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IN VITRO AND IN VIVO BIOCONTROL OF LEAF SPOT DISEASE CAUSED BY *Curvularia lunata* IN MAIZE LANDRACE BY *Bacillus licheniformis* M2-7

BIOCONTROL IN VITRO E IN VIVO DE LA MANCHA FOLIAR CAUSADA POR *Curvularia lunata* EN MAÍZ CRIOLLO POR *Bacillus licheniformis* M2-7

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Key words: bacteria, Elotes Occidentales race, phytopathogen, Zea mays L.

ABSTRACT

Curvularia lunata (Wakker) Boedijn is a phytopathogen that causes leaf spot disease in maize (*Zea mays* L.), a cereal crop of great economic importance. However, the use of biocontrol agents such as *Bacillus* species offers an effective and sustainable alternative to reduce the severity of leaf spot disease. Therefore, our study sought to evaluate the applicability of *Bacillus licheniformis* M2-7 as a potential biocontrol agent against *Curvularia lunata* in maize landrace plants both in vitro and in vivo. The in vitro experiments demonstrated that strain M2-7 inhibited mycelial growth and conidial germination by 73.75 and 78.66%, respectively. In the in vivo tests, 1×10^3 and 1×10^6 CFU concentrations of strain M2-7 significantly reduced disease severity compared to the control. Our findings suggested that *Bacillus licheniformis* M2-7 could be used on a commercial scale as an effective biocontrol agent against leaf spot disease in maize.

Palabras clave: bacteria, elotes occidentales, fitopatógeno, Zea mays L.

RESUMEN

Curvularia lunata (Wakker) Boedijn es un fitopatógeno de importancia económica en el cultivo de maíz (*Zea mays* L.). El uso de agentes de biocontrol como las especies del género *Bacillus* ofrecen una forma alternativa eficaz y sustentable para reducir la severidad de la mancha foliar. En este estudio, se evaluó la acción potencial de biocontrol de *Bacillus licheniformis* M2-7 *in vitro* e *in vivo* sobre *Curvularia lunata* en plantas de maíz criollo. Los resultados de las pruebas de antagonismo *in vitro* mostraron que la cepa M2-7 inhibe el crecimiento micelial y la germinación de conidios en un 73.75 y 78.66 %, respectivamente. En las pruebas *in vivo*, las concentraciones 1×10^3 y 1×10^6 UFC de la cepa M2-7 redujeron significativamente la severidad de la enfermedad con respecto al control. Los resultados obtenidos sugieren el potencial uso a escala comercial de *Bacillus licheniformis* M2-7 como agente de biocontrol contra la mancha foliar en maíz.

INTRODUCTION

Maize (*Zea mays* L.) is a staple food for millions of people worldwide (Mie et al. 2017, Rosenblueth et al. 2018) and is among the three most important cereal crops in the world in terms of annual production (Erenstein et al. 2022). In Mexico, this cereal is cultivated annually in nearly 8,500,000 ha, producing about 24 million tons (SIAP, 2020).

Curvularia lunata (Wakker) Boedijn is a plant pathogenic fungus (anamorph) belonging to the division Ascomycota, class Dothideomycetes, order Pleosporales, and family Pleosporaceae (Tan et al. 2018). *C. lunata* (Wakker) Boedijn is the main causative agent of leaf spot disease. Besides, it participates in the development of the Tar spot complex disease, which has been described to be caused mainly by the interaction of two phytopathogenic fungi, *Pyllachora maydis* and *Monographella maydis*, which are associated with the hyperparasite *Coniothyrium phyllachorae* (Ríos-Herrera et al. 2017) and has become a serious threat to maize production worldwide. The incidence of leaf spot disease has seen an increase in frequency, extent, and severity, leading to significant economic repercussions in tropical regions (Gao et al. 2014, Garcia-Aroca et al. 2018, Zhang et al. 2019, DGSV-CNRF 2020, Barupal et al. 2021). This pathogen mainly infects maize leaves and pods, being the common symptoms of leaf spot disease necrotic spots surrounded by a halo, which then expand into round, oval, fusiform, or striped lesions (Barupal and Sharma 2017). Most leaf spot disease cases have been reported in warm and humid areas worldwide, where it causes significant damage (Munkvold and White 2016), such as reported in Louisiana (Garcia-Aroca et al. 2018), Kentucky (Anderson et al. 2019), Delaware (Henrickson and Koehler 2021), and Mississippi (Jimenez Madrid et al. 2022).

In humans, *C. lunata* has been reported to cause allergies and respiratory and dermal infections (Tóth et al. 2020). Several management strategies have been used to control plant pathogenic fungi in vegetable crops, including using chemical fungicides, breeding resistant cultivars, and other traditional practices (Khan et al. 2020). Several chemical fungicides have been reported to be effective against leaf spot disease, including propineb (2%), thiophanate-methyl, metalaxyl, and mancozeb (Butt et al. 2011). However, their indiscriminate use has been linked to adverse effects on human and animal health, as well as to environmental impacts (Barupal and Sharma 2017). Some places have thus used maize plant varieties improved with pathogen resistance genes. However, this improvement must be continuous because pathogens evolve rapidly, generating resistance and rendering their hosts more susceptible to infection (Khan et al. 2020). Therefore, recent studies have focused on exploring alternative strategies for suppressing phytopathogens in priority crops such as maize, among which biological control agents and their secondary metabolites are considered highly promising (Reyes et al. 2015). In vitro studies have suggested the use of Bacillus sp., Actinomycetes (*Streptomyces* spp.), plant extracts (*Lawsonia inermis* L.), and yeasts (*Torulaspora indica* and *Wickerhamomyces anomalus*) to alleviate the adverse effects of *Curvularia lunata* on maize and rice (Basha and Ulaganathan 2002, Bressan 2002, Limtong et al. 2020, Barupal et al. 2021). However, additional in vivo studies are needed to characterize the potential and efficacy of these biocontrol agents.

The species belonging to the genus Bacillus stand out as uniquely promising biocontrol candidates because they produce a wide range of secondary metabolites

(Villarreal-Delgado et al. 2017). The antifungal effects of *Bacillus licheniformis* M2-7 against *Microdochium, Curvularia*, *Phoma*, *Microsphaeropsis*, and *Fusarium* have been recently demonstrated *in vitro* (Bahena-Oregón et al. 2022). Additionally, a recent study reported that this bacterial strain improves the germination percentage, production, and crop yield of chili, and can be used in agricultural formulations (Bolaños-Dircio et al. 2021). Biocontrol formulations (i.e., biofungicides) prepared from bacteria are increasingly applied in sustainable agriculture (Wu et al. 2015). Particularly, inoculants prepared from endospore-forming *Bacillus* strains have proven as an effective and environmentally friendly alternative due to their long shelf life, which is comparable to that of agrochemicals (Chowdhury et al. 2015, Wu et al. 2015).

Therefore, to develop sustainable and integrated strategies to reduce the severity of leaf spot disease caused by *C. lunata* in maize landrace native to the state of Guerrero, our study sought to evaluate the antagonistic capacity of *B. licheniformis* M2-7 as a biocontrol agent both in vitro and in vivo.

MATERIALS AND METHODS

Biological materials and growth conditions

All experiments were conducted using the *B. licheniformis* M2-7 (Guevara-Luna et al. 2022) and fungal pathogen *C. lunata* SY17, which has been previously isolated and identified by Bahena-Oregón et al. (2022) but whose pathogenicity has not evaluated. The strains are accessible in the collection of the Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Facultad Académica de Ciencias Químico Biológicas of the Universidad Autónoma de Guerrero. The M2-7 bacterial strain was grown in Luria Bertani (LB) liquid medium containing 10.0 g/L casein peptone, 5.0 g/L wash extract, and 10.0 g/L NaCl at pH 7.3, after which the samples were incubated at 180 rpm for 24 hours at 37 °C. The fungal pathogen was grown in potato dextrose agar (PDA) medium containing 4.0 g/L potato extract, 20.0 g/L dextrose, and 15.0 g/L bacteriological agar at a final pH of 5.5 ± 0.2, and the plates were incubated at 30 °C for seven days. Seeds of yellow maize landrace (properly belonging to the group race Elotes Occidentales) were obtained from the fifth mass selection cycle under tropical conditions, provided by the Facultad de Ciencias Agropecuarias y Ambientales of the Universidad Autónoma de Guerrero.

Preparation of pathogen inoculum

All experiments used a 7-day fungal culture grown on PDA at 30 °C for the preparation of a conidia suspension by lightly scraping a colony with a slide and adding 10 mL of sterile distilled water to the PDA medium; the suspension was recovered and filtered with sterile gauze to remove excess mycelia. Conidia of *C*urvularia *lunata* SY17 were then diluted in 2 mL of sterile 0.9% saline solution for counting in a Neubauer chamber under an optical microscope with 40X magnification, after which the concentration was adjusted to 107 conidia/mL in aseptic conditions (Constanza et al. 2012).

Preparation of *B. licheniformis* M2-7 inoculum

Bacterial cells were recovered from a 24 h incubation culture at 37 °C in nutrient broth by centrifugation at 8000 rpm for 10 min. The cells were then washed twice with sterile 0.9% saline solution and resuspended again in 2 mL of 0.9% saline solution for counting in a Neubauer chamber under an optical microscope with 40X magnification. The samples for the bioassays were adjusted until broths were obtained at a concentration in the order of 10^7 CFU/mL (Márquez et al. 2003).

Experimental design and pathogenicity test

Yellow maize landrace seeds (Elotes Occidentales) were sown in polyethylene bags (15 × 17 cm) with sterile substrate and were transferred to a greenhouse at a 16 hours/light and 8 h /dark photoperiod at 25 °C and a 70% relative humidity. The newly sown seeds were initially provided with auxiliary irrigation. After germination, the seeds were irrigated every five days until they reached the V4/V5 stage of corn phenological development (4/5 true leaves). The experiments consisted of two treatments with nine plants each in V4/V5 stage. Treatment 1 consisted of nine plants inoculated with *C. lunata* SY17 in the leaf area, whereas the control treatment consisted of nine plants without fungal inoculum. Each experimental unit consisted of a polyethylene bag with a maize plant. Inoculation was performed by spraying a concentration of 10^7 conidia/mL resuspended in 50 mL of sterile water onto each plant (Garcia-Aroca et al. 2018). The disease incidence rate (% ID) was then calculated 15 days after inoculation (Castillo-Arévalo and Jiménez-Martínez 2020) using Eq. (1):

$$\%ID = \frac{LL}{TL} \times 100 \quad (1)$$

where IL is the number of infected leaves and TL is the total number of leaves.

The disease incidence rate (%) was determined via mean comparison analysis with Student's t-test ($P \leq 0.05$) (Rubio-Hurtado and Berlanga-Silvente 2012).

Re-isolation and microculture technique

To confirm pathogenicity (Agrios 2002), samples were taken from leaves exhibiting symptoms of *C. lunata* infection. The infected tissue was cut, washed with sterile distilled water, cultured on plates with PDA medium, and incubated at 30 °C for seven days. A humid chamber (Ridell 1950) was prepared by pouring sterile distilled water into a tray containing glass rods, under which filter paper was placed to prevent desiccation. A sterile slide was placed on the rods and a 1 cm^2 of PDA was placed on top using a sterile mycological loop. Afterward, the mycelia of *C. lunata* SY17 were collected and sown on the PDA medium by the vertices. The mycelia were then allowed to grow on a coverslip for seven days. Finally, the structures were dyed with a drop of lactophenol blue and observed using an Olympus optical microscope.

Molecular identification

Molecular identification was carried out to corroborate the macroscopic and microscopic identification based on the use of taxonomic keys of the phytopathogenic fungus at the species level. Genomic DNA was extracted from fresh mycelia grown in PDA medium incubated at 30 °C using the Quick- DNATM fungal/bacterial miniprep kit (Zymo research) according to the manufacturer's instructions. Afterward, the polymerase chain reaction (PCR) technique was used to amplify a fragment (650 bp) of the ITS gene using the ITS1 (5'-CTT GGT CAT TTA GAG GAA GTA A-'3) and ITS2 (5'-TCC TCC GCT TAT TGA TAT GC-'3) primer pair (Vancov and Keen 2009). The PCR reactions were conducted using 2.5 µL of 10X reaction buffer, 2 µl of 25 mM dNTPs, 1.5 µL of MgCl2, 2 µL of ITS1 and ITS4 (10 pmol) oligonucleotides, and 0.5 µL of recombinant Taq DNA polymerase (Thermo Scientific, EP0404). The final reaction volume was adjusted to 25 µL with sterile distilled H_2O and each sample was amplified in triplicate. The thermal cycler PCR conditions consisted of an initial denaturation step at 95 °C for 5 min, followed by 30 denaturation cycles at 95 °C for 1 min, 30 alignment cycles at 59 °C for 1 min, 30 elongation cycles at 72 °C for 1 min, and a final elongation cycle at 72 °C for 1 min. The amplification products were separated via 1% agarose gel electrophoresis (Sigma-Aldrich) and visualized in a transilluminator, after which the samples were purified using the GeneJET PCR Purification kit (Thermo Scientific). DNA sequencing was performed using fluorescent dideoxynucleotide terminators via the Sanger method in an automated sequencer at the Instituto de Biotecnología of the National Autonomous University of Mexico (UNAM). The amplified regions were compared with fungal sequences stored in the GenBank database by local research of nucleotide sequence alignments (BLAST).

In vitro antagonism

The PDA agar diffusion method was used to characterize the antagonistic activity of *B. licheniformis* M2-7 against *C. lunata* SY17. Thus, 5 ml of *B. licheniformis* M2-7 culture (10^7 CFU/mL) was added and dispersed into the gelled medium, after which a 5 mm diameter explant of *C. lunata* SY17 mycelium was placed in the center. The plates were incubated at 30 °C for 10 days and the percentage inhibition was calculated with Eq. 2:

$$\text{Inhibition percentage } \% = \frac{[(C-T) \times 100]}{C} \qquad (2)$$

where C is the diameter (mm) of fungal mycelium in the negative control (no bacteria) and T is the diameter (mm) of the mycelium on plates treated with *B. licheniformis* M2-7 (Ahmad et al. 2017).

Inhibition of conidial germination

C. lunata SY17 conidia suspension at 10^7 conidia/mL (see section Preparation of pathogen inoculum) was mixed with 10^7 CFU/mL of *B. licheniformis* M2-7 (1:1 v/v) in 50 mL flasks. Afterward, the samples were incubated at 30 °C at 150 rpm for 24 h. Additionally, 10 µL of the samples was collected and stained with a drop of lactophenol blue for observation under an optical microscope (Olympus). Germination was defined as a germ tube that had developed longer than half of the cell length. Finally, the germination percentage was performed in triplicate by counting the proportion of germinated conidia in a 100 individuals count of each sample under the microscope (Mahadtanapuk et al. 2007).

***In vivo* antagonism test**

Planting was done with two seeds per planting site at 0.40 m distance, and from these two, it was

adjusted to one plant that included 6 plants per treatment and 24 plants per block, equivalent to 96 plants in total. The seeds were watered every three days until they reached the V4/V5 stage of corn phenological development (4/5 true leaves). The phytopathogen was inoculated by spraying a concentration of 10^7 conidia per mL resuspended in 50 mL of sterile water onto the experimental plants. The experimental design was completely randomized with four treatments and five replicates. The treatments were: Treatment 1 (T1, chemical fungicide Metalaxyl at 2 g/L), Treatment 2 (T2, *Bacillus licheniformis* M2-7, 1×10^3 CFU), Treatment 3 (T3, *B. licheniformis* M2-7, 1×10^6 CFU), and Treatment 4 (T4, control of plants treated with sterile water). Disease severity was assessed using the ImageJ software (version 2.0) and the acquired data were analyzed via ANOVA and Tukey's test ($P \leq 0.05$) (Ojendiz-Mata et al. 2023).

RESULTS AND DISCUSSION

Curvularia lunata SY17 is a fungal pathogen of maize plants

The pathogenicity tests of *Curvularia lunata* SY17 were carried out by spraying leaves of yellow maize landrace plants. Fungal growth was confirmed by monitoring disease symptoms (leaf spot) five days after inoculation. The observed symptoms included oval, circular, or elongated spots with an irregular border and gray or dark brown center at the periphery within a yellow 0.5 to 2 mm diameter halo (**Fig. 1A**). Treatment 1 (T1, inoculation with *C. lunata*) exhibited a 99.33% disease incidence, whereas the control treatment (T0, without inoculation) had a nule incidence (**Fig. 1B**). In Mexico, Ríos-Herrera et al. (2017) linked the occurrence of tar spot complex disease on maize in the states of Chiapas and Guerrero with *C. lunata.* To the best of our knowledge, our work is the first to confirm the phytopathogenicity of the strain SY17 of *C. lunata* in maize. Our findings confirmed the symptoms reported by Garcia-Aroca et al. (2018), who described leaf spot disease in maize undergoing the V4/V5 vegetative stage as round to oval, light brown lesions that were 2.0 mm in diameter, with reddish-brown margins and often with chlorotic halos in the middle and upper part of the maize canopy (hybrid DKC 66-19). Furthermore, Jimenez Madrid et al. (2022) described foliar symptoms on hybrid maize plants as small, circular, light brown to tan lesions (0.5 to 3 mm in diameter) with reddish-brown margins (9114VT2P progeny). However, neither of the aforementioned studies reported the incidence rate of this disease. Incidence rates are particularly useful to understand the speed and patterns of disease progression in maize.

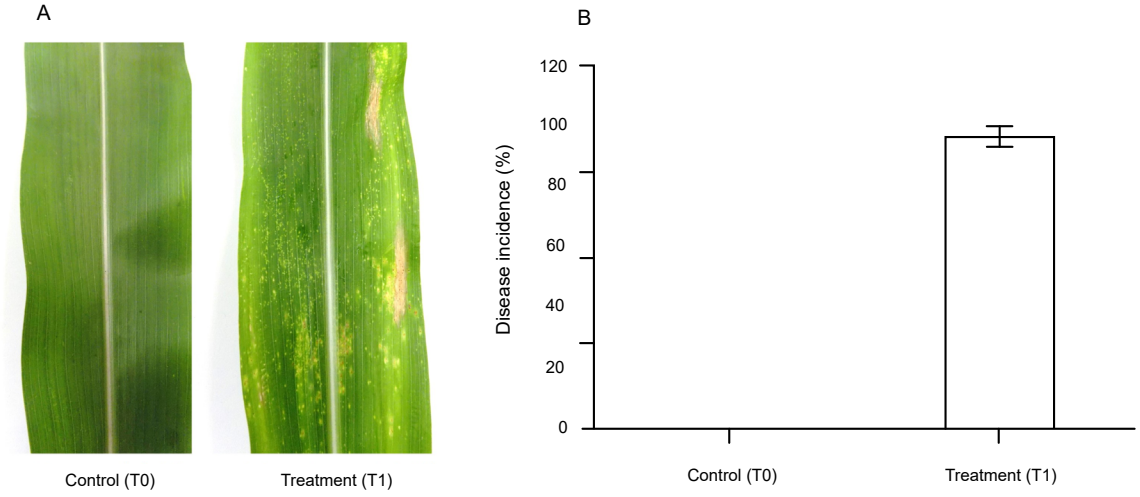

Fig. 1. Incidence of leaf spot disease in yellow maize landrace. **A)** Lesions caused by *C. lunata* SY17 in V4/V5 stage of yellow maize landrace (T1) after 5 days after inoculation in contrast to the control without inoculation (T0). **B)** Disease incidence. *** Indicates significant statistical differences (P<0.05, Student's t-test). Error bars indicate standard deviation (n=9).

The characteristics of strain SY17 coincided with those of *Curvularia lunata*

The macro- and microscopic morphology of the re-isolated fungus was consistent with previous descriptions of the fungus *C. lunata* (Wakker) Boedijn (Bahena-Oregón et al. 2022). The colony exhibited an olive-black coloration, with a cottony texture, a fimbriate margin, and a black reverse side (**Figs. 2A** and **2B**). Microscopic observations confirmed that the fungus developed vegetative septate and branched subhyaline to brownish vegetative hyphae. The conidia were smooth, four-celled, and curved in the third cell from the base, with brown intermediate cells and subhyaline or light brown terminal cells (**Figs. 2C** and **2D**). BLAST analysis of the sequences enabled the identification of the fungus as *C. lunata* with a 100% percentage of identity according to the sequence MK990136 reported in GenBank.

***Bacillus licheniformis* M2-7 inhibits the growth of *Curvularia lunata* SY17 under In vitro conditions**

B. licheniformis M2-7 was confronted against *C. lunata* SY17 on PDA medium via the dilution method. In this trial, we assessed the capacity of the bacterial strain to inhibit the mycelial growth of the phytopathogenic fungus, and the inhibition rates reached up to 73.75% relative to the control ($P \leq 0.05$; **Fig. 3**). Additionally, swelling and hyphal deformation were observed after 10 days of incubation compared to the control (**Fig. S1**). The inoculation of antagonistic bacteria constitutes a promising strategy for disease control in food crops. Among these antagonistic bacteria, several species of the genus *Bacillus* are known to produce a wide range of antagonistic compounds with different chemical structures, devoting up to 5 to 8% of their genome to secondary metabolite biosynthesis (Fira et al. 2018). Lipopeptides, polyketide compounds, bactericides, and siderophores are among the most important bioactive molecules produced by these microorganisms (Ruckert et al. 2011, Zhao et al. 2017). In a recent study, Chen et al. (2020) identified noticeable morphological changes in the hyphae of *Fusarium oxysporum* f. sp. *fragariae* in the presence of *Bacillus licheniformis* X-1 and *Bacillus methylotrophicus* Z-1. The X-1 strain adhered to the hyphae, causing wrinkling and deformities of the hyphae, whereas strain Z-1 caused swelling of the hyphae and extrusion of the protoplasm and micropores. Finally, Albarrán-de la Luz et al. (2022) demonstrated that *Bacillus licheniformis* strains M2-7 and LYA12 affected the occurrence of *Aspergillus oryzae*, *Colletotrichum* sp., and *Aspergillus niger* mycelia, resulting in swelling, malformations, and poor hyphal growth. This effect was detected through the expression of two metacaspases (*casA* and *casB*) that trigger the programmed cell death process in fungi.

***Bacillus licheniformis* M2-7 inhibits germination of *Curvularia lunata* SY17 conidia**

A confrontation assay in nutrient broth confirmed the inhibition of *C. lunata* SY17 conidial germination by *B. licheniformis* M2-7. The bacterial strain successfully inhibited *C. lunata* SY17 growth by up to 78.66% after 24 h of incubation, exhibiting a robust antifungal effect compared to the control treatment ($P \leq 0.05$; **Fig. 4**). Our results were consistent with those reported by Jeong et al. (2017), who demonstrated that *B. licheniformis* MH48 transformed the conidial morphology of *Colletotrichum gloeosporioides* and inhibited its conidial germination, in addition to degrading the mycelia of *Rhizoctonia solani* in the presence of 50 and 200 µg/mL concentrations of benzoic acid. Nawaz et al. (2018) demonstrated that *B. licheniformis* OE-04 impairs the morphology of hyphae

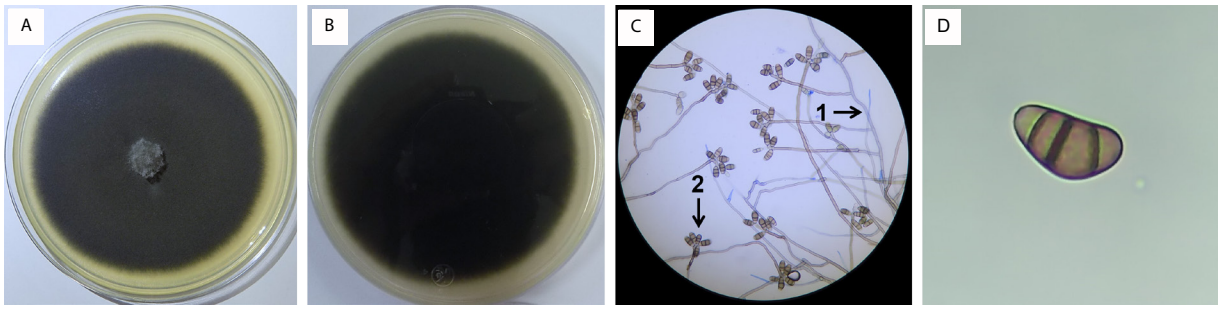

Fig. 2. Morphology de *C. lunata* SY17 reisolated. A) Colony on PDA agar after 7 days of incubation at 30 °C; B) colonial reverse side of the fungus; C) septate vegetative hyphae (1) and conidiophores (2); D) conidium.

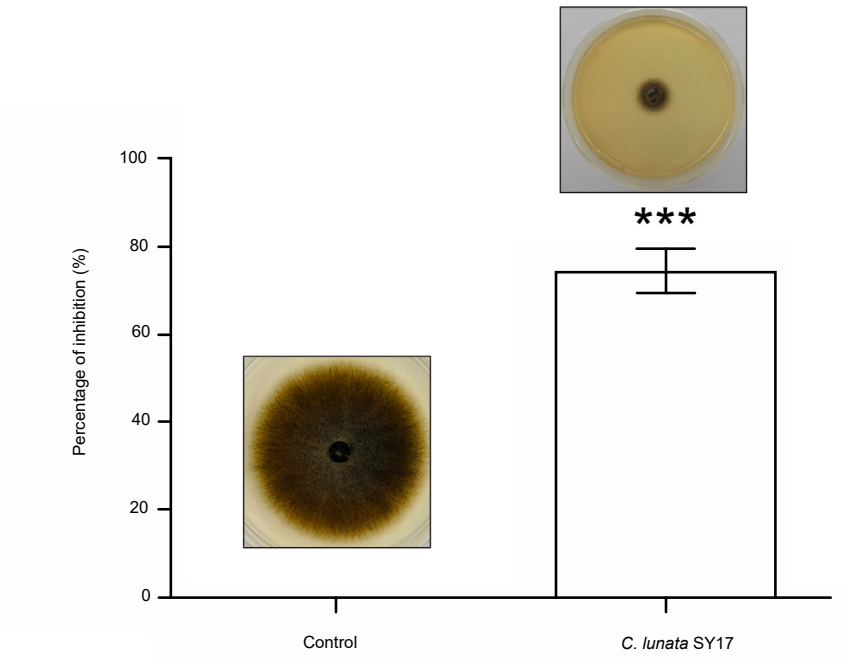

Fig. 3. Inbibition of *B. licheniformis* M2-7 against *C. lunata* SY17. Error bars indicate the standard deviation of three independent experiments (n=3). *** Indicates significant statistical differences (P<0.05, Student's t-test).

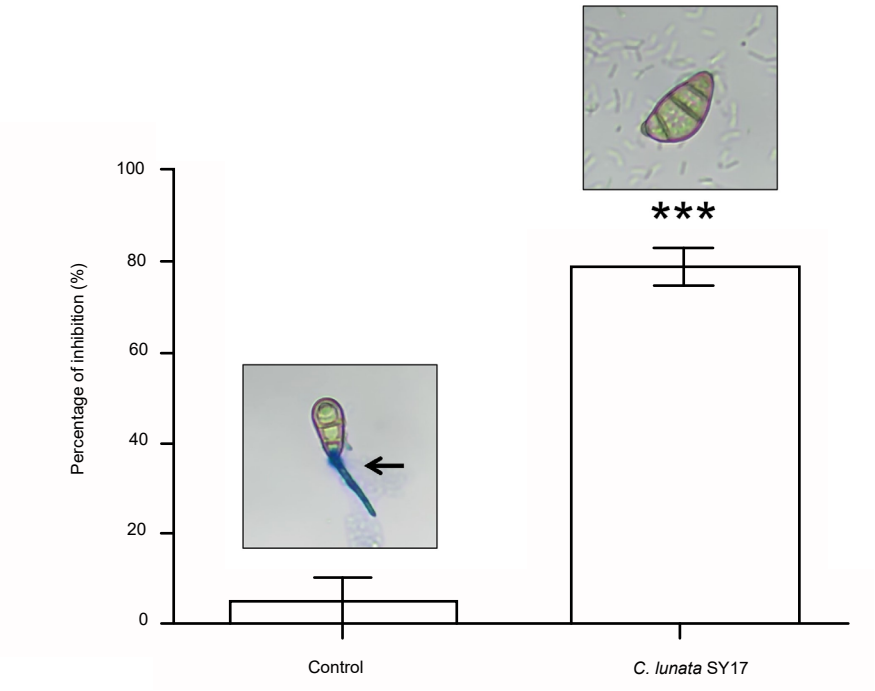

Fig. 4. Inhibition of conidial germination of *C. lunata* SY17 by *B. licheniformis* M2-7. Error bars indicate the standard deviation of three independent experiments (n=3). *** Indicates significant statistical differences (P<0.05, Student's t-test). → It points to the formation of the germ tube of the conidium.

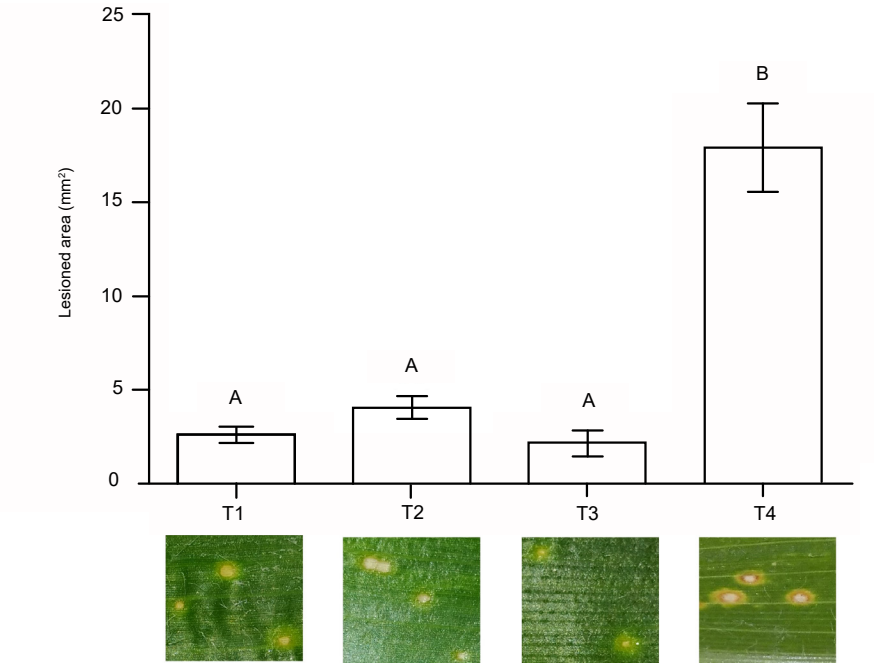

Fig. 5. In vivo effect of *Bacillus licheniformis* M2-7 on the severity of leaf spot disease caused by *Curvularia lunata* SY17 on maize leaves. Error bars indicate standard deviation of five independent experiments (n=30). Different letters indicate significant statistical differences (P<0.05, Tukey's test).

and induces the formation of large vesicles in the phytopathogenic fungus *Colletotrichum gossypii* after 48 h of incubation. This antifungal effect was attributed to the activity of biosurfactants produced by the *B. licheniformis* M2-7 (Bolaños-Dircio et al. 2021).

Bacillus licheniformis M2-7 alleviates leaf spot disease symptoms caused by *Curvularia lunata* SY17 on maize leaves

To evaluate the biocontrol capacity of the bacterial strain, antagonism tests were performed on maize leaves at the V4/V5 stage infected with *C. lunata* SY17. In maize plants, the leaves treated with the two bacterial concentrations (T2: 1×10^3 and T3: 1×10^6 CFU) exhibited a reduction in the symptom-affected areas compared to the untreated plants (T4) at 15 days post-inoculation (**Fig. 5**). However, no significant differences were found between these plants and those treated with the chemical fungicide (T1, P<0.05). Therefore, our findings demonstrated that *B. licheniformis* M2-7 can effectively inhibit the mycelial growth of *C. lunata* SY17 in maize leaves. The two concentrations of the bacterial strain evaluated herein were effective for plant protection compared to the control treatment. Therefore, our results suggest that *B. licheniformis* M2-7 could be used in agricultural formulations for priority crops such as maize.

The antifungal effect of the bacterium may be attributed to nutritional competition and the production of some diffusible molecules that inhibit or reduce pathogen growth (Brader et al. 2014). *B. licheniformis* M2-7 has been reported to produce secondary metabolites such as amylases, proteases, esterases, biosurfactants, and volatile organic compounds, all of which can act as biocontrol agents (Bolaños-Dircio et al. 2021). Our results are consistent with those of Nigris et al. (2018), who demonstrated that *B. licheniformis* GL174 could effectively inhibit the growth of fungal pathogens (*Phaeoacremonium aleophilum*, *Botryosphaeria* spp., *Botrytis cinerea*, *Phytophthora infestans*, and *Sclerotinia sclerotiorum*) and reduce the severity of the infection in *Vitis vinifera* Glera both in vitro and in vivo.

CONCLUSIONS

Our findings showed that *B. licheniformis* M2-7 is a promising biocontrol agent in maize plants, since the inoculation of this bacterium in infected plants reduced the severity of leaf spot disease in vivo. In

vitro, we demonstrated that this reduction in symptoms is due to the ability of *B. licheniformis* to inhibit the growth of *Curvularia lunata* SY17, preventing conidia germination.

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SUPPLEMENTARY MATERIAL

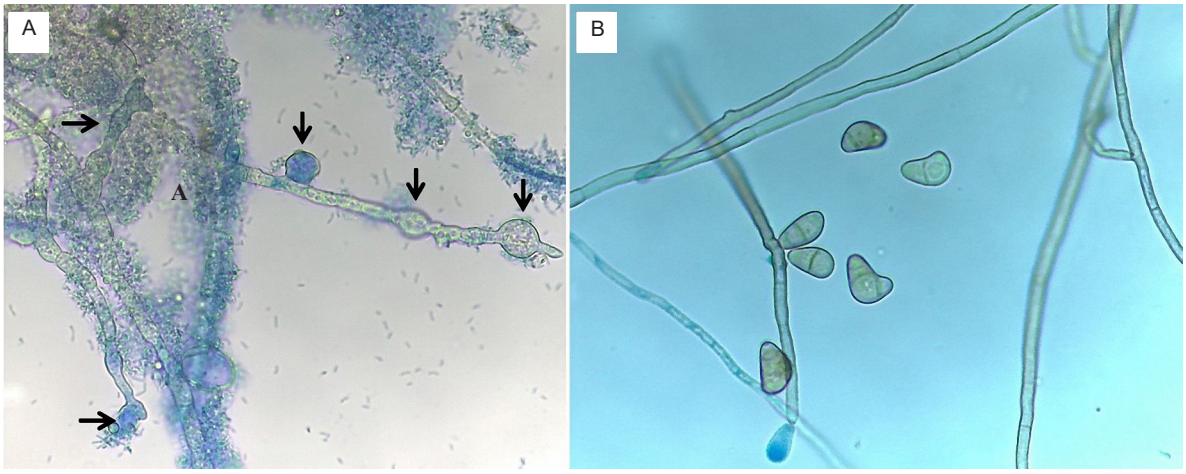

Fig. S1. Hyphal morphology of *Curvularia lunata* SY17 in the presence (A) and absence (B) of *Bacillus licheniformis* M2-7 after 10 days of incubation at 30 °C. Black arrows indicate swelling and deformation of hyphae. Olympus optical microscope, 40 X.