fruit (represented by Index 5) provides further evidence of the inefficiency of the treatments. Treatments 3, 4, and 5 recorded 11%, 9%, and 10% respectively, of severely diseased fruit, which was greater than the 5% recorded for Treatment 1 the 7% recorded for Treatment 2 (Table 4). Overall Treatment 4 recorded the highest incidence of diseased fruit in comparison to the Treatments 3 and 5, which were the only two treatments consisting of or formulated from streptomycete isolates previously shown to be inhibitory to *B. cinerea* growth (Table 4).

Unlike the differences found between the fresh strawberry fruit weights for pot treatments, there were no significant differences ($P > 0.05$) found between shoot, root and total dry plant weights in each treatment when analysed by 2-way ANOVA (Figure 4).

Table 4. Overall incidence of *B. cinerea* infected fruit harvested from each pot treatment.

Pot Treatment	Disease Index	Incidence (%)
Treatment-1	1	91%
	2	2%
	3	0%
	4	2%
	5	5%
Treatment-2	1	75%
	2	9%
	3	2%
	4	7%
	5	7%
Treatment-3	1	70%
	2	6%
	3	8%
	4	5%
	5	11%
Treatment-4	1	62%
	2	9%
	3	12%
	4	8%
	5	9%
Treatment-5	1	70%
	2	8%
	3	6%
	4	6%
	5	10%

Figure 4. Mean shoot, root and total strawberry plant weights.

Bars represent the total mean plant weights (g) of 10 replicate plants (\pm SE), showing contribution of mean shoot and root weights for each treatment.

Even though there were no significant differences, these results follow a similar trend to those obtained for the fresh fruit weights, with the two treatments consisting of soil inoculated with streptomycete isolates, Treatments 4 and 5, recording a higher harvested fruit weight than the other three treatments. Treatments 4 (42.3 g) and 5 (38.1 g) obtained the highest mean total dry plant weights, when compared to the controls Treatments 1 (37.4 g), 2 (35.2 g), and to the treatment consisting of C3-LP-EtOAc extract, Treatment 3 (34.9 g) (Figure 4). In addition, differences existed between plants from Treatments 4 and 5 when visually compared to Treatments 1, 2, and 3 (Figure 4).

4. Conclusions

Fungal infections to strawberry fruit causing significant destruction to crops in the Sunshine Coast region result in significant economic losses to growers. Use of streptomycetes as potential biological control agents might be an alternative way to replace the currently used environmentally-unfriendly pesticides. Assays conducted *in vitro* have indicated that there was a strong antifungal action expressed by the streptomycete isolates against *B. cinerea* supporting the findings of other studies indicating similar antifungal actions by streptomycete species against different phytopathogens [30-33]. Although the ecological relevance and the lack of correlation to field performance of *in vitro* screening procedures utilised for establishing antagonism by the potential biocontrol organism are open to doubt, these procedures have proved useful in identifying potential biocontrol agents warranting further investigations in greenhouse and field studies [34]. For example, Trejo-Estrada *et al.* [31] established that *S. violaceusniger* YCED9 produced at least three antibiotics that inhibited fungal pathogens of turf grass *in vitro*. When greenhouse experiments were conducted, *S. violaceusniger* YCED9 controlled partially a grass seedling disease caused by *Rhizoctonia solani* and a crown-foliar disease caused by *Sclerotinia homeocarpa*. Similarly results obtained from the pot trial demonstrated that the observed antagonism *in vitro* was not reproducible in all cases when assessed under conditions more closely related to field conditions. Furthermore, more diseased fruit obtained in the streptomycete treated pots might indicate that some actinomycete metabolites perhaps convert the pathogen into more aggressive form such as the monosaccharide, flavin-like compound and valine fragments detected in the metabolites identified in the study. Interestingly, Schrey and Tarkka [35] recently documented the suppressive and promoter activity of the streptomycetes on plant pathogens and recommended caution in their selection and use as biological control agents.

Growth promotion of plants grown in a soil medium inoculated with streptomycete spp. has been commonly reported [36,37]. The mechanisms by which beneficial microorganisms promote plant growth include increasing available nutrients for uptake by the plant by recycling and solubilising mineral nutrients and the provision of plant growth substrates by the synthesis of vitamins, amino acids or plant hormone (e.g., auxins and gibberellins) [36,37]. Although a reduction in the incidence of fruit infected by *B. cinerea* by the streptomycete isolates used in this study was not apparent, they were found to significantly stimulate fresh fruit weights harvested from these strawberry plants when compared to fruit weights from plants grown in un-inoculated soil. Nature of the growth promoting compounds from the isolates warrant further investigation, and growth promotion induced by streptomycete species and their metabolites when applied externally to the fruit might be a bonus for the industry during the marketing of the fruit [38].

Acknowledgements

Authors thank Graham and Carol Wood for their support and assistance provided during sample collection at their farm and to Roger Shivas at DPI, Herbarium, Indooroopilly, Queensland for the identification of the *Botrytis cinerea* isolates.

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