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# Antifungal Activity of *Streptomyces* spp. Extracts In Vitro and on Post-Harvest Tomato Fruits against Plant Pathogenic Fungi

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**Abstract:** Plant pathogenic fungi are the most important cause of losses in agriculture. In the context of the overuse of synthetic fungicides, natural products are an encouraging alternative to control these plant pathogenic fungi. In this study, we tested the antifungal capacity of four strains of the genus *Streptomyces* against the plant pathogenic fungi *Alternaria alternata*, *Botrytis cinerea* and *Fusarium oxysporum*. We also investigated the effect of applying the extracts with the highest antifungal activities in a post-harvest setting for the control of *B. cinerea* on cherry tomato fruits. The results revealed the ability of these strains, especially *Streptomyces netropsis* A52M, to inhibit in vitro the growth and, in some cases, the sporulation of plant pathogenic fungi. The protective effect of the *S. netropsis* A52M extracts on post-harvest cherry tomato fruits infected with *B. cinerea* was demonstrated. In addition, when grown in co-culture, we observed an interesting phenomenon in which the *Streptomyces* mycelium physically encapsulated the fungal mycelium, contributing to its inhibition. This outcome offers the potential for research into the role of physical microbial interactions in fungal biocontrol. To sum up, the findings outlined here for the interactions between *Streptomyces* strains and plant pathogenic fungi are a promising, safer, and more sustainable biocontrol alternative to chemicals for agriculture. This is of particular interest in the protection of perishable agricultural products during the post-harvest phase.

**Keywords:** actinomycetes; *Streptomyces*; *Alternaria alternata*; *Botrytis cinerea*; *Fusarium oxysporum*; antifungal activity; post-harvest



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## 1. Introduction

Humanity's most significant challenge today is securing an adequate food supply for an ever-growing world population. This overpopulation, as well as the industrialization of the food sector, has driven an unprecedented intensification of crop cultivation, which puts significant pressure on natural resources and available arable land [1]. A direct negative consequence for soil quality has been observed. This degradation, coupled with the deterioration and the loss of natural resources, is a problem that threatens agriculture's ability to keep pace with the growing demand for food [2].

In addition, plant diseases cause significant losses in crop production worldwide, with their consequent economic impacts being notable [3]. Among the causal agents of these diseases, plant pathogenic fungi are the main cause of agricultural losses, responsible for

infections affecting a wide range of cultivated plants. Among them, *Alternaria alternata*, *Botrytis cinerea*, and *Fusarium oxysporum* stand out as widely distributed pathogens that can cause considerable damage to crops of economic interest, causing root, fruit, and flower rot; vascular wilt; and, ultimately, plant death [4].

Synthetic chemical fungicides are the most widely used substances to combat plant pathogenic fungi. However, the excessive use of these synthetic fungicides has caused a significant impact on human health due to the presence of residues in food and the environment. In this context, natural products or extracts are an encouraging alternative to synthetic chemical fungicides currently used to control plant pathogenic fungi [5]. Many of the losses due to these plant pathogenic fungi occur during the post-harvest stage, a critical stage in the supply chain of agricultural products [6], where the quality and safety of products are key factors. Therefore, the direct application of bacteria poses significant challenges, as they could cause plant infections or raise food safety concerns for consumers, especially when inoculated on raw edible vegetables [7]. Thus, the goal is to adopt approaches that allow the antimicrobial compounds produced by bacteria to be harnessed without the need to apply the bacterial cells themselves to the cultures.

In this context, searching for effective and sustainable methods to control these diseases has prompted research in the fields of microbiology and biotechnology. One of the most promising strategies in this field is the use of beneficial microorganisms such as actinomycetes. These Gram-positive and filamentous bacteria have the genus *Streptomyces* as their primary proponent due to their known ability to produce a wide variety of bioactive compounds with potential pharmaceutical, biotechnological, and agricultural applications, including the inhibition of plant pathogenic fungi [8,9]. There is an impressive diversity of secondary metabolites of interest, including antibiotic, anti-tumor, and immunosuppressive agents, which have been the subject of study and use for decades [10]. Due to their relevance, the search for new *Streptomyces* strains capable of producing new therapeutic agents and bioproducts for various uses is ongoing. Whole-genome sequences have shown that actinomycetes, including *Streptomyces* spp., have larger potential for the biosynthesis of new compounds than those shown in axenic culture. Genomics has shown that a single species has more than 20 gene clusters potentially encoding new compounds. For example, more than 20 clusters for the production of secondary metabolites are detected in the genome of *Streptomyces coelicolor* (8.66 Mb) [11], and 25 clusters are included in the genome of *Streptomyces avermitilis* (9.02 Mb) [12]. However, a significant proportion of these genes remain unexpressed under axenic culture. In this vein, for finding new active compounds, apart from more classical studies analyzing compounds produced by *Streptomyces* when growing in axenic culture, the co-culture of microorganisms has emerged as a valuable approach. Co-culture better simulates ecological habitat interactions and extends the exploration of the impact of microbial interactions on the biosynthesis of new secondary metabolite in *Streptomyces*. Some representative examples from other studies are the co-culturing of *Streptomyces* with *Myxococcus xanthus*, which triggered the production of secondary metabolites of interest [13,14].

The present study is focused on evaluating the extracts' in vitro antifungal capacity, produced by four strains from different species of the genus *Streptomyces* against the plant pathogenic fungi *A. alternata*, *B. cinerea*, and *F. oxysporum*. Furthermore, considering the serious economic losses of fruits and vegetables in the post-harvest phase, the main goal was focused on the study of the viability of these extracts in the post-harvest phase for the fungal control of *B. cinerea* on cherry tomato fruits.

## 2. Materials and Methods

### *2.1. Strains, Culture Growth and Co-Cultures*

The plant pathogenic reference fungi used in this trial were *A. alternata strain* Aa100, *B. cinerea* strain B05.10, and *F. oxysporum* strain 2715, belonging to the strain collection of the University of La Laguna, San Cristóbal de La Laguna, Spain. The actinomycete strains used in the present study were isolated in a previous study from soils from Tenerife (Canary

Islands, Spain) carried out in 2016, following the methodology described previously [9], classified according to their sequences in their closest species, and deposited in the Nertalab S.L. strain collection. These strains were *Streptomyces ardesiacus* P3 and *Streptomyces mauvecolor* P46M, both from pine forest environments; *Streptomyces netropsis* A52M from a coastal environment; and *Streptomyces pratensis* i3E from a high mountain environment. Actinomycetes and fungi were cultured on Trypticase Soy Agar (TSA) and Potato Dextrose Agar (PDA) agar plates. Actinomycetes were grown for five days at 23 °C, forming a homogeneous lawn using a sterile swab. Similarly, fungi strains were grown on PDA agar plates under the same conditions.

Actinomycetes and fungi co-culture was carried out in 250 mL Erlenmeyer flasks containing 50 mL TSB. Two discs of 8 mm in diameter of the chosen actinomycete strain and one disc of the same size of one of the phytopathogenic fungi were added. Controls were inoculated with actinomycetes or fungi strains alone. Each treatment was carried out in triplicate, resulting in three replicates of the extracts for each actinomycete with each fungus. Erlenmeyer flasks were incubated at 23 °C under agitation, and 2 mL aliquots were taken at 24 h and then every 48 h for seven days. They were examined under the light microscope by making a fresh smear, without staining, to observe actinomycete and fungal growth in all treatments. Subsequently, the samples were subjected to two successive centrifugations of 10 min at 13,000 rpm to remove the microorganisms and obtain the supernatant used for the fungal in vitro inhibition assay.

### 2.2. Antifungal Activity of the Media

The antifungal activity of the co-culture media was tested in PDA agar plates. All plates had the same volume (20 mL) and thickness to ensure uniform product diffusion across all plates. Each plate was surface inoculated with 50 µL of a spore suspension at a concentration of $2 \times 10^4$ spores/µL of *B. cinerea* and *F. oxysporum*. The spore suspension was prepared from plates of the different fungi, grown in the dark at 23 °C for 5 to 7 days. Approximately 5 mL of sterile water was poured onto each plate, and the surface was scraped. The resulting suspension was recovered and filtered through sterile cotton wool into a falcon tube. Subsequently, an aliquot of the suspension was taken and quantified in a hemocytometer, allowing the normalization of the spore concentration [15]. In the case of *A. alternata*, as it was impossible to obtain a spore suspension, this was sown by taking sporulated mycelium from a 5–7-day-old plate with a sterile swab.

The agar diffusion method was used to test the antifungal capacity against the indicated fungi [16]. In brief, 8 mm diameter circumferences were removed with a sterile punch, creating wells in the agar, i.e., three per plate. Then, 100 µL of each supernatant from each actinomycete with each fungal was added to each well, using TSB as a negative control. This was performed in triplicate with each replicate of the co-cultures. The plates were incubated at 26 °C in the dark, and inhibition halos were measured after five days. Compared to the paper disc diffusion method [17], this method allows more product volume to be added for evaluation and more uniform diffusion through the agar. The measurements taken were classified according to the type of inhibition observed. Thus, the total growth inhibition, partial or affected inhibition, and inhibition of spore production were evaluated. Partial damage was considered when the mycelium grew abnormally with either different coloring or density.

### 2.3. Media Solvent Extraction and Concentration

After determining the optimal culture duration and confirming the activity ratio of strains, the supernatant was extracted using organic solvents. This process aimed to eliminate the residual culture media and other non-essential substances. The co-culture media were harvested and centrifuged in 50 mL Falcon tubes at 4800 rpm for 15 min to remove cells, hyphae, and debris. The centrifuged media were mixed with petroleum ether, hexane, diethyl ether, dichloromethane, ethyl acetate, and isobutyl alcohol in the ratio of (1:1) (*v*/*v*) and shaken vigorously for 10 min in a solvent extraction funnel. The solvent

phase was separated from the aqueous phase, and this process was repeated three times. The solvent phase was evaporated to dryness in a water bath at 80–90 °C and resuspended in sterile water to the initial volume (30–35 mL). The antifungal activity of the extract was analyzed by following the agar diffusion method again in triplicate [16].

### *2.4. Extract Antifungal Activity on Post-Harvest Cherry Tomatoes*

The extract with the highest antifungal activity in vitro was used for fruit post-harvest treatment against *B. cinerea* infection. Cherry tomato fruits (*Solanum lycopersicum* var. *cerasiforme*) were washed for 15 min in water with Tween 80 and bleach at 0.05% and 0.15%, respectively [18]. Finally, the tomatoes were rinsed thrice (10 min each) in distilled water. This tomato variety has a compact size, which makes it an ideal candidate to be used as a model in laboratory trials, as stated by other authors, such as Fernández-San Millán et al. [4].

Fruits were wounded with a sterile 0.9 mm × 25 mm hypodermic needle. Tomatoes were inoculated with 5 µL of a *B. cinerea* spore suspension at $2 \times 10^4$ spores/µL. After draining the spore suspension, the infected fruits were sprayed with the chosen extract. For this purpose, an atomizer was used to create a film of liquid on the fruit. Two treatments were carried out in parallel: one with the application of the product at the time of inoculation ($A_1$) and the other with the same application and a booster after 24 h ($A_2$). Controls were sprayed with water, and the same protocol was carried out: one control with a single water application ($C_1$) and a second application after 24 h ($C_2$).

Ten fruits were infected for each treatment and incubated at 23 °C for seven days in darkness in closed trays with moistened paper, maintaining a high relative humidity. They were observed every day, and after a week, the disease incidence was measured as the percentage of infected wounds [4]. In addition, we assessed whether the infection was only superficial or internal and the turgidity to the touch of the fruit using tweezers, discriminating between the two results. This trial was carried out 3 times.

### *2.5. Statistical Analysis*

Statistical analyses were performed using SPSS Statistics 25.0 software (Armonk, Westchester County, NY, USA). Homoscedasticity was assessed via Levene's test, while normality was corroborated via the Kolmogorov–Smirnov test. Thus, the different treatments were compared to the control using the Mann–Whitney U-test.

## 3. Results

### *3.1. Actinomycetes Co-Culture Growth Inhibition and Antifungal Activity In Vitro*

Fungal growth inhibition was observed from the beginning of the co-culture (24 h), except for *F. oxysporum*. Actinomycete growth was prominent, while fungi hyphae and spores were covered by actinomycete mycelium with structures like cysts (Figure 1). Within these structures, some fungal hyphae showed a blurred cell wall line, suggesting damage to the fungal wall (Figure 1d,e). Moreover, in the case of the *S. netropsis* strain, the fungal mycelium and spores covered by the actinomycetal mycelium acquired a blue coloration, even though no dye was used (Figure 1a,b,e).

The screening of the media aliquots taken every 24 h from each co-culture allowed the selection of the optimal culture time to obtain the highest activity in vitro antifungal tests. All the Actinomycete species exhibited similar antifungal kinetics in the antifungal activity tests. None of the strains showed activity before 48 h of culture. In the case of *S. mauvecolor* in co-culture with *A. alternaria*, *B. cinerea*, and *F. oxysporum* (Figure 2), the highest activity was shown after 48 h, decreasing progressively after the fifth day. At seven days, the antifungal activity of the aliquots decreased, except for against *F. oxysporum*. Furthermore, the *S. mauvecolor* co-culture extracts against *A. alternaria* showed complete growth inhibition (Figure 2a), while the same extracts tested with *B. cinerea* and *F. oxysporum* only exhibited partial inhibition (Figure 2b,c). In extracts from the co-cultures of the other actinomycete species, the decrease in activity on day 7 was drastic.

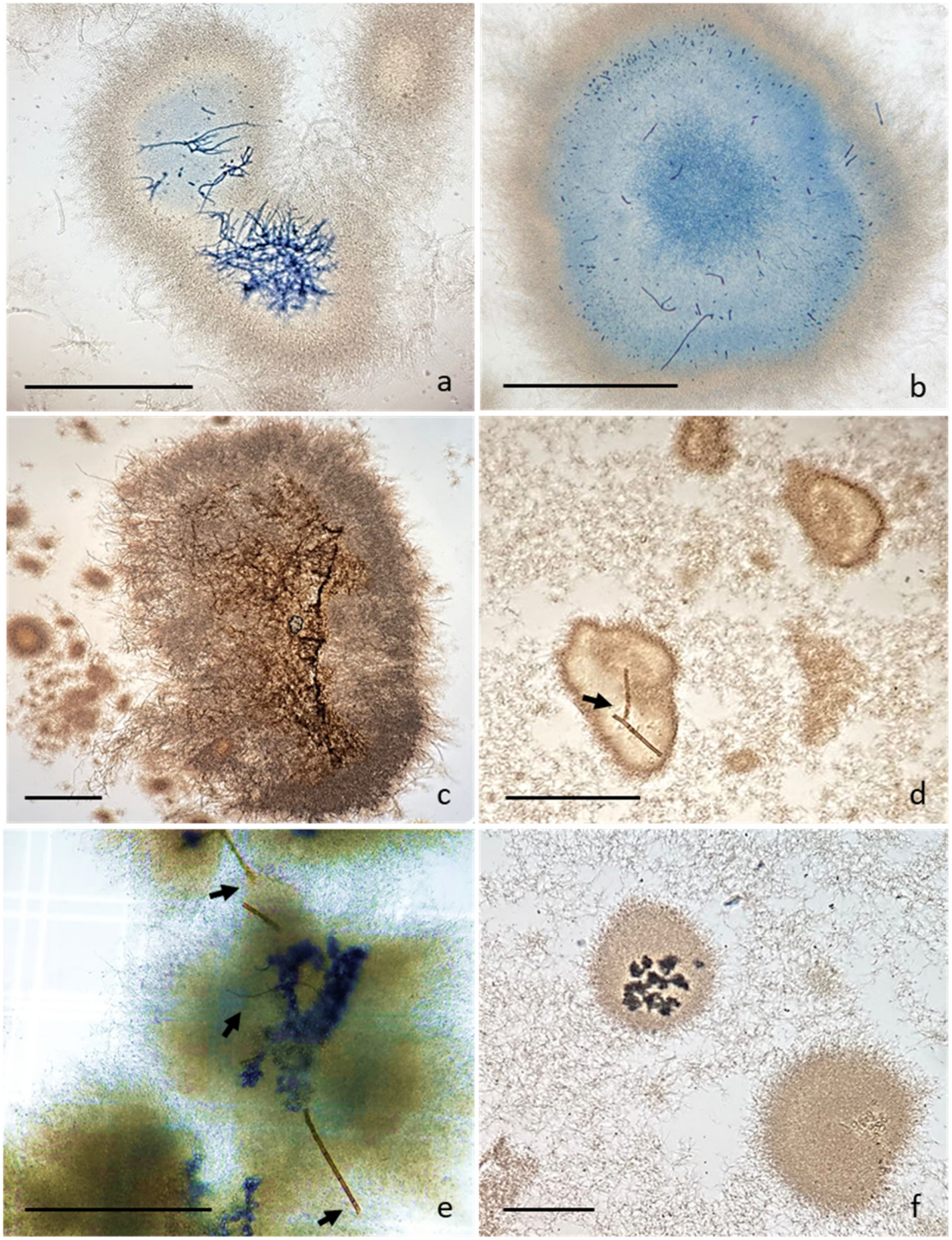


**Figure 1.** Light microscopy photomicrographs of actinomycetes smears after five days. (**a**) Hyphae and spores of *Fusarium oxysporum* encapsulated by *Streptomyces netropsis*. We note the blue staining that is on the encapsulated fungal hyphae but not on the free hyphae. (**b**) Hyphae and spores of *Fusarium oxysporum* encapsulated by *Streptomyces netropsis*. We note the blue staining on the encapsulated fungal hyphae and spores. (**c**) Mycelium of *Alternaria alternata* encapsulated by *Streptomyces pratensis*. (**d**) Hypha of *Botrytis cinerea* encapsulated by *Streptomyces mauvecolor*. We note the 'blurred' appearance of the hyphae (black arrows). (**e**) Hyphae and spores of *Botrytis cinerea* encapsulated by *Streptomyces netropsis*. We note the blue staining of the spores, as well as the 'blurred' appearance of the hyphae (black arrows). (**f**) Spores of *Botrytis cinerea* encapsulated by *Streptomyces ardesiacus*. Graphic scale: 100 μm.

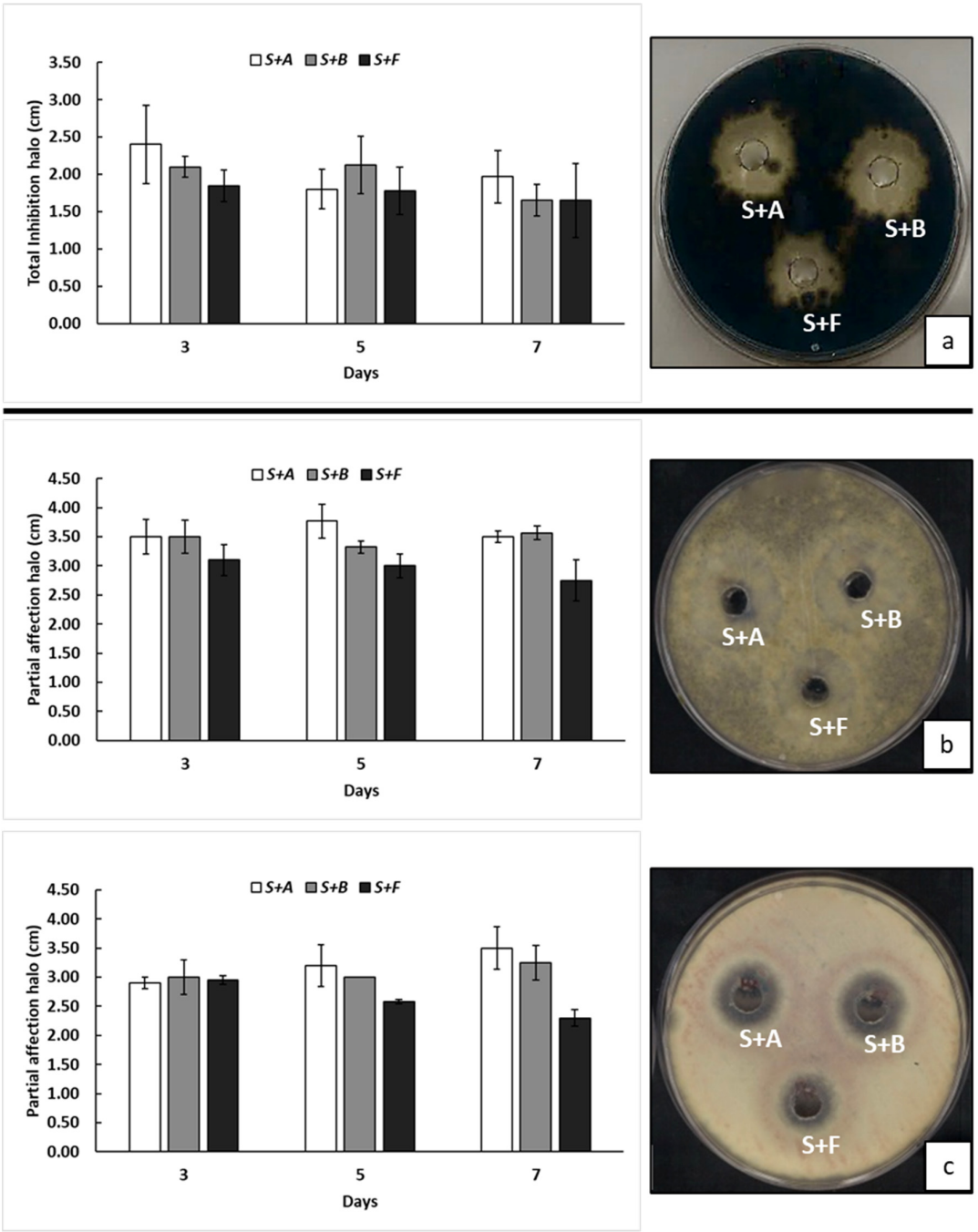


**Figure 2.** Kinetics of the production of compound(s) in supernatants of *Streptomyces mauvecolor* with activity in co-culture with *Alternaria Alternaria* (S + A), *Botrytis cinerea* (S + B), and *Fusarium oxysporum* (S + F) against the three phytopathogenic fungi. (**a**) Kinetics of activity against *Alternaria alternata* and the plate at five days of co-culture. (**b**) Kinetics of activity against *Botrytis cinerea* and the plate at 5 days of co-culture. (**c**) The kinetics and plate growth of the activity against *Fusarium oxysporum* at five days of co-culture. We note that (**b**,**c**) represent partial affection activity.

### *3.2. Actinomycetes Species Antifungal Activity In Vitro*

The antifungal activity was analyzed for the rest of the actinomycetes species using media collected from five days of co-culture. The media with lower activity were found in *S. pratensis* co-culture. Although this actinomycete was able to encapsulate the mycelium of *A. alternata* inhibiting this fungus in co-culture, in vitro, its extract did not inhibit its growth nor *F. oxysporum*. However, it successfully significantly inhibited the growth of *B. cinerea*, as

well as its ability to affect sporulates occurring on the plates in two distinctive concentric halos, i.e., an inner halo without fungal growth and an outer halo where sporulation is inhibited (Figure 3a). For its part, *S. ardesiacus* significantly inhibited the mycelial growth of *A. alternata* and the sporulation of both *B. cinerea* and *F. oxysporum* (Figure 3b–d). Nevertheless, the best antifungal activity was observed for media extracts of *S. netropsis*, which significantly inhibited the sporulation of *F. oxysporum* and the growth of *A. alternata* and *B. cinerea*, showing large halos (Figure 3e–g). Finally, *S. mauvecolor* significantly inhibited the growth of *A. alternata*, although with smaller halo sizes (Figure 3h), while its effects on *B. cinerea* were like those of *S. ardesiacus*, showing a potent inhibition of fungal sporulation but not of mycelial growth (Figure 3i), making its use against this fungus less attractive than *S. netropsis*. In addition, *S. mauvecolor* was the only species that inhibited the sporulation and mycelial growth of *F. oxysporum* (Figure 3j) and the only one that exhibited antifungal activity in axenic culture. However, co-culture with the pathogens enhanced its activity. These good results of *S. netropsis* in co-culture, especially against *B. cinerea*, were responsible for swaying our selection of these extracts, with almost similar activity, for organic solvent extraction and its application in post-harvest trials.

### *3.3. Organic Solvent Extraction and Antifungal Activity*

The use of fewer polar solvents (petroleum ether, hexane, diethyl ether, dichloromethane) for the extraction of actinomycetal compounds exhibited no antifungal activity in vitro. However, the water phase retained similar activity ratios on fungi, as shown above. Consequently, increasing the solvent polarity (ethyl acetate) was necessary to detect the antifungal activity of the extract. However, with ethyl acetate, the highest antifungal activity stayed in the aqueous phase, with a slight reduction in mycelia growth (Figure 4a,b). The highest antifungal activity was obtained using isobutyl alcohol, the most polar solvent immiscible with water. The solvent phase showed almost the same antifungal activity as the crude supernatant media, and hardly any growth inhibition halos were created with the aqueous phase extracted via isobutyl alcohol tests (Figure 4a,c). Therefore, isobutyl alcohol was used to extract the active molecules from the co-culture medium, leaving behind other unwanted substances. The extract was then considered suitable for evaluating its efficacy in post-harvest treatments.

### *3.4. Botrytis Growth Inhibition on Cherry Tomatoes*

The isobutyl alcohol extracts were dissolved in water and tested on cherry tomatoes infected with *B. cinerea*. Seven days later, 100% of the control fruits ($C_1$) and 90% of those subjected to reinforcement with water ($C_2$) showed visible infection with aerial mycelium of the fungus. The area around the infection injury was sunken, and an internal rot was highly developed, resulting in the absence of turgor in all infected fruit (Figure 5a,b). However, fruits sprayed with *S. netropsis* isobutyl alcohol extract showed much lower infection rates. In those subjected to a single application ($A_1$), only 50% of the fruits showed infection by *B. cinerea*, with low internal damage causing the loss of turgor, resulting in a percentage significantly lower than that of the control ($Z = -2.121$; $p = 0.034$) (Figure 5c). Finally, the cherry tomatoes subjected to the reinforcement treatment with the extract ($A_2$) also showed a surface 50% infection, significantly lower than that of the control ($Z = -2023$; $p = 0.043$). However, none of the fruits showed internal rotting, with 100% of them retaining their turgor (Figure 5d). These values were significantly higher than those of the treatment with one application ($A_1$) ($Z = -2121$; $p = 0.034$), highlighting the better results of the reinforcement. For both treatments, the fruits did not show infection but showed scarring tissue, which acquired a dark color. Thus, the treatments resulted in a significant reduction in the percentage of infected fruit (Figure 6).

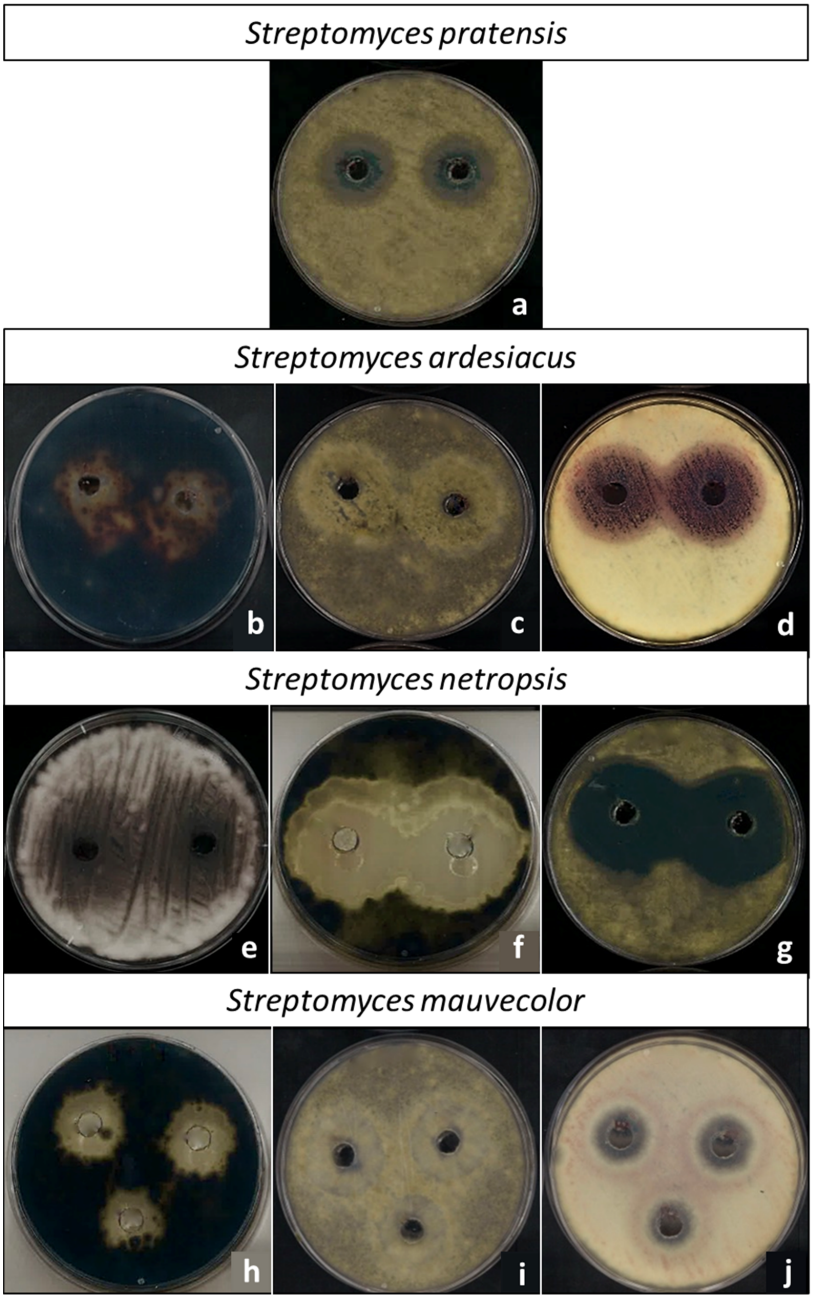


**Figure 3.** Effects of different actinomycetal extracts against three phytopathogenic fungi. (**a**) Plate of *Botrytis cinerea* with *Streptomyces pratensis* supernatant. We note the double halo, an inner halo without fungal growth, and an outer halo where sporulation is inhibited. (**b**–**d**) Phytopathogens with *Streptomyces ardesiacus* supernatant. (**b**) Plate of *Alternaria alternata*. Note the low mycelial density around the wells. (**c**) Plate of *Botrytis cinerea*. We note the halo with affected fungal growth and without conidia formation. (**d**) Plate of *Fusarium oxysporum*. We note the greatly reduced fungal growth around the well and the outer halo with increased mycelial density but no sporulation. (**e**–**g**) Phytopathogens with *Streptomyces netropsis* supernatant. (**e**) *Fusarium oxysporum* plate. We note the low mycelial density around the wells, with no spore formation. (**f**) Plate of *Alternaria alternata*. We note the large fungal growth inhibition halo. (**g**) Plate of *Botrytis cinerea*. We note the large fungal growth inhibition halo. (**h**–**j**) Phytopathogens with supernatants of *Streptomyces mauvecolor*. (**h**) Plate of *Alternaria alternata*. We note the mycelial growth inhibition halo. (**i**) Plate of *Botrytis cinerea*. We note the halo with affected fungal growth and without conidia formation. (**j**) Plate of *Fusarium oxysporum*. We note the total inhibition of fungal growth around the well and the external halo with mycelium development but without sporulation.

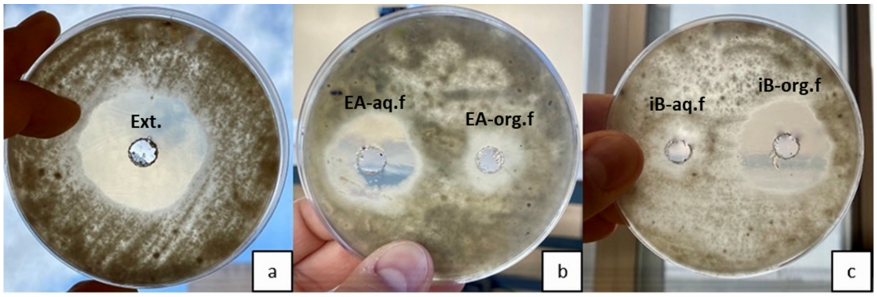


**Figure 4.** The behavior of *Botrytis cinerea* against *Streptomyces netropsis* extracts. (**a**) Crude extract, supernatant from co-culturer. (**b**) Extract in ethyl acetate. The halo on the right shows the organic phase (EA-org.f), while the halo on the left shows the aqueous phase (EA-aq.f). (**c**) Extract in isobutyl alcohol. The halo on the right shows the organic phase (iB-org.f), while the halo on the left shows the aqueous phase (iB-aq.f).

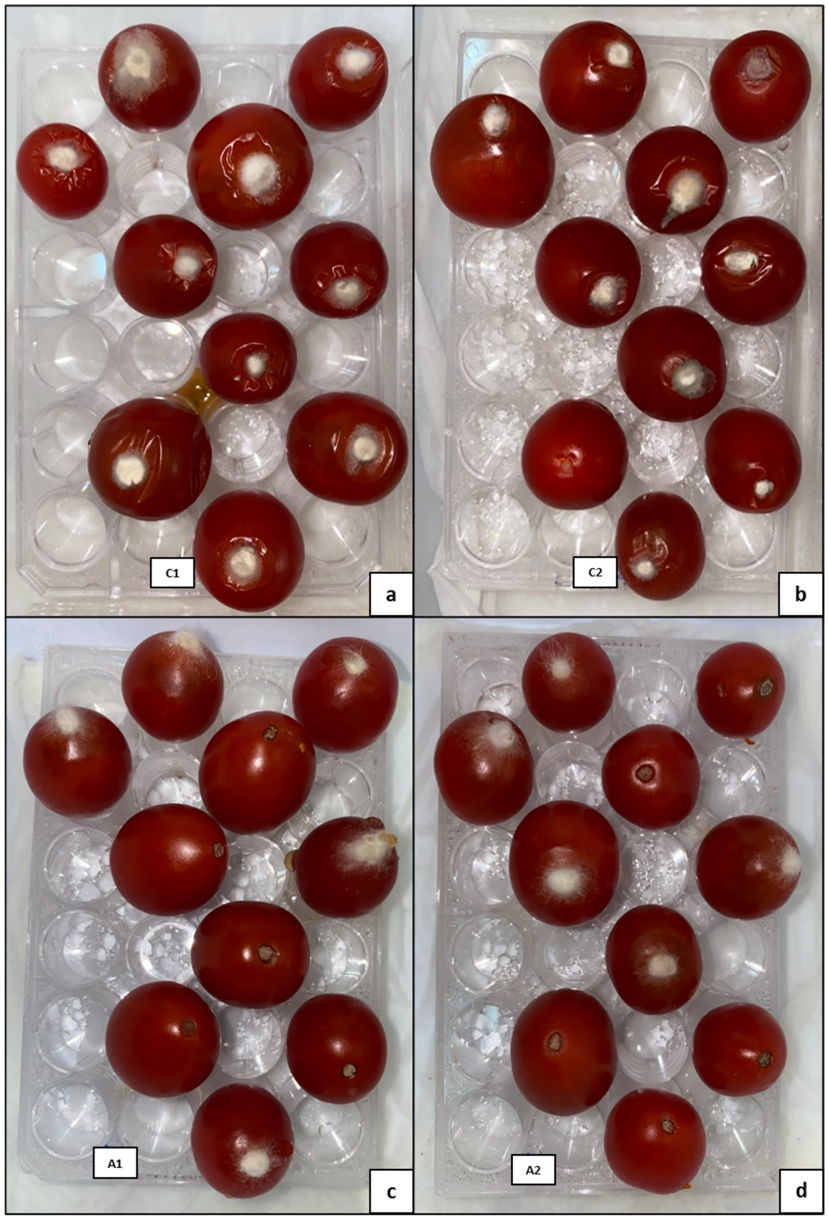


**Figure 5.** Cherry tomato fruits infected with *Botrytis cinerea*. (**a**) Control ($C_1$)—100% infection. (**b**) Control booster ($C_2$)—90% infection. (**c**) Treatment with *Streptomyces netropsis* extract with one application ($A_1$)—50% infection. (**d**) Treatment with *Streptomyces netropsis* extract with booster ($A_2$)—50% infection.

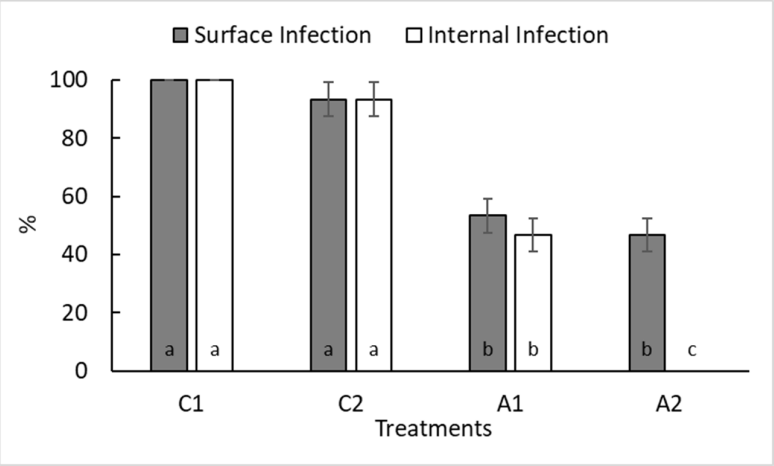


**Figure 6.** Surface and internal infection of cherry tomatoes with *Botrytis cinerea* in percentage terms. ($C_1$) Control, ($C_2$) Control booster, ($A_1$) Treatment with *Streptomyces netropsis* extract with one application, ($A_2$) Treatment with *Streptomyces netropsis* extract with booster. Values with the same letter do not significantly differ ($p \leq 0.05\%$). We note that the percentage of internal infection at $A_2$ is 0, so no bar is observed.

## 4. Discussion

Many previous works have shown the antifungal activity of *Streptomyces* strains against numerous plant pathogenic fungi [19]. This potential seems far from exhausted, and the search for new strains capable of producing new active metabolites remains an open line of research [20]. In this context, in the present study, we also show the good antifungal activity of the culture extracts of four new strains of *Streptomyces* spp. for inhibiting the growth and sporulation of three species of plant pathogenic fungi. However, the anti-fungal activity was different depending on the *Streptomyces* species and the phytopathogenic fungus. The best antifungal activity was recorded for strain A52M of *S. netropsis*, which negatively affected the three fungi tested, namely *F. oxysporum*, *A. alternata*, and *B. cinerea*. The high antifungal potential of *S. netropsis* in co-culture has also been shown by other authors who isolated strain A-ICA, with significant antifungal activity against *Fusarium oxysporum, Botrytis cinerea*, and *Alternaria alternata*, as well as four other fungal species, being recorded [19]. Interestingly, both strains of *S. netropsis* were isolated in harsh environments: A-ICA in the desert of Baja California and our A52M on the arid southern coast of the island of Tenerife. This finding confirms the notion that dry environments can serve as an exceptional reservoir for *Streptomyces* that produce antimicrobial compounds [21].

Regarding the kinetics of the antifungal activity, we found that 3–5 days of co-culture were optimal to reach the highest activity, before decreasing at 7 days. These results were unexpected because secondary metabolism usually happens late when the culture has passed its exponential growth phase. However, several types of this early activity may be part of their ecological strategy of survival due to the limited competitiveness of the slow-growing actinomycete in comparison to faster-growing microorganisms. This early activity during the growth phase coincides with previous research that has documented chitinase activity in supernatants of actinomycetes [22]. Other extracellular hydrolytic enzymes, such as proteases or glucanases, i.e., molecules synthesized during the growth stage that are essential for cell lysis, are also highlighted [23]. Although it is important to note that we do not know the nature of the components responsible for this antifungal activity in our extracts, the polar character of these could suggest that they are hydrolytic enzymes. This could explain the damaged appearance of the hyphae in the smears of the co-cultures (Figure 1d,e). The high polarity of the active compounds was evident during the organic solvent extraction of the supernatants, as isobutyl alcohol was the one for which the activity coefficients were similar to those of the crude supernatants. Nevertheless, further work is needed to elucidate the nature, as well as production kinetics and mode of action,

of these antifungal compounds from our strain, which can be performed by chemically characterizing the extracts.

In addition to the inhibition of growth and the sporulation of the fungi, we show the existence of a physical control mechanism consisting of the "trapping" of the fungus inside the mycelium of the actinomycete. These physical mechanisms, which can be easily observed via a color change on a simple smear of a co-culture sample, open new research areas, which could have implications for understanding how these microorganisms interact in natural environments, as well as how these interactions could be used to design new strategies for agricultural or biotechnological applications. Exploring the nature of these "cysts" and their relationship with antifungal activity could also lead to a better understanding of the microbial defense mechanisms involved in the interaction between these filamentous bacteria and fungi when coexisting in a particular habitat. Although the production of bioactive secondary metabolites by the genus *Streptomyces* is well known [24,25], no similar data have been reported for the encapsulation of fungi by *Streptomyces* mycelium or the compounds related to the color change or staining of fungal mycelium when grown in co-cultures, making these data highly novel. Related results are the induction of the formation of mounds of *M. xanthus* by several *Streptomyces* species [26]. The coloring could be due to compounds produced by the actinomycete or the fungus itself. For example, there are studies in which co-culture induces the production of metabolites by the actinomycete, as in the case of *Streptomyces coelicolor* when co-cultured with *M. xanthus*, which produces curiously blue-colored actinorhodin [13]. In contrast, in other studies, such as with *Streptomyces peucetius*, co-culture has induced the synthesis of DK-Xanthene by *M. xanthus*, which is yellow in color [26]. Therefore, the coloring of the fungal mycelium could be due to this fungal stimulation, as secondary metabolites of fungi with blue coloring, such as azulenes or atrovenetin produced by *Penicillium herquei* or *Lactarius* sp., have also been reported [27]. The literature seems to indicate that one of the crucial aspects of this interaction is the competition for iron, which induces the production of siderophores, triggering the activation of biosynthetic pathways related to the synthesis of secondary metabolites by the actinomycete [14], while small molecules of less than 3 kDa have been identified in the stimulation of *M. xanthus* [26]. In our case, further studies are needed to determine the nature and origin of this staining.

Our finding that *Streptomyces* strains are able to control plant pathogenic fungi such as *A. Alternaria* and *B. cinerea* are remarkable, as they are responsible for causing significant losses in the production and marketing of fresh fruits, vegetables, and ornamentals worldwide. Therefore, post-harvest treatment is a crucial aspect of the supply chain of agricultural products, as it directly influences the quality, shelf life, and food safety of fruits [5,28]. Post-harvest fungus control using synthetic fungicides allows the control of losses due to plant rot. However, their adverse effects on environmental and human health make it necessary to search for safer and more environmentally friendly alternatives [29]. Numerous research fronts are currently open in this field, and they constantly rely on bioactive compounds produced by bacteria for fungal biocontrol [30–32]. *F. oxysporum* is not a problem in post-harvest but in the field, where it causes Panama disease in banana trees. This result is exciting in a region like the Canary Islands, since more than 9000 hectares of land are used to grow banana crops. At the beginning of the century, this crop accounted for 25% of the gross value of agricultural production, representing 6% of the employment of the active population in the Canary Islands [33]. This percentage has decreased over the last two decades. The highest production is found on the islands of Tenerife (43%) and La Palma (35%). However, it is on the second island where the relative importance of the crop compared to other economic sectors is more prominent. This is due to the large surface area that this crop covers and the fact that, in previous decades, there was no other crop with such a high demand that it can cover its production costs [34].

Thus, the promising results of *S. netropsis* and its lack of pathogenicity in humans and plants make this bacterium an excellent and promising alternative in the fight against fungal

biocontrol. In particular, the extract derived from *S. netropsis* stands out as particularly compelling for use in post-harvest applications.

The inhibition of *B. cinerea* growth on cherry tomatoes assay by *S. netropsis* extracts is relevant, as this fungus infect fruits and leaves, generating considerable losses in agricultural yield before and after harvest [28]. Treatment with *S. netropsis* extracts not only significantly reduced the rate of superficially infected fruit but also showed an ability to slow or stop the development of infection inside the fruit ($A_2$). This was evidenced by preserving the turgor of affected fruit. Both a single and booster application of these extracts showed positive effects in terms of preventing infection and preserving fruit quality, showing a marked improvement compared to control groups. The fact that tomato is one of the most consumed vegetables in the world, with more than 5 million hectares being cultivated [35], makes controlling biotic stress a priority nowadays. The results obtained with *S. netropsis* increase the interest in this species for future research in the fight against phytopathogens, both pre- and post-harvest. The most suitable dosage, mode of application, molecule identification method, and safety of treated foods for human consumption still need to be established through detailed studies.

## 5. Conclusions

In conclusion, the positive outcomes of antifungal activities obtained using crude extracts of *Streptomyces* co-cultured with pathogenic fungi could have a significant impact on improving agricultural production. This can be achieved by controlling fungal infections during crop growth or post-harvest by inoculating plants with these Streptomyces strains or spraying their purified active products. This strategy would reduce the dependence on chemical fungicides, promoting more environmentally friendly practices and food safety. Specifically, the extract of a single strain of *S. netropsis* greatly inhibited the sporulation of *F. oxysporum* and the growth of *A. alternata* and *B. cinerea*. Furthermore, it showed promising results for post-harvest application on tomato fruits against *B. cinerea*, as it halved the number of infected fruits. Other *Streptomyces* strains showed more specific effects on the pathogenic fungi, with the extract of *S. mauvecolor* being the only one able to completely inhibit *F. oxysporum*. Extracts of *S. pratensis* and *S. ardesiacus*, were also able to inhibit at least totally or partially one of the three phytopathogenic fungi. Remarkable was the observation of the formation of actinomycetal "cysts" surrounding and trapping the fungal mycelium, an easily distinguishable physical encapsulation caused by the specific acquired blue coloration of the fungal mycelium, which resulted in the inhibition of fungal growth for *F. oxysporum*. These novel results are of interest for new lines of research, which could significantly impact the understanding of interactions between microorganisms in natural environments. Finally, the extracts of these new strains of *Streptomyces* could be new sources of environmentally friendly antifungal controls.

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