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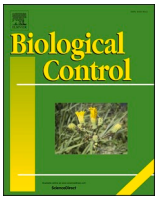

Exploring bacterial cell-free supernatants, unfiltered ferments and crude bacteria uses of *Xenorhabdus* and *Photorhabdus* (Morganellaceae) for controlling *Botrytis cinerea* (Helotiales: Sclerotiniaceae)

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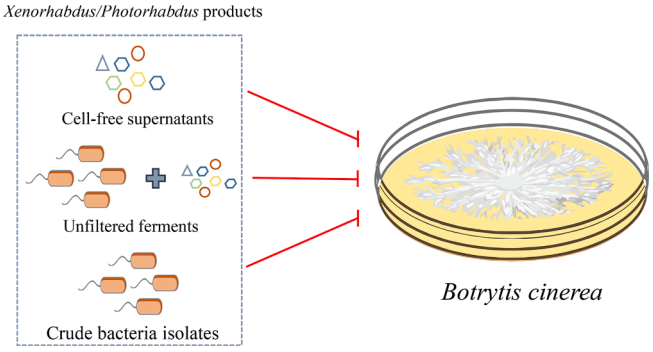
HIGHLIGHTS

- *Xenorhabdus/ Photorhabdus* bacteria/ products explored against *Botrytis cinerea.*
- *X. nematophila* cell-free supernatant inhibited 82% *B. cinerea* growth.
- *X. nematophila* unfiltered ferments inhibited 100% *B. cinerea* growth.
- *P. laumondii*-bacteria inhibited the growth of *Botrytis* in *in-vitro* conditions.
- *P. laumondii* bacteria and *Bacillus amyloliquefaciens* had similar control capability.

GRAPHICAL ABSTRACT



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ABSTRACT

The pathogen *Botrytis cinerea* (Helotiales: Sclerotiniaceae) is a wound necrotrophic fungus that causes significant losses in fruits and vegetables worldwide. The entomopathogenic nematode (EPN) symbiotic bacteria, *Xenorhabdus* spp. and *Photorhabdus* spp., are well-known associated biological control agents that produce a diverse range of natural antifungal compounds. This study aimed to evaluate the efficacy of different control strategies against *B. cinerea* using: (*i*) EPN symbiotic bacterial cell-free supernatants, (*ii*) unfiltered ferments and (*iii*) crude bacteria isolates. The antifungal efficacy of *X. bovienii*, *X. nematophila, X. kozodoii* and *P. laumondii* subsp. *laumondii* cell-free supernatants obtained after the bacterial fermentations were tested *in vitro* at two different concentrations (10% and 20%). Furthermore, the antifungal effect of *X. nematophila* and *P. laumondii* unfiltered ferments were tested *in vitro*, and their dissuasive effect was also tested over tomato leaves. Finally, the antifungal capacity of the crude *X. nematophila* and *P. laumondii* isolate was tested comparing their effect with the fungicide effect of the commercial *Bacillus amyloliquefaciens* (Serenade® ASO fungicide). The results showed that employing the bacterial cell-free supernatants or the unfiltered ferments could have different antifungal efficacy against this pathogen. Applying *X. nematophila* cell-free supernatant and unfiltered ferments at 20% concentration resulted in the highest inhibition effect compared with the control (distilled water), 82% and 100%, respectively. Furthermore, *P. laumondii*-isolate can control the growth of *Botrytis* in *in vitro* conditions, showing

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no significant differences with the efficacy of *Bacillus amyloliquefaciens* in a four-day experiment. Overall, this study builds on a better understanding of the effects of these novel biocontrol agents against *B. cinerea* and helps to develop an innovative formulation of these bacterial products as an efficient biocontrol tool.

1. Introduction

The pathogen *Botrytis cinerea* (Helotiales: Sclerotiniaceae) is a wound necrotrophic fungus that causes grey mold on more than two hundred species of plants, including agricultural crops, leading to significant losses in fruits and vegetables worldwide. Despite the wide range of hosts, it is most destructive on mature or senescent tissues of dicotyledonous plants (Yigal et al., 2004). It usually entry to such tissues at an earlier stage in crop development. Then, it remains quiescent for a considerable period (as mycelia and/or conidia) before rapidly rotting tissues when the humidity and temperature conditions are favourable and the host physiology changes (Elad et al., 2007; Williamson et al., 2007). The fungus *B. cinerea* causes massive losses in some field- and greenhouse-grown horticultural crops (i.e. tomato crops) before the harvest, or even at the seedling stage in some hosts. Moreover, *B. cinerea* is the main cause of postharvest fruit and vegetable decay during the supply chain (Elad et al., 2007). Fungal spores are generally present on the surface of pre-harvest fruit and vegetables, and during post-harvest handling, and a suitable environment can drive to spore germination. The *Botrytis* costs are diffuse because its damage occurs in different stages of the production and retail chain and are difficult to estimate. Nevertheless, global expenses of *Botrytis* control (cultural measures, botryticides, broad-spectrum fungicides, biocontrol) easily surmount 1000 million € per year, which highlight the exceptional importance of this pathogen (Dean et al., 2012).

The control of *B. cinerea* is challenging because it has a variety of modes of attack, diverse hosts as inoculum sources, and it can survive as mycelia and/or conidia or for extended periods as sclerotia in crop debris (Yigal et al., 2004). The extended use of synthetic fungicides has been the most common control method during the past decades (Oliver and Hewitt, 2014). However, the European Green Deal aims to change the crop protection paradigm, reducing the use of chemical pesticides by half in 2030, enhancing the use of different control treatments and diverse active principles (European Commission, 2020). In recent decades, biocontrol agents are one of these tools promoted in pest management as possible alternatives to synthetic pesticides. Furthermore, it has been demostrated that under field conditions, micro-organisms have more potential for use than macro-organisms as biocontrol agents because they are easier to store and transport, and can be bulked up under laboratory conditions (Veres et al., 2020).

So far, most commonly biological control agents studied against *B. cinerea* are filamentous fungi from the genera *Trichoderma*, *Ulocladium* and *Gliocladium*, bacteria from the genera *Bacillus* and *Pseudomonas* and yeasts from the genera *Pichia* and *Candida* (Jacometti et al., 2010). However, the search for new control agents remains a priority and new organisms are beginning to be investigated (Raymaekers et al., 2020). Previous studies proved that the bacteria symbiont of entomopathogenic nematodes (EPNs), *Xenorhabdus bovienii* YL002 and *X. nematophila* ALL produces antimicrobial compounds with potential for controlling grey mould rot on tomato plants and leaf scorch of pepper (Fang et al., 2011, 2014). Likewise, Vicente-Díez et al (submitted) showed that the volatile organic compounds (VOCs) emitted by *X. nematophila* ALL and *P. laumondii* subsp. *laumondii* 102 inhibit ~100 % of *botrytis* mycelial growth on the postharvest *Vitis vinifera* var Tempranillo for winemaking-grapes. These bacterial VOCs have direct (fungicide activity) and preventive antagonistic effect on the pathogen. Thus, the EPN symbiotic bacteria has become a potential alternative for controlling *B. cinerea*. However, the efficacy of the different EPN symbiotic bacteria species or strains and their different ways of use for fungal control has not been thoroughly studied.

Based on those previous works, we hypothesize that different *Botrytis* control strategies can be developed from the EPN symbiotic bacteria whose efficacy will depend on the bacterial species and the bacterial products used. Therefore, the aim of this study was to evaluate the efficacy of different EPN symbiotic bacterial products in controlling *Botrytis* mycelial growth. The specific objectives were: (*i*) to evaluate the antifungal efficacy of bacterial cell-free supernatants (CFSs) of four EPN symbiotic bacteria species (*X. bovienii*, *X. nematophila, X. kozodoii* and *P. laumondii* subsp. *laumondii*) *in vitro* conditions at two concentrations (10% and 20%); (*ii*) to investigate the antifungal effect of *X. nematophila* and *P. laumondii* unfiltered ferments (UFs) at *in vitro* conditions and their dissuasive effect over tomato leaves; and (*iii*) to study the antifungal capacity of the crude isolated *X. nematophila* and *P. laumondii* bacteria, comparing their effect with the fungicide effect of the commercial *Bacillus amyloliquefaciens* (Serenade® ASO fungicide).

2. Material and methods

2.1. Biological resources

We isolated the strain of *B. cinerea* from a contaminated tomato from an organic cultivar in La Rioja (Spain) and we transferred it to Potato Dextrose Agar (PDA) (VWR, Leuven, Belgium) medium. We molecularly confirmed identification as *B. cinerea* following the approach described by Bueno-Pallero et al. (2020). We compared the sequences using Blast (https://blast.ncbi.nlm.nih.gov) and those submitted to Genbank (Accession number MZ544643). For the bioassay, the pathogenic fungi were grown in Petri dishes with PDA medium by seeding a plug of agar with mycelium in active growth and we let the *B. cinerea* growth at 25 °C for three days. From these plates, the conidia were removed, and a suspension was prepared with sterile phosphate-buffering saline (PBS, pH 0 7,4) at a concentration of 1×10^7 conidia/mL via cell counting method in Neubauer counting chamber.

We isolated three *Xenorhabdus* and one *Photorhabdus* species from their symbiotic entomopathogenic nematode (EPN) to test their antifungal activity against *B. cinerea* (Table 1). We performed the isolation process following the protocol described by Vicente-Díez et al. (2021). For all the bioassays described in the present work, we obtained the secondary metabolites by Tryptone Soya Broth (TSB) fermentation during 3 d at 150 rpm, in darkness and at 25 °C. After this time, we kept the bacterial cultures at 4 °C for their use as unfiltered ferment (UF). We obtained the cell-free supernatant (CFS) by centrifuging this bacterial culture at 68.905 × g (g-force or relative centrifugal forcé, rcf). (Thermo Scientific™ Sorvall LYNX 4000 Superspeed Centrifuge, Fisher Scientific SL, Madrid, Spain) for 20 min at 4 °C. Then, we filtered the supernatant through a 0.22 μm sterile pore filter (Nonsterile, PES Syringe Filters, Branchia, Labbox LabwarE, S.L. Barcelona, Spain). After this process, we

Table 1
Xenorhabdus and *Photorhabdus* species and their symbiotic nematodes associate.

Bacterial species	EPN species	Population
Xenorhabdus bovienii	*Steinernema feltiae*	RM-107
Xenorhabdus nematophila	*Steinernema carpocapsae*	All
Xenorhabdus kozodoii	*Steinernema riojaense*	RM-30
Photorhabdus laumondii subsp. *laumondii*	*Heterorhabditis bacteriophora*	RM-102

cultured 50 μL of this filtrate on Nutrient Agar (NA, VWR, Dorset, UK), Bromothymol blue (Alfa Aesar, Kandel, Germany), 2,3,5-Triphenyl Tetrazolium chloride (TTC, VWR, Chemicals, Barcelona, Spain) (NBTA plates) in duplicate to verify the absence of bacteria. Finally, we recovered bacterial pellet obtained after the centrifugation with a 1 μL seed loop to test the antifungal capacity of crude bacteria isolate as it is explained in the section 2.4. We filtered the TSB to be used as controls to maintain all treatments under the same conditions.

The *Bacillus amyloliquefaciens* (former *subtilis*) QST 713 strain used in the present study was isolated from the commercial product Serenade®ASO. This product is characterized to have a broad-spectrum biofungicide action and has been approved for use in the European Union (Reg. (EC) No. 839/2008). First, we took 1 mL from the commercial product in one Eppendorf tube and centrifuged the bacterial suspension. Then, we removed the supernatant and suspended the pellet with 1 mL of PBS (the whole process was repeated three times). Then, we inoculated 1 mL of the bacterial suspension in 250 mL of Nutrient Broth (NB) in one 500 mL Erlenmeyer, and the NB bacterial fermentation was performed during 3 d at 150 rpm in darkness at 25 °C. Finally, we recovered the crude isolate bacteria following the protocol described before for EPN symbiotic bacteria.

The tomato plants *Solanum lycopersicum* L. Money Maker variety were grown from seeds under greenhouse conditions (22 ± 1 ∘C and 60 ± 10% RH, with 16:8, L:D photoperiods), at the Institute of Grapevine and Wine Sciences (ICVV, Logroño, La Rioja, Spain) during three weeks. Then, the leaves were cut and arranged for the experiment as described in section 2.3.

2.2. Antifungal activity of entomopathogenic nematodes bacterial symbionts cell-free supernatants

We evaluated the effects of CFSs of four EPN bacteria symbionts (*X. bovienii*, *X. nematophila, X. kozodoii* and *P. laumondii* subsp. *laumondii*) on the mycelial growth of *B. cinerea*. We mixed 10 or 20 mL of each bacterial cell-free supernatants with 90 mL (10%, proportion 1:10 final) or 80 mL (20%, proportion 1:5 final) of autoclaved PDA that cooled down to 60 °C, respectively. Then, we pooled 10 mL of each mix into Petri dishes (9 cm diam.). Once the media solidified, we pipetted 20 μL suspension of 10^7 spores/mL of *B. cinerea* prepared in Gamborg B-5 (Sigma-Aldrich, St. Louis, MO, USA) in the middle of the plate. The controls were mixed with distilled autoclaved water and with TSB at the same proportion that the CFSs. All the experimental units were incubated at 60% RH, 22 ± 1 °C, 16:8 L: D photoperiod. A total of n = 15 plates were studied for each treatment in two different trials (n total = 30). We assessed the mycelial growth area (mm^2) by measuring the fungus growth using image analysis with the Image J® program (v. 1.50i, MD, USA) four days after starting the experiment (the day in the mycelial growth of the controls occupy the plate completely).

2.3. Antifungal activity of Xenorhabdus nematophila and Photorhabdus laumondii unfiltered ferments and their dissuasive effect over tomato leaves

We evaluated the antifungal activity of *X. nematophila* and *P. laumondii* UFs on the mycelial growth of *B. cinerea*. We mixed the bacteria fermentation suspension into Petri dishes (9 cm diam.) with PDA (20%). Then, we pipetted 20 μL suspension of 10^7 spores/mL of *B. cinerea* prepared in Gamborg B-5 in the middle of the plate. The controls comprised the mixture of distilled autoclaved water and TSB at the same proportion that the UFs. The mycelial growth area (mm^2) of each treatment was calculated as described before.

For the dissuasive assay over tomato leaves, the second and third leaf from the tip were detached from three weeks old plants following the protocol described by Miazzi et al. (2010). We disinfected the leaves surface by dipping them in 3 % (v/v) of sodium hypochlorite (NaOCl) solution for 1 min, washed them with distilled water and then air-dried them for ~2 h. Then, we placed singly Petri dishes (9 cm diam.) containing 10 mL of the Agar medium, immersing the petiole in the substrate. Using a steel needle, we spot-inoculated each leaf in 10 points, avoiding veins, and we placed 1 cm^2 PDA with *Botrytis* from four days old colonies of a single fungal isolate. We incubated in the same conditions described above. Four days later, we detached new leaves and performed the disinfected protocol. After that, we dipped them in the *X. nematophila* or *P. laumondii* unfiltered ferments or in TSB as control. Then, we placed them in the same Petri dishes with the infected tomato leaves. Fifteen leaves per treatment were used, and the experiment was performed twice. We assessed the mycelial growth area (mm^2) by measuring the fungus growth compared with the size of the leave using image analysis with the Image J® program four days after starting the experiment. The infection rate was calculated by dividing the infected area by the total leaf area.

2.4. Antifungal activity of isolated bacteria compared with commercial Bacillus amyloliquefaciens QST 713 (Serenade)

One centimeter from the edge of the PDA plate (5.5 cm diam.), 0.1 mL of crude bacterial isolated were inoculated with a seeding loops and 20 μL of the pathogen were applied in the opposite direction. The plates were incubated at 25 °C for three days. As control, we left a plate without any application. We assessed the mycelial growth area (mm^2) by measuring the fungus growth using image analysis with the Image J® program four days after pathogen inoculation. The experimental unit was each Petri dish, and the experimental design was completely randomized with ten replicates. The whole experiment was performed twice.

2.5. Statistical analysis

We analyzed the inhibitory effect of bacterial CFSs, UFs and bacteria-isolate on mycelial growth of *B. cinerea* by one-way analysis of variance (ANOVA). Means are shown with standard errors of the mean (SEM), and a comparative analysis was performed using a Tukey test for significance analysis (HSD) at $P < 0.05$. We performed these analyses with SPSS 25.0 (SPSS Statistics, SPSS Inc., Chicago, IL, USA). All figures were built using Graph Pad 8.

3. Results

3.1. Antifungal effect of bacterial cell-free supernatants on Botrytis cinerea mycelial growth

The bacterial CFSs showed statistically significant inhibitory effect on the mycelial growth of *B. cinerea* at 10% ($F_{5,\ 174} = 90.835$, $P < 0.001$) and 20% ($F_{5,\ 239} = 102.71$, $P < 0.001$) concentrations four days after pathogen inoculation (Fig. 1). In particular, we found that *X. nematophila* reduced ~60 and ~80 % of *Botrytis* mycelial growth compared with water at 10% and 20% concentration in the media, respectively. Likewise, *P. laumondii* showed an inhibitory effect (~40 %) for both concentrations compared to water treatment. TSB control inhibited the fungal mycelial growth, even though there was not more inhibition at higher concentration applied. Therefore, we selected *X. nematophila* and *P. laumondii* as the most efficient bacterial strains for subsequent bioassays.

3.2. Antifungal effect of unfiltered bacterial ferments on Botrytis cinerea mycelial growth and their dissuasive effect in tomato leaves

The UFs showed statistically significant inhibitory effect on the mycelial growth of *B. cinerea* at 20% ($F_{3,\ 76} = 273.53$; $P < 0.001$) four days after pathogen inoculation (Fig. 2A.). All the treatments and the TSB control showed an inhibitory effect compared with the distilled water. In particular, we found that *X. nematophila* reduced ~100 % of

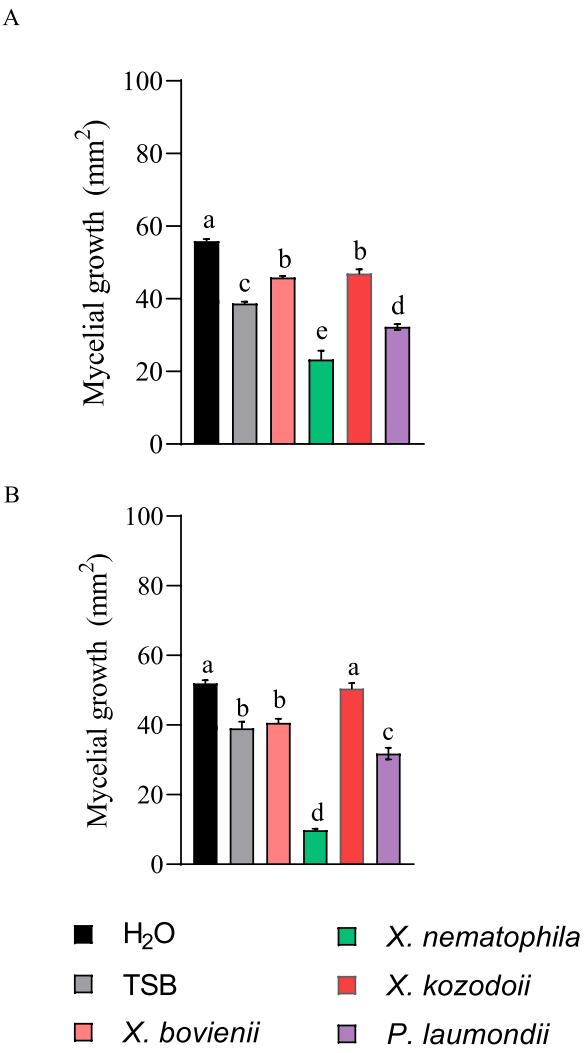

Fig. 1. Mycelial growth (mm^2) of *Botrytis cinerea* induced by *Xenorhabdus bovienii*, *X. nematophila, X. kozodoii* and *Photorhabudus laumondii* cell-free supernatants (CFS) in Potato Dextrose Agar (PDA). **(A)** Antifungal effect of bacterial cell-free supernatants 1:10 (CFS:PDA). **(B)** Antifungal effect of bacterial cell-free supernatants 1:5 (CFS:PDA). Data are expressed as mean ± SEM. Different lowercase letters represent statistically significant differences between treatments according to Tukey's multiple comparison test ($P < 0.05$).

botrytis mycelial growth at 20% of concentration in the media. Likewise, *P. laumondii* showed an inhibitory effect (~60 %) compared with the distilled water control at this concentration.

The UFs showed a significant dissuasive effect on the mycelial growth of *B. cinerea* over tomato leaves ($F_{2,\ 86} = 20.47$; $P < 0.001$) (Fig. 2B.). In particular, *X. nematophila* and *P. laumondii* unfiltered ferments reduced the *B. cinerea* mycelial growth over treated tomato leaves (Fig. 2C.) by 88% and ~100%, respectively.

3.3. Antifungal effect of crude bacteria isolates on Botrytis cinerea mycelial growth

The resuspended bacteria showed a significant antifungal effect on the mycelial growth of *B. cinerea* ($F_{3,\ 86} = 220.683$; $P < 0.001$) four days after pathogen inoculation (Fig. 3A). In particular, we found that *P. laumondii* and *B. amyloliquefaciens* seeded over the plate reduced ~75 and 88% of *botrytis* mycelial growth, respectively (Fig. 3B.). Nevertheless, the bacterium *X. nematophila* did not show inhibitory capacity on the fungus. Indeed, the presence of the bacteria on the plate promoted the mycelial growth in four days (Fig. 3A,B).

4. Discussion

In agreement with our hypothesis, employing the bacterial CFSs or the UFs could result on different antifungal efficacy against *B. cinerea*. In addition, different species showed variability in the control potential against this fungus, with *X. nematophila* and *P. laumondii* being the bacteria with the highest potential for growth inhibition among the four evaluated. Furthermore, *P. laumondii*-isolate controlled the growth of *Botrytis* in *in vitro* conditions, showing in four days no significant differences with the efficacy of *B. amyloliquefaciens* commercial product. These results are in agreement with the enormous potential of the *Xenorhabdus* and *Photorhabdus* bacteria, and their expected production of a variety of toxins with antifungal activity against the phytopathogen *B. cinerea*. As shown by Fang et al. (2011, 2014), these natural compounds have a great potential to control *B. cinerea* in different fruit and vegetable crops, but still, developing an adequate formulation of these bacterial products, including CFSs, UFs or crude cell isolate, is crucial to get an efficient biological control tool.

The secondary metabolites released by bacteria of the genera *Xenorhabdus* and *Photorhabdus* into the media and used as CFS are well-known active compounds with antifungal capacity (Chacón-Orozco et al., 2020; Hazir et al., 2016). Indeed, similar results were obtained by Hazir et al. (2016) using four *Xenorhabdus* species and two *P. luminescens* strains against *Fusicladium carpophilum* (peach scab), *F. effusum* (pecan scab), *Monilinia fructicola* (brown rot), *Glomerella cingulate* (anthracnose) and *Armillaria tabescens* (root rot), concluding that, overall, *Xenorhabdus* spp. exhibited a stronger antifungal effect compared with supernatant of *Photorhabdus* spp. The reason for differential suppressive abilities between the different EPN symbiotic bacteria species and strains may lie in production of different active compounds among the genus *Xenorhabdus* and *Photorhabdus* and the different species and strains (Bode, 2009).

The use of the unfiltered ferments, including the bacteria and the secondary metabolites, released to the media resulted as the most efficient formulation against the *B. cinerea.* Similar pattern was observed against the insect *Lobesia botrana* (Lepidoptra: Tortricidae), where the UF resulted more efficient against the larvae than the CFS (Vicente-Díez et al., 2021). However, these results contrast with the study by Bussaman et al. (2012), who performed a screening in the acaricidal activities of *X. stokiae* UFs and CFSs against *Luciaphorus* sp. Then, Bussaman et al. (2012) showed that the UFs were slightly less effective than its CFS, suggesting that *X. stokiae* was more likely to release its metabolites with acaricidal activities to the surrounding culture media. Whether these differences were due to the bacteria species, the application method or the differences in the host (acari vs insect) are unknown. Also, in agreement with Fang et al. (2014), *X. nematophila* and *P. laumondii* UFs showed a dissuasive effect against *B. cinerea* protecting the surface of the treated tomato leaves. Therefore, the use of the whole product generated in the UFs has the great potential for their use in integrated control of this pathogen in tomato crops.

Finally, we evaluated the potential use of the crude bacteria isolated in an approach similar to the current commercial product based on *Bacillus* spp. We observed that *P. laumondii*-isolate inhibited *Botrytis* mycelial growth while *X. nematophila*-isolate did not control the growth of the fungi in comparison with the control. Few researchers have examined the effect of the free-living forms of *Xenorhabdus* and *Photorhabdus* as control agents (Sandhi and Reddy, 2019). Until recent findings, scientific observations suggested that there was little likelihood that the symbiotic bacteria could effectively control pests when used alone (Burnell and Stock, 2000). Nevertheless, recent studies have proved the phenotypic heterogeneity of bacteria of the genus *Photorhabdus* that have a second putative lifestyle out of their nematode symbiont and remain in the soil (Eckstein and Heermann, 2019). Furthermore, current transcriptomic analysis has proved that *P. luminescens* responds to the external signals (e.g. root exudates) driving the expression of various genes, for example, those involved in chitin degradation, biofilm regulation, flagella formation, and type VI

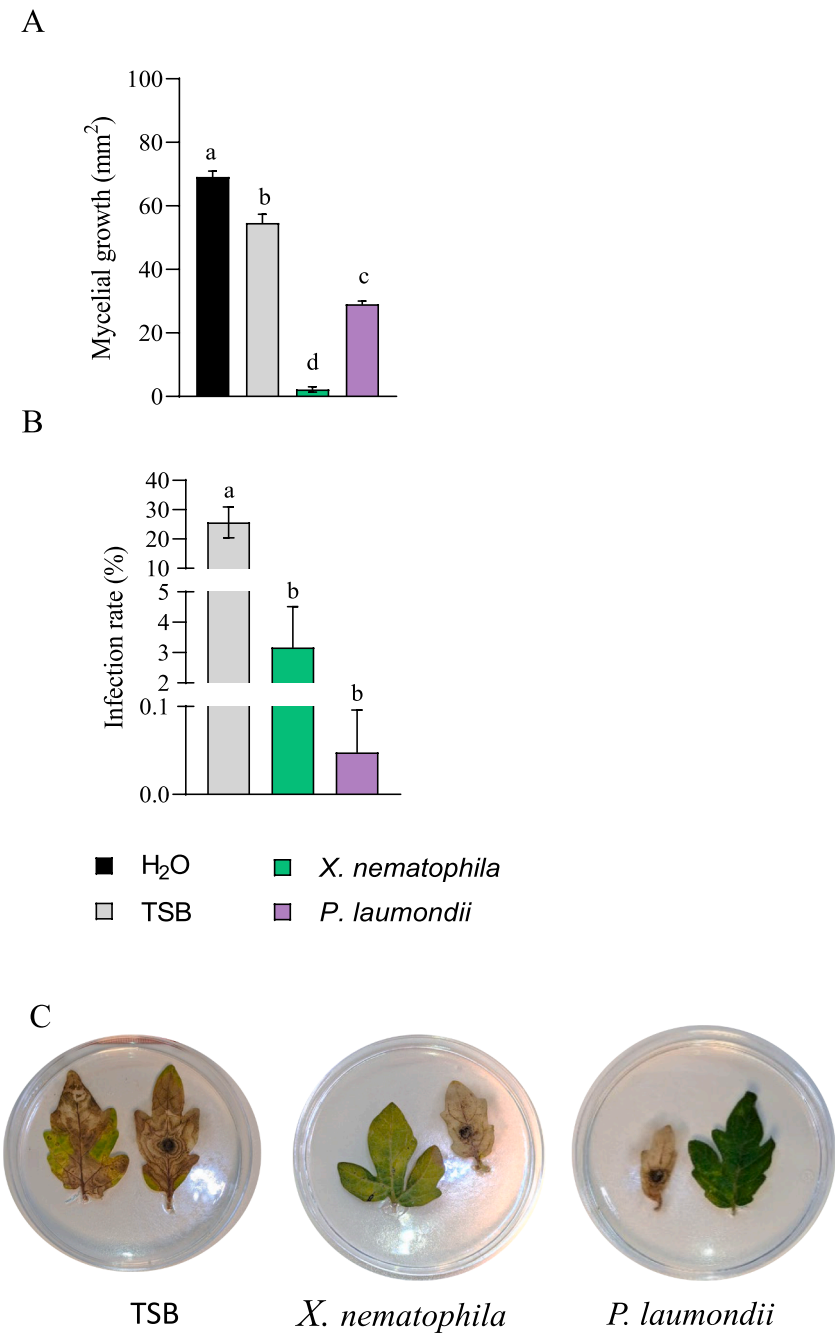

Fig. 2. Mycelial growth (mm2) and Infection rate (%) of *Botrytis cinerea* induced by *Xenorhabdus nematophila* and *Photorhabdus laumondii* unfiltered ferments (UFs) after 4 days post-inoculation in Potato Dextrose Agar (PDA). **(A)** *B. cinerea* mycelial growth induced by *X. nematophila* and *P. laumondii* TSB unfiltered ferments 1: 5 (UFs:PDA). **(B)** Dissuasive effect of *X. nematophila* and *P. laumondii* against *B. cinerea* over tomato leaves. **(C)** Display of the fungal growth in each treatments four days after infection. Data are expressed as mean ± SEM. Different lowercase letters represent statistically significant differences between treatments according to Tukey's multiple comparison test ($P < 0.05$).

secretion system (Regaiolo et al., 2020). These recent evidence help to understand the positive results found by the use of *P. laumondii*-isolate against *B. cinerea*. On the other hand, in agreement with Bussaman et al. (2012), up to date, there is no evidence that any *Xenorhabdus*-isolate can control pests or pathogens when applied to free-living cells.

5. Conclusions

Different forms of use of the EPN symbiotic bacteria fermentation have variable effectivity controlling the mycelial growth of *B. cinerea*. On the one hand, among the screened bacteria studied herein, *X. nematophila* UFs showed the highest antifungal effectiveness. On the other hand, *P. laumondii*-isolate could control the fungal growth during four days as the commercial produced based on *B. amylolyquefancies*. This study illustrates the possible uses of the bacteria with various formulations as a plant-protecting organism in agriculture. Indeed, the development of these novel bacteria-based tools can be used as an eco-friendly and economical alternative in the integrated control of diseases by reducing the amount and number of chemical fungicide applications in agricultural crops, in line with the current paradigm of reduction of pesticide such as the EU Green Deal (European Commission, 2020). More greenhouse and field application studies are required to determine the possible modulation effects in the plant and under natural conditions.

Archive of data

The data presented in this study will be archived in https://digital.csic.es/, to ensure that we compile with the FAIR mandate, to ensure accessibility to any researcher.

CRediT authorship contribution statement

Ignacio Vicente-Díez: Conceptualization, Methodology,

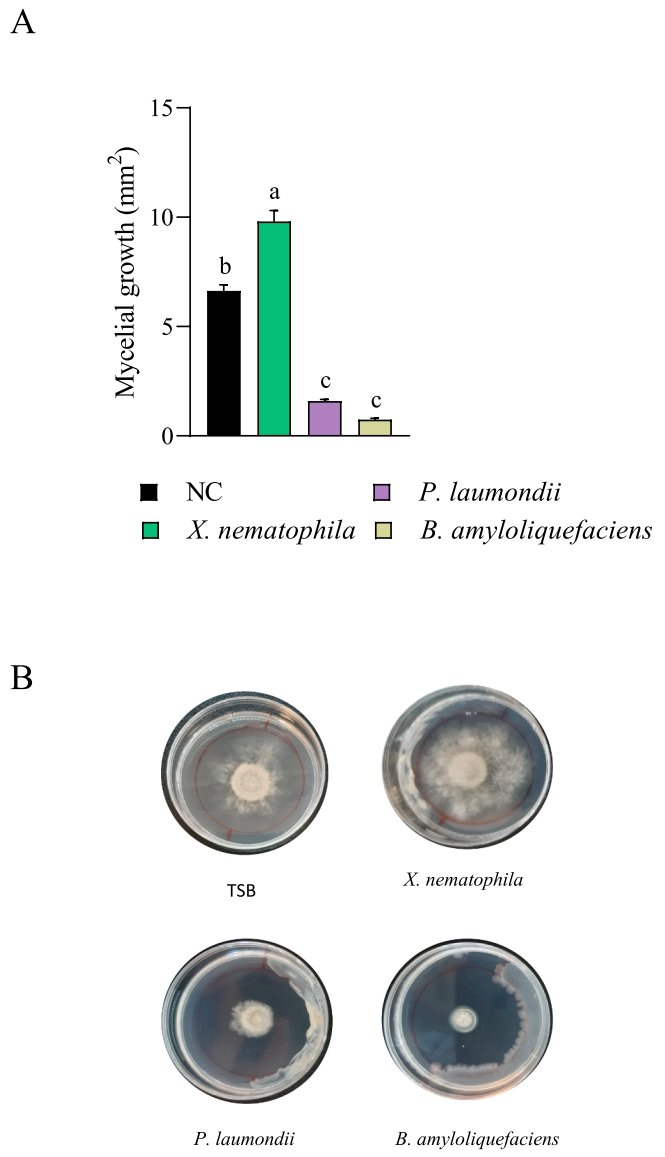

Fig. 3. Impact of *Xenorhabdus nematophila, Photorhabdus laumondii*, *Bacillus amyloliquefaciens* isolated bacteria in the *Botrytis cinerea* growth. **(A)** Mycelial growth (mm^2) of *B. cinerea* in the presence of *X. nematophila, P. laumondii*, and *B. amyloliquefaciens* isolated bacteria. **(B)** Display of the fungal growth in each treatments four days after infection. Data are expressed as mean ± SEM. Different lowercase letters represent statistically significant differences between treatments according to Tukey's multiple comparison test ($P < 0.05$).

Investigation, Writing – original draft, Visualization, Data curation, Resources, Writing – review & editing. **Elizabeth Carpentero:** Methodology, Writing – review & editing. **Alicia Pou:** Resources, Writing – review & editing, Funding acquisition. **Raquel Campos-Herrera:** Conceptualization, Resources, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

Conceptualization, IVD and RCH; Methodology, IVD and EC; Analysis, Investigation, and Data Curation: IVD; Resources, IVD, AP and RCH; Writing—Original Draft Preparation and Visualization, IVD; Writing—Review and Editing, IVD, EC, AP and RCH; Funding, Acquisition and Administration, AP and RCH. All authors have read and agreed to the published version of the manuscript.

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