Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers – 12th Special issue on Grapevine Trunk Diseases

Heritage grapevines as sources of biological control agents for Botryosphaeria dieback pathogens

Carmen Sanjuana DELGADO-RAMÍREZ[1], Edgardo SEPÚLVEDA[2], Edelweiss Airam RANGEL-MONTOYA[3], Cesar VALENZUELA-SOLANO[4], Rufina HERNÁNDEZ-MARTÍNEZ[3,*]

[1] *Posgrado Ciencias de la Vida, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Carretera Ensenada-Tijuana No. 3918, C.P. 22860 Zona Playitas, Ensenada, Baja California, México*
[2] *CONACYT-Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Ensenada, Baja California, 22860, Mexico*
[3] *Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Ensenada, Baja California, 22860, Mexico*
[4] *Sitio Experimental Costa de Ensenada. INIFAP. Ensenada, Baja California, Mexico*
*Corresponding author. E-mail: ruhernan@cicese.mx

Summary. Grapevine trunk diseases cause severe damage in grapevines. Management strategies focus on protection of grapevine pruning wounds using chemical fungicides or biological control agents. Botryosphaeria dieback, caused mainly by *Lasiodiplodia* spp., is one of the main trunk diseases in northwest Mexico. This study obtained endophytic bacteria and fungi from the heritage grapevine *Vitis vinifera* cv. 'Mission' for potential biological control of Botryosphaeria dieback. A collection of 135 bacterial and 37 fungal isolates were obtained and initially tested for antagonistic activity against *Lasiodiplodia brasiliensis*. The most promising isolates belonging to *Trichoderma* and *Bacillus* spp. were selected and characterized to determine their modes of action. *Bacillus* isolates produced volatile organic compounds that inhibited growth of *Neofusicoccum parvum*, and diffusible organic compounds with antifungal effects against *L. brasiliensis* and *N. parvum*. *Trichoderma* isolates produced diffusible organic compounds and were mycoparasites. In greenhouse assays, plants inoculated with three *Trichoderma asperellum* isolates (T20BCMX, EF09BCMX, and EF11BCMX), *B. amyloliquefaciens* (BEVP26BCMX) or *Bacillus* sp. (rbES015), applied preventively in soil, gave up to 50% smaller necrotic lesions when compared with the plants inoculated only with *L. brasiliensis*. In the field, plants inoculated with three *Bacillus* isolates (BEVP02BCMX, BEVP26BCMX, BEVP31BCMX) or five *Trichoderma* (T11BCMX, T15BCMX, T17BCMX, T20BCMX, and EF11BCMX) had lesions up to four times smaller than control plants inoculated only with *L. brasiliensis*. This study has demonstrated the potential of heritage grapevines to provide biological control agents for Botryosphaeria dieback.

Keywords. Grapevine trunk diseases, *Botryosphaeriaceae*, *Bacillus*, *Trichoderma*.

OPEN ACCESS

Citation: C.S. Delgado-Ramírez, E. Sepúlveda, E.A. Rangel-Montoya, C. Valenzuela-Solano, R. Hernández-Martínez (2023) Heritage grapevines as sources of biological control agents for Botryosphaeria dieback pathogens. *Phytopathologia Mediterranea* 62(2): 115-134. doi: 10.36253/phyto-14154

Accepted: March 17, 2023

Published: May 12, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: José R. Úrbez Torres, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada.

ORCID:
CD: 0000-0003-2387-3050
ES: 0000-0002-2413-8261
ER: 0000-0002-2362-9229
CV: 0000-0003-3030-6038
RH-M: 0000-0002-0914-3732

INTRODUCTION

Grapevine trunk diseases (GTDs) cause severe problems in vineyards. These are a complex of diseases that include black foot, Esca, Eutypa dieback, Petri disease, and Botryosphaeria dieback (Úrbez-Torres *et al.,* 2012). Approx. 133 fungal species in 34 genera have been reported as causal agents of GTDs (Gramaje *et al.,* 2018). Among them, Botryosphaeria dieback is considered to be the most important (Billones-Baaijens and Savocchia, 2019). Almost 30 species of *Botryosphaeria, Diplodia, Dothiorella, Lasiodiplodia, Neofusicoccum, Neoscytalidium, Phaeobotryosphaeria* and *Spencermartinsia* have been identified as causal agents of Botryosphaeria dieback (Gramaje *et al.,* 2018).

Among the fungi associated with Botryosphaeria dieback, *Lasiodiplodia* and *Neofusicoccum* contain the most virulent species identified in different countries, including the United States of America (Úrbez-Torres and Gubler, 2009), Iraq (Abdullah *et al.,* 2012) and Mexico (Rangel-Montoya *et al.,* 2021). *Lasiodiplodia* spp. have white colonies in culture, that later become dark gray with abundant mycelium, and their conidia can be aseptate hyaline or septate pigmented, with longitudinal striations. *Neofusicoccum* spp. have initially white colonies, and some species as *N. luteum* and *N. australe* produce yellow pigments. Their abundant aerial mycelium later turns gray, and conidia are hyaline, unicellular, and aseptate, with a subtruncate bases (Zhang *et al.,* 2021).

Botryosphaeria dieback symptoms usually appear in grapevines several years after pathogen infections, and the symptoms include wedge-shaped perennial cankers, wood discolourations, brown streaking on the wood under the bark, and premature plant death (Bertsch *et al.,* 2013; Spagnolo *et al.,* 2014; Gramaje *et al.,* 2018; Niem *et al.,* 2020). The pathogen enters host plants through wounds, made mostly during pruning (Gramaje *et al.*, 2018).

Management of GTDs is complex, and no curative treatments are known to date. Therefore, strategies commonly focus on implementation of cultural and preventive treatment measures, with each depending on plant damage, the pathogens involved, and the geographic region (Gramaje *et al.,* 2018). These include the protection of pruning wounds with fungicides or biological control agents (BCAs), and curative surgery (Mondello *et al.,* 2018).

The search for endophytic microorganisms for biological control of GTDs pathogens has increased, due to their potential to have antagonistic activities against different species of fungi and, in some cases, to also promote plant growth (Dini-Andreote, 2020; Jacob *et al.,* 2020). Different endophytic microorganisms have been reported as antagonists of GTDs pathogens. These include fungi (*Aspergillus, Chaetomium, Clonostachys*, *Cladosporium, Epicoccum, Fusarium,* and *Trichoderma*), which have shown antagonistic *in vitro* activity against *Diplodia* and *Neofusicoccum* spp. (Almeida *et al.,* 2020; Silva-Valderrama *et al.,* 2021). As well, bacteria (*Bacillus*, *Burkholderia, Paenibacillus*, *Pseudomonas,* and *Streptomyces*) have been recognized as antagonists of *Diplodia, Lasiodiplodia,* and *Neofusiccocum* (Haidar *et al.,* 2016). Among the organisms with potential as BCAs, *Bacillus* and *Trichoderma* show *in vitro* antagonistic activity through different mechanisms of action, including competition for space, production of volatile and non-volatile compounds, or mycoparasitism. In greenhouse and field trials, these organisms have shown efficacy for control of GTDs fungi when applied to grapevine pruning wounds (Almeida *et al.,* 2020).

In Mexico, the Spanish introduced grapevine (*Vitis vinifera*) in the 16th century, and Jesuit and Dominican friars disseminated its cultivation (Crowley, 1989). 'Listan Prieto', now known as 'Mission', was introduced in Mexico and in the United States of America (Walker *et al.,* 2019). In the Baja California peninsula, the first grapevines were established in the San Francisco Javier mission, located in Loreto (Magoni, 2009). From then on, grapevine cultivation continued to expand. Currently in Baja California, 'Mission' grapevines are over 40 years old, and have been propagated from the first vines introduced in the state by the Jesuit missionaries, and these plants are considered to be heritage grapevines. Approx. 38 ha of this variety are grown with minimal management in Baja California, with yields of approx. 3.5 tons ha^{-1} (SEFOA, 2011; Andrade *et al.,* 2013).

Different reports have shown that plants growing in arid conditions associate with microorganisms (bacteria and fungi). These can enhance plant drought resistance through various mechanisms, including improved water and nutrient absorption by inducing changes in root morphology, protecting against oxidative damage, regulating phytohormone levels, and suppressing phytopathogens such as those responsible of GTDs (Poudel *et al.,* 2021; Riseh *et al.,* 2021).

Considering the time since they were established and the complex climatic conditions in which the 'Mission' grapevines have been planted (dry to very dry climate with average rainfall of 200 mm p.a. (INEGI, 2017), it is likely that these plants maintain associations with beneficial microorganisms which allow them to survive in the adverse conditions and resist plant pathogens. Therefore, the objective of the present study was to obtain endophytic bacteria and fungi from the cultivar 'Mission' with the potential as BCAs of Botryosphaeria dieback fungi,

thus providing sustainable alternatives for the control of this disease in commercial vineyards of Baja California.

MATERIALS AND METHODS

Sampling and isolation of microorganisms from heritage grapevines

Microorganisms were isolated from lignified 1-year-old branches of heritage grapevines cultivar 'Mission', growing in local vineyards in the Guadalupe valley (31.994722, -116.683896) and Ejido Uruapan (31.628436, -116-434295), Baja California. Small tissue fragments were cut from each branch sample, and after bark removal, these were surface sterilized by flaming (Rangel-Montoya *et al.,* 2021). Subsequently, for bacteria isolations, branch fragments were transferred to plates containing LB Agar (ATCC media No.1065), YPD Agar (1245) or King's medium B Agar (1213), or PY medium (tryptone 5.0 g, yeast extract 3.0 g, $CaCl_2$ 0.9 g, pH 6.8), supplemented with cycloheximide (final concentration 100 μg mL^{-1}). For fungal isolations, tissue fragments were inserted in plates containing Potato Dextrose Agar (PDA), or water agar supplemented with chloramphenicol (final concentration 25 μg mL^{-1}). The isolation plates were incubated at 30°C until microorganism growth was observed, and the resulting bacterial and fungal colonies were recovered and subcultured to obtain pure cultures. Fungal strains were preserved at 4°C in 20% glycerol, and bacteria strains in 35% glycerol solution at -20°C. Additionally, *Bacillus* sp. rbES015 and 35 strains of *Trichoderma* were obtained from the collection of the Phytopathology Laboratory of CICESE.

Screening for antifungal activity

The GTD fungi *Lasiodiplodia brasiliensis* MXBCL28 (Rangel-Montoya *et al.,* 2021) and *Neofusicoccum parvum* 14P4MX (Rangel-Montoya, 2021) were used to test the biological control potential of the obtained fungal and bacterial isolates. Using a flame-sterilized 7 mm cork borer, an agar plug with mycelium was obtained from a 4-day-old culture, from each fungus grown on PDA. The plug was then placed on the centre of a fresh PDA plate, and incubated at 25°C. When the fungus colony reached 1 cm diam. 5 μL of four different potential BCA bacterial cultures or one mycelium plug from potential fungal BCAs, were inoculated at the edges, as described by Guevara-Avendaño *et al.* (2018). Plates with only GTD fungi were used as experimental controls. These assays were each carried out in triplicate. After 7 d incubation at 30°C, the inhibition of radial growth of *L. brasiliensis* mycelium was assessed. In total, 135 bacterial and 39 fungal isolates were screened in these assays.

After discarding isolates with low or no inhibition activity, quantitative fungal inhibition assays were carried out for 58 strains (21 bacteria and 37 fungi), as described for the qualitative assays (above). For each of these quantitative assays, the *L. brasiliensis* mycelium plug was placed at the edge of the Petri plate, and only one bacterial or fungi strain was inoculated directly opposed to it. The following formula (Méndez-Bravo *et al.,* 2018) was used to calculate the percentage of inhibition of mycelial growth: %inhibition = $[(R\text{-}r)/R] \times 100$, where R is the colony radius of the pathogenic fungus growing alone in the control plates, and r is the colony radius of the fungus growing in the plate in confrontation with a tested isolate. All *in vitro* antagonistic assays were performed in triplicate.

These procedures allowed selection of the most promising BCAs for used in the experiments described below.

Evaluation of antagonistic effects by volatile organic compounds

Antagonistic effects of volatile organic compounds produced by eleven selected strains (four bacteria and seven fungi) were evaluated against *L. brasiliensis* and *N. parvum*, using the two sealed base plate method of Rangel-Montoya *et al.* (2022). For each potential fungal BCA isolate, a mycelial plug was placed in the centre of a PDA plate, and for each bacterium, 20 μL of culture was spread in a PY plate. The lid of each plate was the replaced by a second PDA plate with a mycelial plug of the pathogen at the centre. The two plates were sealed with tape and incubated at 30°C for 4 d, with mycelium growth assessed every 24 h. As experimental controls, non-inoculated PDA or PY plates were used as the covering plate.

Evaluation of the antagonistic effects by diffusible organic compounds

The antagonistic activity of diffusible organic compounds produced by eleven selected BCAs strains was evaluated against *L. brasiliensis* and *N. parvum*. For bacteria, 5 mL of liquid PY medium was inoculated with a single colony, and then incubated at 30°C and 110 rpm in a shaker incubator. After 7 d, cultures were each centrifuged at 10,000 rpm for 20 min, and the resulting supernatant was filter-sterilized using a 20 μm syringe filter. PDA plates containing 15% (v/v) of sterile bacterial

supernatant were then prepared, and a mycelial plug of each pathogen was placed in the centre of each test plate (Salvatierra-Martinez *et al.,* 2018). The plates were then incubated at 30°C for 4 d, registering mycelium growth every 24 h. For evaluation of fungal isolates, cellulose membrane assays were used (Mayo-Prieto *et al.,* 2020). A mycelial plug disc of each fungus was inoculated in the centre of each PDA plate. The plug was then covered with a sterile cellulose membrane, and the plates were incubated at 30°C for 48 h. The membrane with the mycelial growth was then removed, and a mycelial plug of the pathogen was placed in the centre of the plate. Plates were incubated at 30°C for 4 d, and mycelium growth was assessed every 24 h.

Evaluation of mycoparasitism activity

Mycoparasitism activity of seven selected *Trichoderma* isolates was assessed against *L. brasiliensis* and *N. parvum*, using the pre-colonized plate method described by Bailey *et al.,* (2008). A mycelial plug of each pathogenic fungus was inoculated at the edge of a PDA plate, and after 5 d incubation at 30°C, an agar strip (4.0 × 0.5 cm) from a colony of a *Trichoderma* isolate was placed at the opposite side. The plates were incubated for 28 d at 30°C in darkness. Ten mycelial plugs were then collected from each plate in a straight line beginning near the agar strip and extending towards the opposite edge of the plate. The mycelial plugs were then inoculated into PDA plates and incubated for 24 h at 30°C in darkness, followed by 5 d incubation under white light at room temperature. As experimental controls, cultures of *L. brasiliensis* and *N. parvum* were used, grown without *Trichoderma* and maintained under the same conditions. Mycoparasitism was determined by assessing the presence of *Trichoderma* and the mycoparasitic fungi in the ten mycelial plugs collected. Micoparasitic activity was also assessed under a microscope. For each of these assessments, a dual culture assay was performed in water agar plates, and after 3 d incubation at 30°C, a fragment of agar was cut from the centre of the plate. The obtained samples were observed with inverted microscope (Zeiss Axiovert 200), and the obtained images were analyzed using Zeiss AxioVision SE64, Rel. 4.9.1 software.

In vitro *screening for plant growth promoting traits*

Coluorimetric tests were carried out to determine plant growth promotion by the potential BCA bacteria and fungi strains. For these assays, the strains were recovered from glycerol stock cultures, and bacteria were inoculated into PY liquid medium, and fungi onto PDA medium. Bacterial cultures were incubated for 2 d at 30°C and 100 rpm in a shaker incubator. Fungi were incubated at 30°C for 7 d, Coluorimetric tests were carried out in triplicate, in 35 mm diam. Petri dishes for solid media or 10 mL capacity tubes for liquid media, as described in the sections below. Petri plates or tubes with the corresponding media but without BCAs were used as experimental controls.

Mineral solubilization assays

Some microorganisms are capable of hydrolyzing organic and inorganic insoluble mineral compounds to soluble forms, that can be assimilated by plants, acting as biofertilizers or plant grow-promoters. Phosphate, potassium, and zinc solubilization assays were performed for the potential BCAs. In each case, 5 µL of bacterium culture or a 7 mm diam. mycelial plug of fungus were inoculated at the centre of each assay plate.

Inorganic phosphate solubilization was evaluated on modified Pikovskaya agar (0.5 g L^{-1} yeast extract, 10 g L^{-1} glucose, 5 g L^{-1} $Ca_3(PO_4)_2$, 0.5 g L^{-1} $(NH_4)_2SO_4$, 0.2 g L^{-1} KCl, 0.1 g L^{-1} $MgSO_4$, 0.1 mg L^{-1} $MnSO_4$, 0.1 mg L^{-1} $FeSO_4$, 10 mg L^{-1} of bromocresol purple, 15 g L^{-1} agar, pH 7.2). After inoculation, Petri dishes were incubated for 72 h at 30°C. Colour change from purple to yellow indicated a positive phosphate solubilizing strain (Gupta *et al.,* 1994; Zheng *et al.,* 2018).

Inorganic potassium solubilization was determined on modified Pikovskaya agar (Pikovskaya, 1948), using KNO_3 and bromocresol green. After inoculation, Petri plates were incubated for 72 h at 30°C. Colour change from blue to yellow indicated a positive potassium solubilizing strain.

Zinc solubilization was assessed using zinc-supplemented Pikovskaya medium complemented with 1.2 g L^{-1} ZnO and bromothymol blue. Inoculated Petri dishes were incubated in the dark for 72 h at 30°C. Colour change from blue to yellow indicated a positive zinc solubilizing strain (Bapiri *et al.,* 2012).

Indole Acetic Acid (AIA) production assays

Indole acetic acid (IAA) is one of the most physiologically active auxins. It induces production of long roots and root hairs, and lateral roots, which are involved in nutrient uptake by plants (Datta and Basu, 2000). For determination of IAA production by bacterial strains, 5 µL of 1-d-old cultures were reinoculated in 96 well micro-

plates, with 200 µL PY liquid medium supplemented with tryptophan to a final concentration of 500 µg mL^{-1}. Microplates were incubated for 48 h at 30°C and 110 rpm in a shaker incubator. Subsequently, 100 µL of Salkowski reagent (50 mL, 35% $HClO_4$, 1 mL 0.5 M $FeCl_3$) (Ahmad *et al.,* 2008) were then added per well, and the microplates were each covered with aluminum foil and incubated for 30 min. For each fungal strain, three 7 mm diam. mycelial plugs were inoculated into 5 mL of PDB medium, and these cultures were incubated for 7 d at 30°C and 110 rpm in a shaker incubator. Two hundred µL of the culture were then placed in 96 well microplates, and 100 µL of Salkowski reagent were immediately added. Microplates were each covered with aluminum foil and incubated for 30 min. For the bacteria and fungi, change of colour to pink indicated a positive result.

Hydrogen cyanide production assays

Hydrogen cyanide (HCN) is produced by some BCAs, and its toxicity to phytopathogens makes the BCAs suitable for biocontrol. After inoculating the bacteria onto solid PY and the fungi onto PDA in Petri plates, a filter paper moistened with a solution of 0.5% sodium carbonate in 0.5% picric acid (Ahmad *et al.,* 2008) was fixed to each Petri plate cover. The plates were subsequently sealed with parafilm and incubated in dark at 30°C for 4 d. Development of orange-red colour indicated positive hydrogen cyanide producer strains.

Siderophore production assays

Siderophores are competitive traits used for BCAs to sequester iron, depriving pathogens of this element required for their growth and pathogenesis. To test for siderophore production, chrome azurol S agar (CAS) medium (Schwyn and Neilands, 1987) was prepared as described by Lynne *et al.* (2011). In the centre of each assay plate was inoculated 5 µL of bacterial culture or a 7 mm diam. mycelial plug of fungus. The Petri dishes were incubated in the dark for 96 h at 30°C. Colour changes from blue to yellow indicated siderophore producing strains.

Chitinase production assays

Chitin is an important component of the cell walls of fungi, and chitinolytic microorganisms are likely to act as biocontrol agents and pathogen antagonists. Chitinase determination basal medium (0.3 g L^{-1} $MgSO_4{\cdot}7H_2O$, 3 g L^{-1} NH_4 (SO_4), 2 g L^{-1} KH_2PO_4), 1 g L^{-1} citric acid, 15 g L^{-1} agar, 0.2 g L^{-1} Tween-80, pH 4.7) was supplemented with 4.5 g L^{-1} colloidal chitin and 0.15 g L^{-1} bromocresol purple (Agrawal and Kotasthane, 2012). After inoculation, the Petri dishes were incubated for 48 h at 30°C. Colour changes from yellow to purple indicated chitinase producer strains.

Molecular identification of selected isolates

Bacterial isolates were identified by sequencing of the 16S rRNA genes. Genomic DNA was purified using the Gentra Puregen kit (Qiagen). The 16S rRNA gene was amplified using the 27F and 1492R primers (Frank *et al.,* 2008). PCR reactions were each prepared in a final volume of 25 µL, containing 1 µL of genomic DNA (25 ng μL^{-1}), 2.5 µL of Taq Buffer 10×, 0.5 µL of dNTP mix (10 mM), 0.5 µL of primer 27F (10mM), 0.5 µL of primer 1492R (10mM), 0.2 µL of Taq DNA polymerase (5 units μL^{-1}) (Thermo Fisher), and 19.8 µL of ultrapure water to complete the volume. Amplifications were each carried out in a MiniAmp Plus Thermal Cycler (Thermofisher), under the following conditions: a 3 min initial denaturation step of 95°C, followed by 30 cycles of 95°C for 30 sec, 48°C for 30 sec, 72°C for 1 min, and a final cycle 72°C for 10 min.

Fungal isolates were identified by sequencing of the elongation factor *tef-1α* gene. Total genomic DNA was extracted from mycelia using cetyltrimethylammonium bromide (CTAB), as described by Wagner *et al.* (1987). The *tef-1α* gene was amplified using EF1and EF2 primers (O'Donnell *et al.,* 1998). PCR reactions were each prepared in a final volume of 25 µL, containing 1 µL of genomic DNA (25 ng μL^{-1}), 2.5 µL of Taq Buffer 10×, 0.5 µL of dNTP mix (10 mM), 0.62 µL of primer EF1 (10 mM), 0.62 µL of primer EF2 (10 mM), 0.125 µL of Taq DNA polymerase (5 units μL^{-1}) (Thermo Fisher) and 19.6 µL of ultrapure water to complete the volume. Amplification was carried out in a MJ Mini Gradient Thermal Cycler (BioRad) under the following conditions: a 3 min initial denaturation step of 95°C, followed by 35 cycles of 95°C for 1 min, 57°C for 1 min, 72°C for 1 min, and a final cycle 72°C for 10 min.

All obtained PCR products were verified by electrophoresis on 1% agarose gels, purified using the GenElute PCR Clean-Up Kit (Sigma-Aldrich), and sent to Eton Bioscience Inc. for sequencing.

The resulting sequences were aligned using MEGA XI (Kumar *et al.,* 2018), with the multiple alignment program MUSCLE. the bacteria sequences were blasted against the GenBank 16S Ribosomal RNA sequences database (Table 1), and the *Trichoderma* spp. sequences were compared with the GenBank elongation factor 1α gene sequences database (Table 2), and the clos-

est matches were used to construct each alignment. A Maximum-Parsimony method was used with Bootstrap values based on 1,000 replicates. New sequences were deposited in the GenBank (Tables 1 and 2).

Greenhouse biocontrol assays of Lasiodiplodia brasiliensis

Grapevine plants (*Vitis vinifera* 'Cabernet Sauvignon') obtained from 1-year-old cuttings were used to determine the biocontrol activity of selected bacterial and fungal strains and a rhizosphere strain rbES015 obtained in a previous study (Delgado-Ramírez *et al.*, 2021). Grapevine shoots were submerged in a 3 g L^{-1} solution of rooting agent ROOTEX (Cosmocel SA), and were then planted in tubs containing Cosmopeat substrate (Cosmocel SA). After 45 d, the plants were transplanted into 3.78 L plastic pots. Two weeks after transplanting, 50 mL of a solution (1×10^6 CFU) of each potential beneficial microorganism was applied at the base of the plant stem, followed by a second application 7 d later. Control treatments were inoculated with sterile water. For each tested isolate, ten replicates were used. Immediately after the second application of potential BCA, inoculations of the plants with *L. brasiliensis* were carried out through mechanical wounds in the woody tissues, each made with a drill bit (2 mm diam.), followed by insertion of a mycelium plug inside each hole. After inoculation, the wounds were each covered with parafilm. Plugs of sterile PDA were used as experimental control inoculations. The plants were then kept under greenhouse conditions for 60 d, and necrotic lesions generated in the stems were measured. Attempts were also made to recover the inoculated microorganisms.

Vineyard biocontrol assays of Lasiodiplodia brasiliensis

A field biocontrol trial was carried out in a 2-year-old 'Chenin Blanc' vineyard, in Ejido el Porvenir, Baja

Table 1. GenBank and culture accession numbers of bacterium species used in the present study for phylogenetic analyses.

Species	Isolate	Isolate source	Origin	GenBank accession number 16S rRNA
Bacillus amyloliquefaciens	NBRC 15535	Soil	Japan	NR_112685
B. amyloliquefaciens	W9	Marine water sample	India	MH188056
B. amyloliquefaciens	AB-525	Rice cake	China	KJ879953
B. amyloliquefaciens	BsA3MX	Strawberry rhizosphere	Mexico	MW651769
B. amyloliquefaciens	BsC11MX	Strawberry rhizosphere	Mexico	MW651770
B. amyloliquefaciens	**BEVP26BCMX**	**Grapevine**	**Mexico**	**OQ073757**
B. amyloliquefaciens	**BEVP31BCMX**	**Grapevine**	**Mexico**	**OQ073762**
B. axarquiensis	CIP 108772	River-mouth sediments	Spain	DQ993670
B. axarquiensis	**BEVP02BCMX**	**Grapevine**	**Mexico**	**OQ073758**
B. cereus	ATCC 14579	Unknown	Unknown	AE016877
B. circulans	IAMI 12462	Soil	Unknown	D78312
B. coagulans	NBRC 12583	Evaporated milk	Unknown	AB271752
B. licheniformis	ATCC 14580	Unknown	Unknown	CP000002
B. mojavensis	IFO 15718	Soil	USA	AB021191
B. mojavensis	**BEVP01BCMX**	**Grapevine**	**Mexico**	**OQ073759**
B. mycoides	ATCC 6462	Soil	Unknown	AB021192
B. siamensis	PD-A10	Poo-dong	Thailand	GQ281299
B. siamensis	RET2912	Landfill soil	India	MN530054
B. siamensis	LFS1715	Landfill soil	India	MN519261
B. subtilis	DSM10	Unknown	Unknown	AJ276351
B. subtilis subsp. *spizizenii*	NBRL B-23049	Tunisian desert	Tunisia	AF074970
B. thuringiensis	IAM 12077	Mediterranean flour moth	Unknown	D16281
B. vallismortis	DSM 11031	Soil	USA	AB021198
B. velezensis	CR-502	Brackish water	Spain	AY603658
Alicyclobacillus acidocaldarius	DSM 446	Acid hot spring	USA	AJ496806

Isolates from this study are highlighted in bold font.

Table 2. List of GenBank and culture accession numbers of fungal species used in the present study for phylogenetic analyses.

Species	Isolate	Isolate source	Origin	GenBank accession number tef-1α
Trichoderma asperellum	Th047	Soil	Colombia	AB568381.1
T. asperellum	cds	Not available	Brazil	KP696459.1
T. asperellum	ST1	Not available	Spain	KJ677260.1
T. asperellum	**T11BCMX**	**Carnation**	**Mexico**	**OQ161180**
T. asperellum	**T15BCMX**	**Grapevine**	**Mexico**	**OQ161181**
T. asperellum	**T20BCMX**	**Grapevine**	**Mexico**	**OQ161182**
T. asperellum	**EF09BCMX**	**Grapevine**	**Mexico**	**OQ161183**
T. asperellum	**EF11BCMX**	**Grapevine**	**Mexico**	**OQ161184**
Trichoderma atroviride	DAOM 238037	Not available	Thailand	KJ871093
T. atroviride	PARC1011	Not available	Italy	MT454114
Trichoderma guizhouense	DAOM 231412	Not available	Not available	AY605764
T. guizhouense	DAOM 231435	Not available	Not available	EF191321
T. guizhouense	PARC1022	Prunus persica	Italy	MT454125
Trichoderma harzianum	DAOM 233986	Not available	Not available	EF392749
T. harzianum	DAOM 242937	Not available	Not available	KX463434
T. harzianum	PARC1019	Prunus persica	Italy	MT454122
T. harzianum	**T06BCMX**	**Grapevine**	**Mexico**	**OQ161179**
Trichoderma koningiopsis	Arak-96	Soil	Iran	KP985652
T. koningiopsis	ITCC 7291	Soil	India	LN897322
T. koningiopsis	PARC1024	Prunus persica	Italy	MT454127
Trichoderma longibrachiatum	DAOM 234103	Not available	Not available	DQ125467
T. longibrachiatum	CIB T13	Not available	Colombia	EU280033
T. longibrachiatum	PARC1015	Not available	Italy	MT454118
T. longibrachiatum	**T17BCMX**	**Grapevine**	**Mexico**	**OQ161184**
Trichoderma paraviridescens	BMCC:LU786	Not available	New Zealand	KJ871271
T. paraviridescens	KX098484	Not available	New Zealand	KX098484
T. paraviridescens	PARC1016b	Not available	Italy	MT454119

Isolates from this study are highlighted in bold font.

California. Fifty plants, which did not show symptoms associated with wood diseases, were chosen per row on five vineyard rows, leaving an interval of three to five plants between each selected vine. The experimental design was completely randomized with ten grapevines per treatment. Putative BCAs evaluated included five bacteria (BEVP01BCMX, BEVP02BCMX, BEVP26BCMX, BEVP31BCMX, and rbES015) and six fungi (T06BCMX, T11BCMX, T15BCMX, T17BCMX, T20BCMX, and EF11BCMX). In each selected plant, a pruning cut was made in a woody branch, and 10 µL of a 1×10^6 CFU suspension of the selected biocontrol organism were inoculated, and 20 plants were treated with each isolate. Five days later, a second inoculation of the biocontrol agent was made in the same wound. One hour later, 10 µL of a $1x10^5$ suspension of fragmented mycelium of *L. brasiliensis* was applied to ten of the plants. Negative controls were inoculated only with sterile distilled water. The inoculated branches were each sealed with parafilm and then covered with a paper bag (Figure S1). One month later, the treated branches were cut, the length of the lesions produced by *L. brasiliensis* was measured, and a tissue fragment from each branch was inoculated onto PDA to assess if the pathogen and the inoculated BCA was present.

Statistical analyses

Data obtained from the greenhouse and vineyard biocontrol experiments were analyzed using one-way ANOVA, with post-hoc LSD analysis, and an $\alpha < 0.05$ test for statistical significance, using the STATISTICA 8.0 package.

RESULTS

Isolation, screening and molecular identification of microorganisms from heritage grapevines

A total of 135 isolates of bacteria from the heritage grapevine tissues were characterized by morphological characteristics as *Bacillus, Paenibacillus* and *Pseudomonas*. Isolates of fungi included two *Trichoderma* spp., and *Alternaria*, *Chaetomium, Sordaria* and *Diplodia* spp. Given the small number of potential beneficial fungal isolates recovered, 35 uncharacterized *Trichoderma* strains from our laboratory collection were included in this study.

From the 172 evaluated strains, 37 fungal and 21 bacterial isolates showed antagonistic activity in the qualitative antagonism assays (Figure 1). Quantitative dual culture assays and screening for plant growth promotion traits were performed only for those 58 isolates. Results showed that mean inhibition proportions for these BCAs against *L. brasiliensis* were between 3.4% to 52.8%, and that the isolates had different plant growth promotion characteristics (Table S1).

Based on inhibition proportions, and possession of at least one growth promoting trait, four bacteria (BEVP01BCMX, BEVP02BCMX, BEVP26BCMX, and BEVP31BCMX) and seven fungi (EF09BCMX, EF11BCMX, T06BCMX, T11BCMX, T15BCMX, T17BCMX, and T20BCMX) were selected. Molecular identification of these isolates confirmed that all the bacteria were *Bacillus* spp. (Figure S2), and all the fungi were

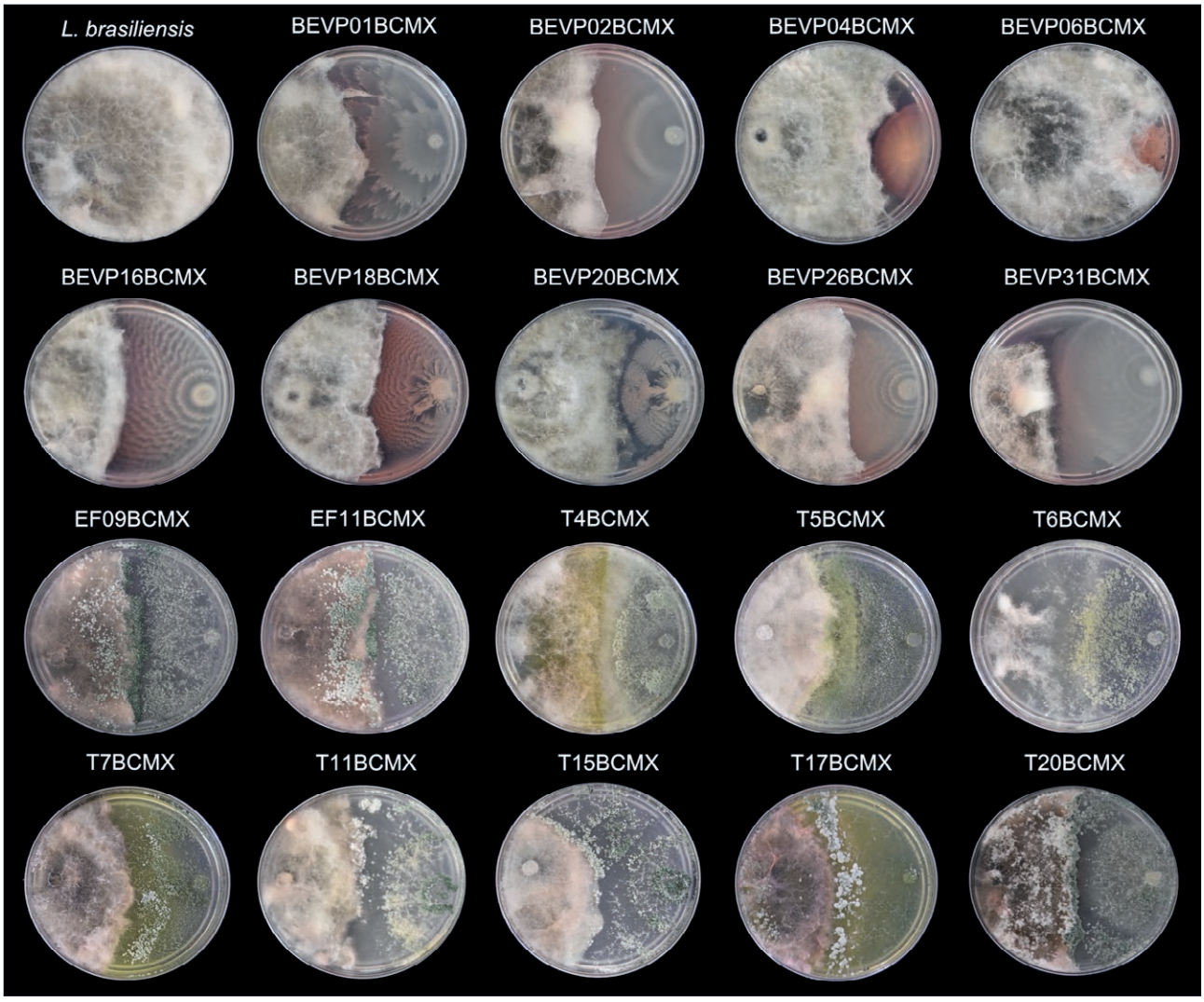

Figure 1. Representative images of dual culture assays of selected *Bacillus* and *Trichoderma* isolates against *Lasiodiplodia brasiliensis.*

Table 3. Mean percent inhibition of *Lasiodiplodia brasiliensis* by different potential biocontrol microorganisms, and their respective production of plant growth promotion compounds, for selected *Bacillus* and *Trichoderma* isolates.

Isolate	Mean inhibition %	Production				Solubilization		
		SID	CHI	HCN	IAA	P	K	ZN
B. mojavensis BEVP01BCMX	51.3	+	+	-	+	-	-	-
B. axarquiensis BEVP02BCMX	17.1	-	+	-	+	+	+	-
B. amyloliquefaciens BEVP26BCMX	38.0	+	+	-	+	+	-	-
B. amyloliquefaciens BEVP31BCMX	50.6	-	-	-	+	-	-	-
T. asperellum EF09BCMX	51.8	+	+	-	+	+	-	-
T. asperellum EF11BCMX	51.7	+	+	-	+	+	-	-
T. harzianum T06BCMX	41.0	+	+	-	+	-	-	-
T. asperellum T11BCMX	29.1	+	+	-	+	-	-	-
T. asperellum T15BCMX	25.1	+	+	-	+	-	-	-
T. longibrachiatum T17BCMX	52.8	+	+	-	+	-	-	-
T. asperellum T20BCMX	39.3	+	+	-	-	-	-	-

+ positive result, - negative result. SID, siderophore production; CHI, chitinase production; HCN, HCN production; IAA, indole acetic acid production; P, phosphate solubilization; K, potassium solubilization; Zn, zinc solubilization.

Trichoderma spp. (Figure S3). Mean inhibition proportions ranged from 17.1% to 51.8%. Almost all the selected isolates (except *T. asperellum* T20BCMX) produced AIA, and (except *B. amyloliquefaciens* BEVP31BCMX) produced chitinase. Most of the isolates (except *B. axarquiensis* BEVP02BCMX and *B. amyloliquefaciens* BEVP31BCMX) produce siderophores. Four isolates (*B. axarquiensis* BEVP02BCMX, *B. amyloliquefaciens* BEVP26BCMX, *T. asperellum* EF09BCMX and *T. asperellum* EF11BCMX) solubilized phosphate, and one isolate (*B. axarquiensis* BEVP02BCMX) solubilized potassium. None of the isolates solubilized zinc, or produced HCN (Table 3).

Evaluation of antifungal effect of volatile and diffusible organic compounds

The eleven isolates were further screened for the antifungal activity from diffusible and volatile organic compounds. None of the assessed *Trichoderma* or *Bacillus* isolates produced volatile organic compounds with suppressive effects on *L. brasiliensis* (Figure 2; Table 4). However, all the *Bacillus* isolates affected growth of *N. parvum*, with mean inhibition percentages ranging from 22.6% to 34.0%. Isolate BEVP31BCMX gave the greatest inhibition (Figure 2; Table 4). In contrast, the 11 isolates affected the growth of both pathogenic fungi by the production of diffusible organic compounds. The *Bacillus* isolates gave mean inhibition percentages from 40.4% to 62.1% against *L. brasiliensis*, and from 66% to 78% against *N. parvum,* while the *Trichoderma* strains gave 61.2% to 81.1% inhibition of *L. brasiliensis* and close to 100% inhibition of *N. parvum* (Figure 3; Table 4). While *T. harzianum* T06BCMX did not affect radial colony growth of either of the pathogenic fungi, this isolate caused a significant decrease in aerial mycelium (Figure 3).

Characterization of mycoparasitic activity of Trichoderma *strains*

The pre-colonized plate experiments showed that all the assessed *Trichoderma* isolates had vigorous mycoparasitic activity, with colonization percentages ranging from 70% to 100% (Table 5). When the colonization percentage was 100%, the inoculated phytopathogenic fungus could not be recovered, indicating total suppression. Microscope observations from dual culture assays indicated that all the *Trichoderma* isolates coiled around, and cause morphological deformations, of *L. brasiliensis* hyphae, while the isolates *T. asperellum* T15BCMX and *T. longibrachiatum* T17BCMX also induced lysis of mycelium walls (Table 5; Figure 4).

Evaluation of biocontrol activity of selected bacterial and fungal isolates in greenhouse trials

The preventative application to soil of the *Trichoderma* and *Bacillus* isolates, for suppression of *L. brasiliensis* infection revealed the following. While the untreated

Table 4. Mean percent inhibition of *Lasiodiplodia brasiliensis* and *Neofusicoccum parvum* from volatile organic compounds and diffusible organic compounds by different *Bacillus* and *Trichoderma* isolates.

Strain	Mean inhibition (%) from volatile organic compounds (%)		Mean inhibition (%) from diffusible organic compounds	
	L. brasiliensis	*N. parvum*	*L. brasiliensis*	*N. parvum*
Bacillus mojavensis BEVP01BCMX	0	31.2	62.1	78.2
B. axarquiensis BEVP02BCMX	0	23.6	31.6	76.4
B. amyloliquefaciens BEVP26BCMX	0	26.2	40.4	73.8
B. amyloliquefaciens BEVP31BCMX	0	34.0	49.3	66.0
Trichoderma asperellum EF09BCMX	0	0	63.5	100
T. asperellum EF11BCMX	0	0	61.2	98
T. harzianum T06BCMX	0	0	0	0
T. asperellum T11BCMX	0	0	67.1	100
T. asperellum T15BCMX	0	0	81.1	100
T. longibrachiatum T17BCMX	0	0	66.8	98
T. asperellum T20BCMX	0	0	63.5	100

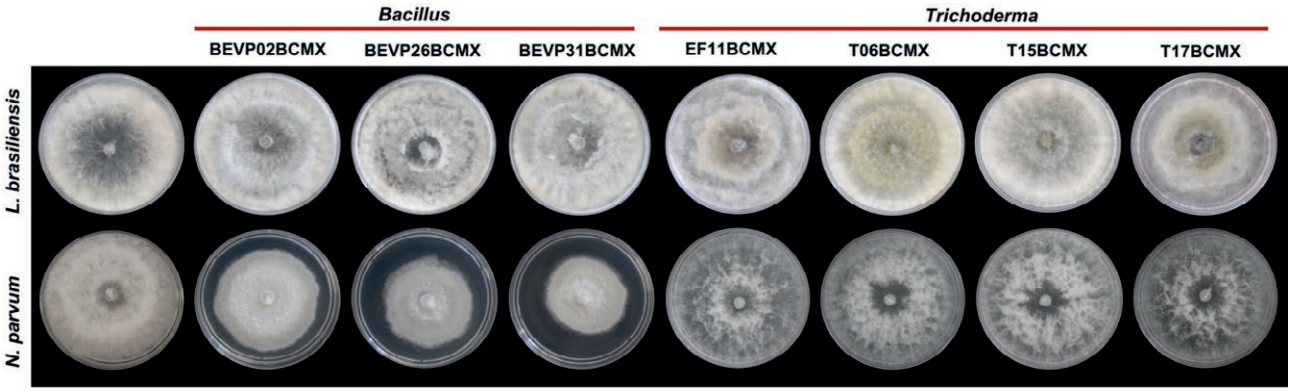

Figure 2. Representative images of the antifungal effects of volatile organic compounds produced by *Bacillus* and *Trichoderma* isolates against *Lasiodiplodia brasiliensis* and *Neofusioccum parvum.*

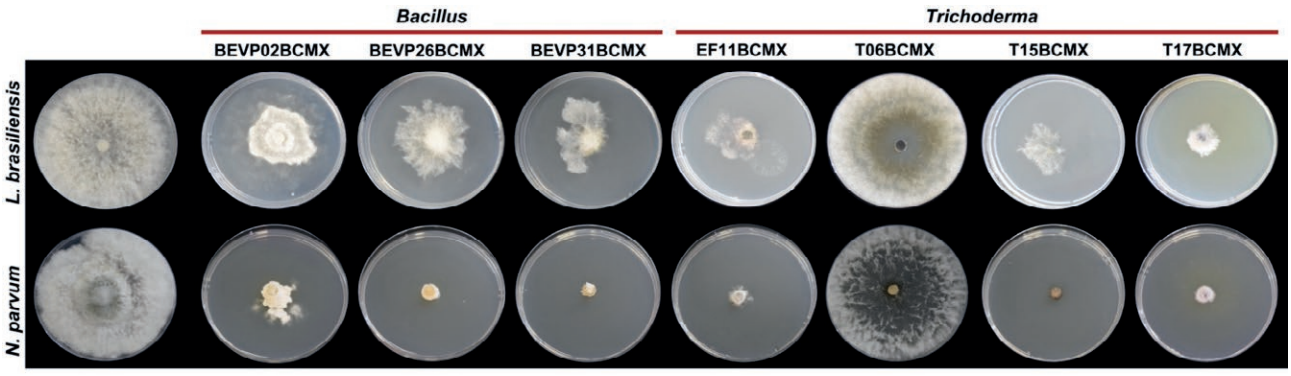

Figure 3. Representative images of the antifungal effect of diffusible organic compounds produced by *Bacillus* and *Trichoderma* isolates against *Lasiodiplodia brasili*ensis and *Neofusicoccum parvum*.

Table 5. Mean colonization percentages from pre-colonized plate assays, and microscope observations, indicating mycoparasitism activity of six *Trichoderma* isolates against *Lasiodiplodia brasiliensis*.

Isolate	Mean colonization percentage	Type of effect of mycoparasitism		
		Coiling	Hyphae deformation	Cell lysis
T. asperellum EF09CMX	100	+	+	-
T. asperellum EF11BCMX	70	+	+	-
T. harzianum T06BCMX	80	+	+	-
T. asperellum T11BCMX	90	+	+	-
T. asperellum T15BCMX	100	+	+	+
T. longibrachiatum T17BCMX	90	+	+	+
T. asperellum T20BCMX	86	+	+	-

+ positive result, - negative result.

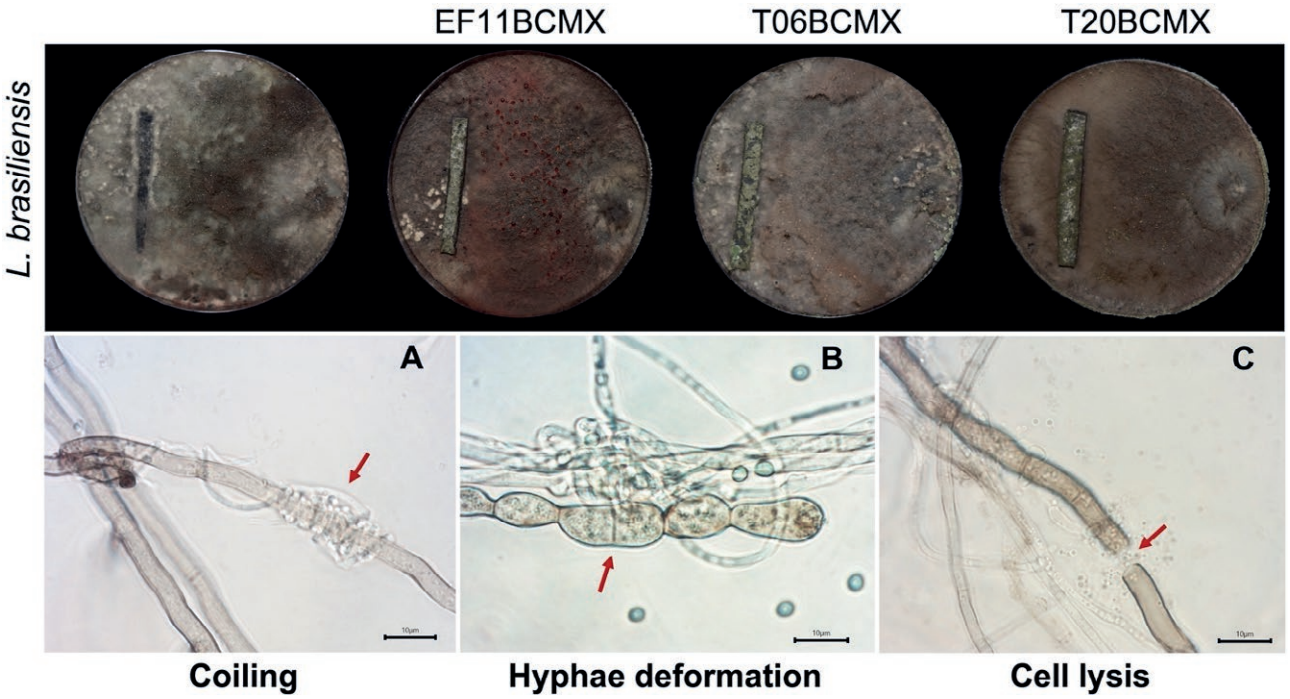

Figure 4. Representative microscope images (captured after 28 d incubation at 30ºC) from pre-colonization assays of *Trichoderma* isolates against *Lasiodiplodia brasiliensis* showing mycoparasitism activity of the *Trichoderma* isolates (red arrows indicate effects caused by *Trichoderma*). A, Hyphal coiling of *T. asperellum* EF11BCMX against *L. brasiliensis*. B, *T. harzianum* T06BCMX causing deformation in *L. brasiliensis* hyphae. C, Lysis of the hyphal wall of *L. brasiliensis* induced by *T. longibrachiatum* T17BCMX.

grapevine plants inoculated with *L. brasiliensis* developed wounds of mean length up to 10.0 cm, the plants treated with three isolates of *T. asperellum* (T20BCMX, EF09BCMX, and EF11BCMX), *B. amyloliquefaciens* BEVP26BCMX and *Bacillus* sp. rbES015 showed significantly shorter necrotic lesions (Figures 5 and 6). Plants inoculated with *L. brasiliensis* and treated with *T. harzianum* T6BCMX developed larger lesions than plants inoculated only with *L. brasiliensis* (Figures 5 and 6). This isolate was the only *Trichoderma* isolate that gave no effect in the diffusible compounds assays (Figure 3). In the greenhouse tests, all the *Bacillus* and *Trichoderma* isolates were recovered from the root tissues of the inoculated plants, and *L. brasiliensis* was re-isolated from the stems of the plants.

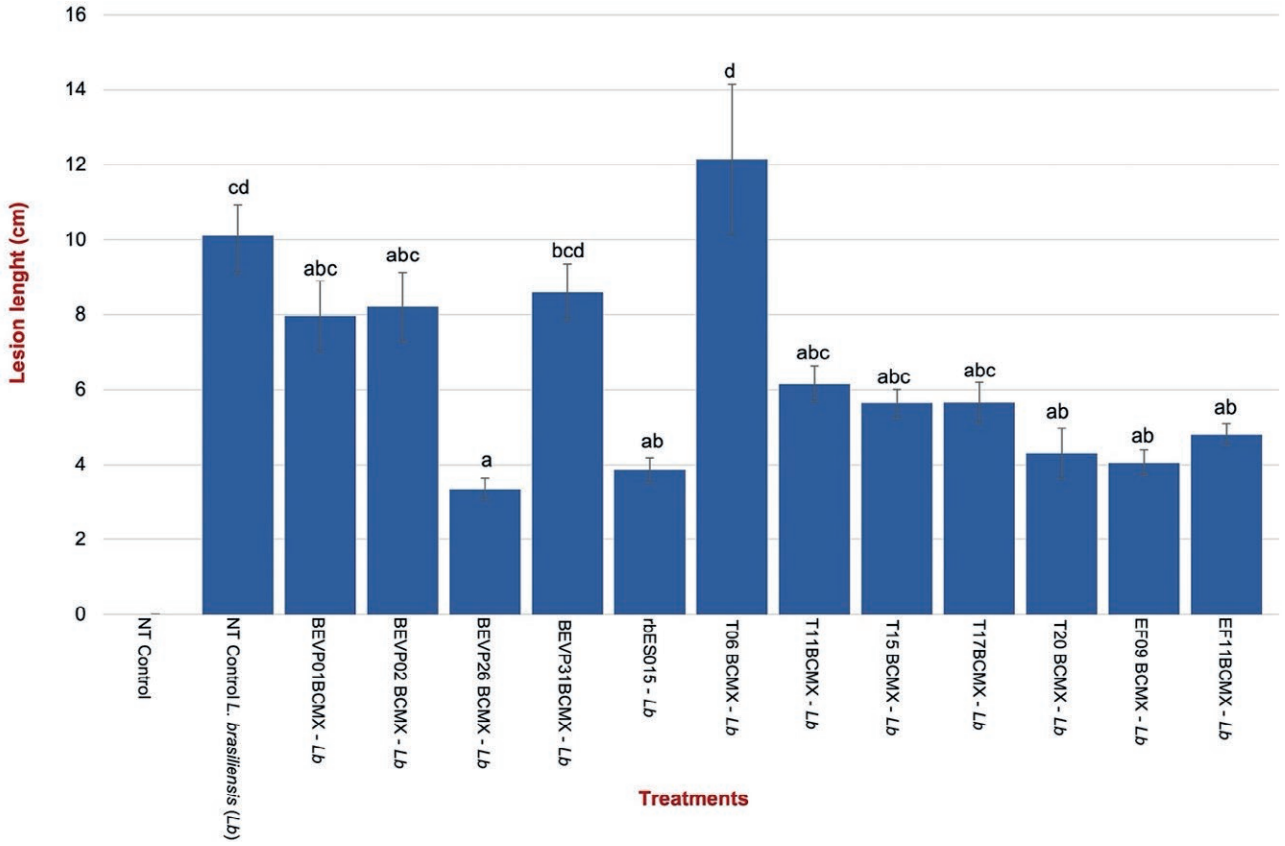

Figure 5. Mean internal necrotic lesion lengths caused by *Lasiodiplodia brasiliensis (Lb)* in grapevine plants, after treatments with different *Bacillus* (BEVP02BCMX, BEVP26BCMX and BEVP31BCMX) or *Trichoderma* isolates (T11BCMX, T15BCMX, T17BCMX, T20BCMX, and EF11BCMX). NT**,** Non-treated Control. Each bar represents the mean for ten (± SE). Different letters indicate differences ($P < 0.05$), according to LSD tests after ANOVA.

Figure 6. Images of grapevine stems after preventive soil inoculations with *Bacillus* or *Trichoderma* isolates and *Lasiodiplodia brasiliensis*. The yellow lines indicate the lengths of necrotic lesions caused by *L. brasiliensis* in 'Cabernet Sauvignon' stems.

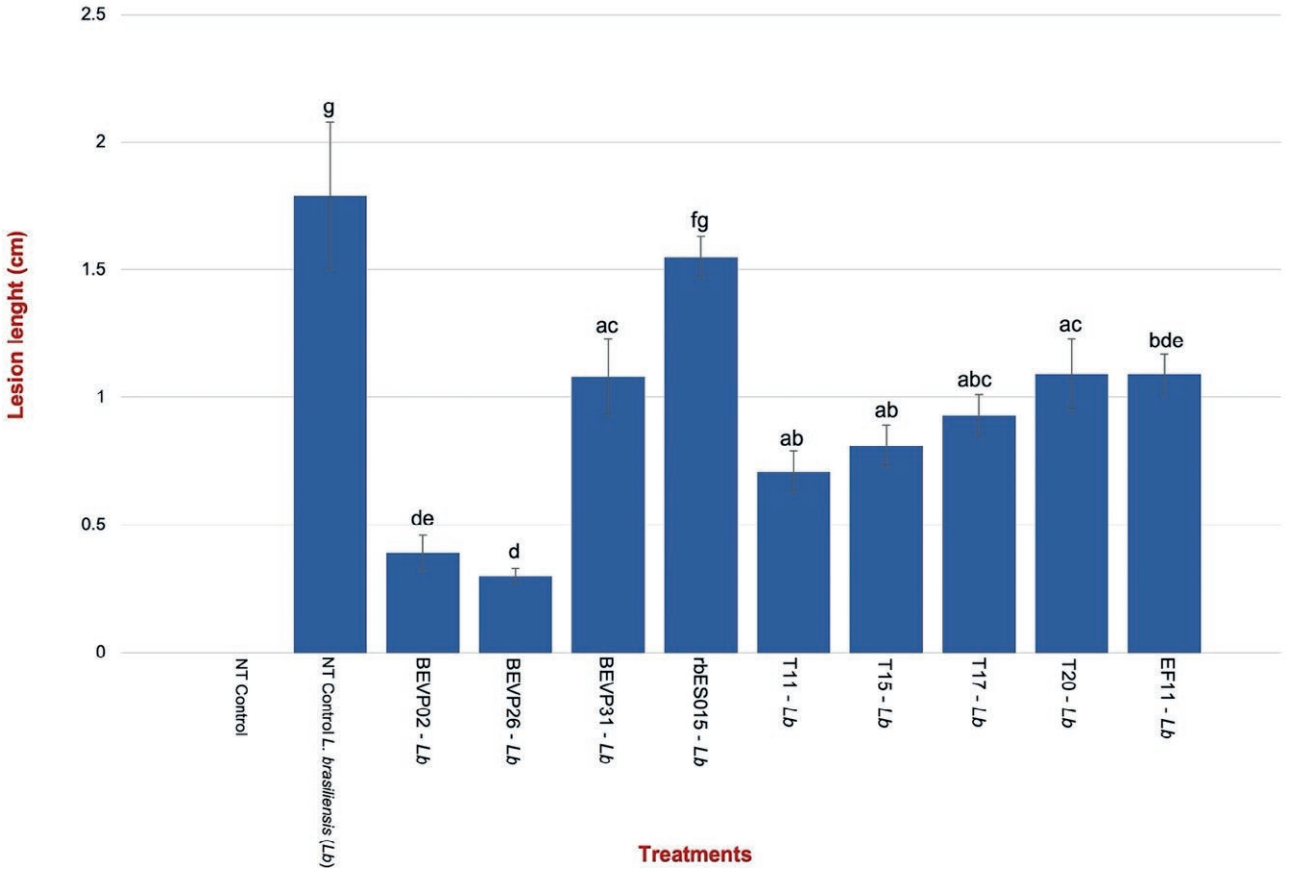

Figure 7. Mean internal necrotic lesion lengths in grapevines, caused by *Lasiodiplodia brasiliensis* after treatments with isolates of *Bacillus* (BEVP02BCMX, BEVP26BCMX, and BEVP31BCMX) or *Trichoderma* (T11BCMX, T15BCMX, T17BCMX, T20BCMX, and EF11BCMX). NT, Non-treated control. Each bar represents the mean for ten plants (± SE). Different letters over bars indicate differences ($P < 0.05$), according to LSD tests after ANOVA.

Evaluation of biocontrol activity of selected isolates under vineyard conditions

In the field assessments, grapevine branches preventively treated with most of the evaluated *Bacillus* and *Trichoderma* isolates showed two to five times shorter lesions than the untreated branches inoculated only with *L. brasiliensis* (Figure 7). Between the *Trichoderma* isolates no statistically significant differences were detected, while among *Bacillus* isolates, *B. axarquiensis* BEVP02BCMX and *B. amyloliquefacines* BEVP26BCMX gave significant differences from *B. amyloliquefacines* BEVP31BCMX. Isolate *B. amyloliquefaciens* BEVP26BCMX gave the strongest effect, with a 5-fold reduction on the length of the necrotic lesions (Figures 7 and 8). All the beneficial organisms were re-isolated from the respective treated branches, while *L. brasiliensis* was only re-isolated from the experimental controls and the *Bacillus*-treated, vines but not from the *Trichoderma*-treated branches.

DISCUSSION

This study has identified endophytic microorganisms associated with heritage grapevines that biocontrol *L. brasiliensis and N. parvum*, which are two of the most virulent fungi associated with Botryosphaeria dieback (Gramaje *et al.,* 2018; Rangel-Montoya *et al.,* 2021). The isolates used in this research were originally obtained from GTD symptomatic grapevines growing in the Guadalupe Valley in Baja California, Mexico (Rangel-Montoya *et al.,* 2021). Considering the conditions in which heritage vines grow in this region, with no irrigation and with little cultural management, the study has identified beneficial microorganisms that could be useful in commercial vineyards. This is the first study focusing on biological control potential of microorganisms associated with heritage grapevines.

Under *in vitro* conditions, several *Bacillus* isolates obtained from heritage grapevines inhibited *L. brasilien-*

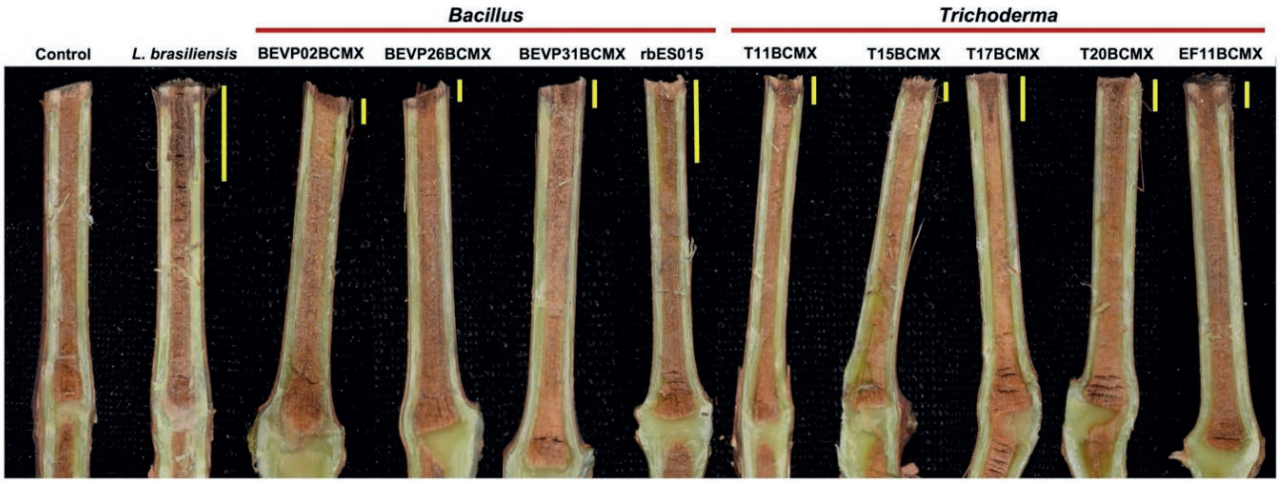

Figure 8. Images field-grown grapevine stems after treatments of pruning wounds with different *Bacillus* or *Trichoderma* isolates against *Lasiodiplodia brasiliensis* infections. The images are of ten replicates representative plants, taken of 30 d after *L. brasiliensis* inoculations.

sis and *N. parvum*, and showed additional characteristics associated with plant growth promotion, including production of siderophores and indole acetic acid, and solubilization of phosphate and potassium. In dual culture assays five *Bacillus* strains inhibited growth of *L. brasiliensis* by up to 51%, showing that these assays were useful for the initial screening and selection of strains with antagonistic activity. This method was previously shown to be useful for identifying two *Bacillus* strains with antagonistic effects against *Diaporthe ampelina, Diplodia seriata, Eutypa lata* and *N. parvum* (Blundell *et al.,* 2021), and two strains of *Bacillus velezensis* against eight different fungi, including *L. theobromae, D. seriata* and *N. parvum* (Bustamante *et al.,* 2022).

While *Bacillus* spp. inhibited the growth of *N. parvum* by up to 34% through the production of organic volatile compounds, *L. brasiliensis* was not affected. However, a decrease in formation of aerial mycelium was observed, indicating a slight antagonistic effect on this fungus. However, diffusible compounds had strong antifungal effects against both pathogenic fungi, although these were less against *L. brasiliensis* (from 40% to 62%), indicating that diffusible compounds were the main antagonistic mechanisms of these *Bacillus* isolates. The production of volatile antifungal compounds, such as ketones, alcohols, esters, pyrazine, acids, hydrocarbons, heterocycles, aldehydes, phenols, thioalcohols, and thioesters, has been reported for *Bacillus* spp. For example, *B. amyloliquefaciens* CPA-8 produced 1,3 pentadiene, acetoin (3-hydroxy-2 butanone), and thiophene, that reduced *in vitro* mycelial growth of *Monilina laxa, M. fructicola,* and *Botrytis cinerea* (Gotor-Vila *et al.,* 2017). Diffusible compounds with antifungal activity have also been identified from *Bacillus* spp., including iturin, fengycin, macrolactin, surfactin. *Bacillus* INECOL-6004, INECOL-6005, and INECOL-6006 showed antagonistic activity against *Fusarium kuroshium* by the production of iturin, fengycin, and surfactin (Guevara-Avedaño *et al*., 2020). Identifying the metabolites produced by the *Bacillus* isolates obtained in the present study, and their role in disease suppression, would be worthwhile.

All of the seven selected *Trichoderma* strains showed *in vitro* antagonistic activity. Additionally, they all produced siderophores, chitinase, and indole acetic acid. Several previous studies have identified *Trichoderma* spp. with activity against GTD pathogens. Úrbez-Torres *et al.,* (2020) evaluated the antagonistic activities of sixteen *Trichoderma* strains against *D. seriata, E. lata* and *N. parvum,* with *T. atroviride* PARC1018 giving the greatest inhibition of *D. seriata* and *E. lata,* and *T. koningiopsis* against *N. parvum*. Similarly, Blundell *et al.,* (2021) reported that *T. asperellum* UC8360 inhibited *D. seriata, E. lata, N. parvum and D. ampelina,* with *D. ampelina* being the most inhibitory, while a *T. harzianum* isolate gave high rates of inhibition of *N. parvum* (Langa-Lomba *et al.* 2022). In the present study, three strains showed inhibition proportions greater that 50%. Two of these (*T. asperellum* EF09BCMX and *T. asperellum* EF011BCMX) were isolated from heritage grapevines, indicating that the heritage grapevine cv. 'Mission' contains beneficial microorganisms that can be used as BCAs with additional benefit of plant growth promotion. Different *Trichoderma* strains inhibit growth of phytopathogenic fungi through the production of volatile compounds (Zhao *et al.,* 2022), In the present study, however, the selected strains did not inhibit the mycelial

growth of *L. brasiliensis* or *N. parvum* by the production of volatile compounds, although decreases in the formation of aerial mycelium were observed.

Mycoparasitism is considered to be an important biocontrol mechanism of *Trichoderma* (Sood *et al.,* 2020). However, previous studies showed that this mode of action is not always present. For example, from 50 *Trichoderma* isolates evaluated against *Moniliophthora roreri,* mycoparasitism varied between 0% and 100%, with only nine isolates reaching 100% (Reyes-Figueroa *et al.,* 2016). Leiva *et al.* (2020) also found that for 199 *Trichoderma* isolates, mycoparasitism rates varied from 32% to 100%. Isolates with this capacity parasitize and colonize phytopathogens, reducing the fungal inoculum and alleviating the intensity of the diseases they cause (Nusaibah and Musa, 2019, Mukherjee *et al.,* 2022). The pre-colonized plate assays of the present study showed a colonization rates of *L. brasiliensis* from 70% to 100%. When *T. asperellum* EF09BCMX and *T. asperellum* T15BCMX were evaluated, *L. brasiliensis* was not recovered from the plates, indicating total elimination of the pathogen, as expected by the 100% colonization obtained. In contrast, the microscopical observations showed that hyphae from seven *Trichoderma* isolates coiled around hyphae of *L. brasiliensis*. This ability has been extensively reported. For example, *T. asperellum* UDEAGIEM-H01 formed coils around hyphae of *F. oxysporum* and *Macrophomina phaseolina* (Díaz-Gutiérrez *et al.,* 2021), *T. harzianum* KMISO2-2-19A around *Fusarium virguliforme* hyphae (Pimentel *et al.,* 2020), and *T. koningiopsis* around hyphe of *Phytophthora xcambivora* (Frascella *et al.,* 2022). Coiling around hyphae is the first step of *Trichoderma* mycoparasitic activity, and is followed by production of hydrolytic enzymes that allow *Trichoderma* to penetrate the hosts and absorb their contents (Rocha-Ramirez et al., 2002). *Trichoderma* isolates produce extracellular cell wall degrading enzymes such as endochitinases, b-1,3- glucanases, and proteases, that lyse pathogen mycelium (Harman *et al.,* 2004; Druzhinina *et al.,* 2011). Although the production of cell wall degrading enzymes was not evaluated in the present study, *T. asperellum* T15BCMX and *T. longibrachiatum* T17BCMX caused deformation and the cell wall lysis of *L. brasiliensis* hyphae, indicating production of enzymes that damaged cell walls, and potential as BCAs.

Although the *in vitro* tests provided information on the antagonistic potential of the evaluated strains, low inhibition proportions may not indicate that isolates will perform poorly when applied as biocontrol agents under field conditions. Effectiveness of biocontrol in the field often depends on capacity to colonize plant tissues, establish compatible interactions, prevail in the hosts, and tolerate abiotic factors (Finkel *et al.,* 2017; Afzal *et al.,* 2019). For example, isolate *B. axarquiensis* BEVP02BCMX showed low inhibition in dual culture assays against *L. brasiliensis*, but when it was applied as a preventive pruning protectant, it reduced the size of the necrotic lesions. Effectiveness of this strain could be related to its ability to colonize grapevine tissues, which is possibly expected considering its endophytic nature. However, beneficial microorganisms do not always reduce damage caused by pathogens, as was observed here with *T. harzianum* T06BCMX, since plants showed longer necrotic lesions compared to those inoculated only with *L. brasiliensis*. This effect has been observed previously. Leal *et al.* (2021) reported that plants inoculated with *T. atroviride* SC1 and *B. subtilis* PTA-271, applied in soil against *N. parvum*, developed longer necrotic lesions than experimental controls. This is a good reason why potential biological control agents should be thoroughly assessed.

In plants, beneficial microorganisms have been evaluated using different methods, including preventative applications in substrates or directly applied to pruning wounds (Haidar *et al.,* 2016). In the present study, selected isolates were first evaluated by direct application to soil, and then in a vineyard by applying them to pruning wounds. In the greenhouse assay, two *Bacillus* isolates (*Bacillus* sp. rbES015 and *B. amyloliquefaciens* BEVP26BCMX) and three *T. asperellum* isolates (EF09BCMX, EF11BCMX, and T20BCMX) reduced necrotic lesion lengths caused by *L. brasiliensis*. Since the beneficial microorganisms were applied in soil without direct contact with the pathogen, the observed effect could be due to activation of host systemic response. Previous studies have indicated that non-pathogenic bacteria and fungi have capabilities to reduce damage caused by pathogens through activation of induced host systemic resistance. Haidar *et al.* (2016) and Zehra *et al.* (2021) identified different bacteria isolates with biocontrol activity against *P. chlamydospora*, when applied preventatively as drenches. Similarly, Haidar *et al.* (2021) identified different bacteria isolates that reduced necrotic lesions caused by *N. parvum* in grapevines when inoculated in soil. This opens the possibility for applying selected biocontrol isolates during irrigation or as drenches, diminishing the costs of biocontrol applications.

Pathogens causing GTDs enter grapevines mainly through pruning wounds (Gramaje *et al.*, 2018), so control strategies should focus on wound protection. Few studies have been carried out in field conditions, and they generally used commercial formulations and specific strains. Martínez-Diz *et al.* (2020) evaluated the *Trichoderma* isolates SC1 and 1-1237 against *D. seriata*

and *P. chlamydospora*, observing low efficacy. The low effectiveness of commercial formulations based on biocontrol agents is common, and has been mainly attributed to microorganism failure to colonize plant tissues (Mutawila *et al.,* 2016). In the present study, eight isolates applied directly to grapevine wounds reduced necrotic lesions caused by *L. brasiliensis,* and only *Bacillus* sp. rbES015 failed. This isolate was obtained from soil so may be incapable of colonizing grapevine tissues. The microorganisms obtained in the present study showed strong biocontrol activity, even though they were applied without the addition of carriers and protectants. Although comprehensive evaluation needs to be carried out, it may be possible to transfer the selected strains to a company or association to develop a formulation based on the strains to improve its usability and stability for grape producers.

In this research, a strain of *T. longibrachiatum* was shown to be a good candidate as a biological control agent. However, this species has been reported as an opportunistic human pathogen of immunosuppressed patients (Myoken *et al.,* 2002; Lipový *et al.,* 2021; Vasiliki *et al., 2021*). Therefore, the use of *T. longibrachiatum* T17BCMX as biological control agent should be restricted, though it showed excellent antagonistic activity in assays carried out under *in vitro* and *in planta* conditions. Most of the other identified *Trichoderma* strains were *T. asperellum*, which is widely used as a biological control agent, without reported human risks.

The antagonistic activity of the *Bacillus* isolates was mainly due to production of diffusible compounds, while in the *Trichoderma* spp. it resulted from production of diffusible compounds and the mycoparasitism. However, volatile compounds may also have contributed to the biological control activity of the isolates, since all the selected strains produced these compounds that inhibited *Neofusicoccum in vitro*.

In the present study strains with antagonistic activity were tested separately. Combination of isolates of *Bacillus* and *Trichoderma* have been previously shown to be successful. For example, *B. subtilis* PTA-271 and *T. atroviride* SC1 were evaluated individually and together against *N. parvum* Bt67 in two grapevine varieties ('Tempranillo' and 'Chardonnay'). 'Tempranillo' plants inoculated with either *T. atroviride* SC1 or the consortium had fewer internal lesions caused by *N. parvum* (Leal *et al.,* 2021). In the future, compatibility among the isolates identified in the present study could be determined for the development of a consortium that takes advantage of the strengths of different isolates.

In conclusion, this study has identified *Bacillus* and *Trichoderma* isolates with biocontrol activity against *L. brasiliensis* when applied preventatively to soil and to the pruning wounds. Therefore, heritage grapevines of Baja California have been shown to be a reservoir of beneficial microorganisms, which can be potentially utilized in commercial grapevine varieties to help reduce damage caused by grapevine trunk disease fungi.

ACKNOWLEDGMENTS

Delgado-Ramírez C.S received a scholarship from Consejo Nacional de Ciencia y Tecnología (México). The authors thank the OIV for funding this research, through the OIV research grant 2022 program. Special thanks are given to the students of the Phytopathology Laboratory for helping in the establishment of the different assays of this study, to Dr Frédérique Reverchon for her valuable comments and to Consejo Mexicano vitivínicola and the grapevine growers of Baja California for their support.

LITERATURE CITED

Abdullah H.A.S., Mohanad K.M.A., Muhammed A.H.A.B., Zulfiqar A., 2012. First report of grapevine dieback caused by *Lasiodiplodia theobromae* and *Neoscytalidium dimidiatum in* Basrah, Southern Iraq. *African Journal of Biotechnology* 11(95): 16165–16171. DOI: 10.5897/AJB12.010

Afzal I., Shinwari Z.K., Sikandar S., Shahzad S., 2019. Plant beneficial endophytic bacteria: mechanisms, diversity, host range and genetic determinants. *Microbiological Research* 221: 36–49. DOI: 10.1016/j.micres.2019.02.001

Agrawal T., Kotasthane, A.S.K., 2012. Chitinolytic assay of indigenous *Trichoderma* isolates collected from different geographical locations of Chhattisgarh in central India. *Springer Plus* 1(73): 1–10. DOI: 10.1186/2193-1801-1-73

Ahmad F., Ahmad I., Khan M.S., 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research* 163: 173–181. DOI: 1016/j.micres,2006.04.001

Almeida A.B., de Concas J., Campos M.D., Materatski P., Varanda, C., … Félix M.D.R., 2020. Endophytic fungi as potential biological control agents against grapevine trunk diseases in Alentejo region. *Biology* 9(420): 1–23. DOI: 10.3390/biology9120420

Andrade S.G., Arón N., Flores F., 2013. Matriz de insumo producto vitivinícola de Baja California México. *Revista de Economía* 30(81): 57–57.

Bailey B.A., Bae H., Strem M.D., Crozier J., Thomas S.E., … Holmes K.A., 2008. Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. *Biological Control* 46(1): 24–35. DOI: 10.1016/j.biocontrol.2008.01.003

Bapiri A., Asgharzadeh A., Mujallali H., Khavazi K., Pazira E., Graduated P.D., 2012. Evaluation of zinc solubilization potential by different strains of fluorescent Pseudomonads. *Journal of Applied Sciences and Environmental Management*. 16(3): 295–298.

Bertsch C., Ramirez-Suero M., Magnin-Robert M., Larignon, P., Chong, J., … Fontaine, F., 2013. Grapevine trunk diseases: Complex and still poorly understood. *Plant Pathology* 62: 243–265. DOI: 10.1111/j.1365-3059.2012.02674.x

Billones-Baaijens R., Savocchia S., 2019. A review of Botryosphaeriaceae species associated with grapevine trunk diseases in Australia and New Zealand. *Australasian Plant Patho*logy 48: 3–18. DOI: 10.1007/s13313-018-0585-5

Blundell R., Arreguin M., Eskalen A., 2021. *In vitro* evaluation of grapevine endophytes, epiphytes and sap microorganisms for potential use to control grapevine trunk disease pathogens. *Phytopathologia Mediterranea* 60(3): 535–548. DOI: 10.36253/phyto-12500

Bustamante M.I., Elfar K., Eskalen A., 2022. Evaluation of the antifungal activity of endophytic and rhizospheric bacteria against grapevine trunk pathogens. *Microorganisms* 10(10): 2035. DOI: 10.3390/microorganisms10102035

Crowley W.K., 1989. The Growth of the Mexican wine industry. *Yearbook of the Association of Pacific Coast Geographers* 51: 25–47.

Datta C., Basu P.S., 2000. Indole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub, *Cajanus cajan*. *Microbiological Research* 155: 123–127. DOI: 10.1016/S0944-5013(00)80047-6

Delgado-Ramírez C.S., Hernández-Martínez R., Sepúlveda E., 2021. Rhizobacteria Associated with a Native Solanaceae Promote Plant Growth and Decrease the Effects of *Fusarium oxysporum* in Tomato. *Agronomy* 11(3): 579. DOI: 10.3390/agronomy11030579

Díaz-Gutiérrez C., Arroyave C., Llugany M., Poschenrieder C., Martos S., Peláez C. 2021. *Trichoderma asperellum* as a preventive and curative agent to control Fusarium wilt in *Stevia rebaudiana*. *Biological Control* 155: 104537. DOI: 10.1016/j.biocontrol.2021.104537

Dini-Andreote F., 2020. Endophytes: The second layer of plant defense. *Trends in Plant Science* 25(4): 319–322. DOI: 10.1016/j.tplants.2020.01.007

Druzhinina I., Seidl-Seiboth V., Herrera-Estrella A., Horwitz B.A., Kenerley C.M., … Kubicek C.P., 2011. *Trichoderma*: the genomics of opportunistic success. *Nature Reviews Microbiology* 9: 749–759. DOI: 10.1038/nrmicro2637

Finkel O.M., Castrillo G., Herrera-Paredes S., Salas González I., Dangl J.L., 2017. Understanding and exploiting plant beneficial microbes. *Current Opinion in Plant Biology* 38: 155–163. DOI: 10.1016/j.pbi.2017.04.018

Frank J.A., Reich, C.I., Sharma S., Weisbaum J.S., Wilson B.A., Olsen G.J., 2008. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Applied and Environmental Microbiology* 74(8): 2461–2470. DOI: 10.1128/AEM.02272-07.

Frascella A., Sarrocco S., Mello A., Venice F., Salvatici C., … Della Rocca G., 2022. Biocontrol of *Phytophthora xcambivora* on *Castanea sativa*: Selection of local *Trichoderma* spp. isolates for the management of Ink disease. *Forests* 13: 1065. DOI: 10.3390/fl3071065

Gotor-Vila A., Teixidó N., Di Francesco A., Usall J., Ugolini L., … Mari M., 2017. Antifungal effect of volatile organic compounds produced by *Bacillus amyloliquefaciens* CPA-8 against fruit pathogen decays of cherry. *Food Microbiology* 64: 219–225. DOI: 10.1016/j.fm.2017.01.006

Gramaje D., Úrbez-Torres J.R., Sosnowski M.R., 2018. Managing grapevine trunk diseases with respect to etiology and epidemiology: Current strategies and prospects. *Plant Disease* 102: 12–39. DOI: 10.1094/PDIS-04-17-0512-FE

Guevara-Avendaño E., Carrillo J.D., Ndinga-Muniania C., Moreno K., Méndez-Bravo A., … Reverchon, F., 2018. Antifungal activity of avocado rhizobacteria against *Fusarium euwallaceae* and *Graphium* spp., associated with *Euwallacea* spp. nr. *fornicatus*, and *Phytophthora cinnamomi*. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology* 111(4): 563–572. DOI: 10.1007/s10482-017-0977-5.

Guevara-Avendaño E., Bravo-Castillo K.R., Monribot-Villanueva J.L., Kiel-Martínez A.L., Ramírez-Vázquez M., … Reverchon F., 2020. Diffusible and volatile organic compounds produced by avocado rhizobacteria exhibit antifungal effects against *Fusarium kuroshium*. *Brazilian Journal of Microbiology* 51(3): 861–873. DOI: 10.1007/s42770-020-00249-6.

Gupta R., Singal R., Shankar A., Kuhad R.C., Saxena R.K., 1994. A modified plate assay for screening phosphate solubilizing microorganisms. *Journal of General and Applied Microbiology* 40(3): 255–260. DOI: 10.2323/jgam.40255

Haidar R., Roudet J., Bonnard O., Dufour M.C., Corio-Costet M.F., … Fermaud M., 2016. Screening and modes of action of antagonistic bacteria to control the fungal pathogen *Phaeomoniella chlamydospora* involved in grapevine trunk diseases. *Microbiological Research* 192: 172–184. DOI: 10.1016/j.micres.2016.07.003

Haidar R., Amira Y., Roudet J., Marc F., Patrice R., 2021. Application methods and modes of action of *Pantoea agglomerans* and *Paenibacillus* sp. to control the grapevine trunk disease-pathogen, *Neofusioccum parvum*. *OENO One* 55(3): 1–16. DOI: 10.20870/oeno-one.2021.55.3.4530

Harman G.E., Howell C.R., Viterbo A., Chet I., Lorito M., 2004. *Trichoderma* specie opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology 2*: 43–56. DOI: 10.1038/nrmicro797

INEGI, 2017. Anuario Estadístico y Geográfico de Baja California 2017. Available at https://www.inegi.org.mx/contenido/productos/prod_serv/contenidos/espanol/bvinegi/productos/nueva_estruc/anuarios_2017/702825094874.pdf. Accessed July 20, 2022

Jacob J., Krishnan G.V., Thankappan D., Amma D.K., 2020. Endophytic bacterial strains induced systemic resistance in agriculturally important crop plants. In: *Microbial Endophytes* (Ajay K., Radhakrishnan E.K. ed.), Elsevier, Duxford, Cambridge, United Kingdom, 75–105. DOI: 10.1016/B978-0-12-819654-0.00004-1

Kumar S., Stecher G., Li M., Knyaz C., Tamura K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35 (6): 1547–1549. DOI: 10.1093/molbev/msy096.

Langa-Lomba N., Martín-Ramos P., Casanova-Gascón J., Julián-Lagunas C., González-García V., 2022. Potential of native *Trichoderma* strains as antagonists for the control of fungal wood pathologies in young grapevine plants. *Agronomy* 12 (2): 336. DOI: 10.3390/agronomy12020336

Leal C., Richet N., Guise J.F., Gramaje D., Armengol J., … Trotel-Aziz P., 2021. Cultivar contributes to the beneficial effects of *Bacillus subtilis* PTA-271 and *Trichoderma atroviride* SC1 to Protect Grapevine Against *Neofusicoccum parvum*. *Frontiers in Microbiology* 12: 726132. DOI: 10.3389/fmicb.2021.726132

Leiva S., Oliva M., Hernández E., Chuquibala B., Rubio K., … Torres de la Cruz M., 2020. Assessment of the potential of *Trichoderma* spp. strains native to Bagua (Amazonas, Peru) in the biocontrol of frosty pod rot (*Moniliophthora roreri*). *Agronomy* 10 (9): 1376. DOI: 10.3390/agronomy10091376

Lipový B., Raška F., Kocmanová I., Hanslianová M., Hladík M., … Macháček C., 2021. *Trichoderma longibrachiatum* and *Aspergillus fischeri* Infection as a cause of skin graft failure in a patient with critical burns after liver transplantation. *Journal of Fungi* 7(6):487. DOI: 10.3390/jof7060487

Lynne A.M., Haarmann D., Louden B.C., 2011. Use of blue agar CAS assay for siderophore detection. *Journal of Microbiology & Biology Education* 12: 51–53. DOI: 10.1128/jmbe.v12i1.249

Magoni C., 2009. *Historia de la vid y el vino en la Península de la Baja California*. 1st ed. Universidad Iberoamericana, Tijuana, Mexico, 283 pp.

Martínez-Diz M. del P., Díaz-Losada E., Díaz-Fernández Á., Bouzas-Cid Y., Gramaje D. 2020. Protection of grapevine pruning wounds against *Phaeomoniella chlamydospora* and *Diplodia seriata* by commercial biological and chemical methods. *Crop Protection* 143: 105465. DOI: 10.1016/j.cropro.2020.105465

Mayo-Prieto S., Campelo M.P., Lorenzana A., Rodríguez-González A., Reinoso B., … Casquero P.A., 2020. Antifungal activity and bean growth promotion of Trichoderma strains isolated from seed vs soil. *European Journal of Plant Pathology* 158: 817–828. DOI: 10.1007/s10658-020-02069-8

Méndez-Bravo A., Cortazar-Murillo E.M., Guevara-Avendaño E., Ceballos-Luna O., Rodríguez-Haas B., … Reverchon F., 2018. Plant growth-promoting rhizobacteria associated with avocado display antagonistic activity against *Phytophthora cinnamomi* through volatile emissions. *PLOS ONE* 13(3): e0194665. DOI: 10.1371/journal.pone.0194665

Myoken Y., Sugata T., Fujita Y., Asaoku H., Fujihara M., Mikami Y. 2002. Fatal necrotizing stomatitis due to *Trichoderma longibrachiatum* in a neutropenic patient with malignant lymphoma: a case report. *International Journal of Oral and Maxillofacial Surgery* 31(6): 688-691. DOI: 10.1054/ijom.2001.0211.

Mondello V., Larignon P., Armengol J., Kortekamp A., Vaczy K., … Fontaine F., 2018. Management of grapevine trunk diseases: Knowledge transfer, current strategies and innovative strategies adopted in Europe. *Phytopathologia Mediterranea* 57(3): 369–383. DOI:10.14601/Phytopathol_Mediterr-23942

Mukherjee P. K., Mendoza-Mendoza A., Zeilinger S., Horwitz B.A., 2022. Mycoparasitism as a mechanism of Trichoderma-mediated suppression of plant diseases. *Fungal Biology Reviews* 39: 15–33. DOI: 10.1016/j.fbr.2021.11.004

Mutawila C., Halleen F., Mostert L., 2016. Optimisation of time of application of *Trichoderma* biocontrol agents for protection of grapevine pruning wounds. *Australian Journal of Grape and Wine Research* 22(2): 279–287. DOI: 10.1111/ajgw.12218

Niem J.M., Billones-Baaijens R., Stodart B., Savocchia S., 2020. Diversity profiling of grapevine microbial endosphere and antagonistic potential of endophytic pseudomonas against grapevine trunk diseases. *Frontiers in Microbiology* 11: 477. DOI: 10.3389/fmicb.2020.00477

Nusaibah A., Musa H., 2019. A review report on the mechanism of *Trichoderma* spp. as biological control agent of the basal stem rot (BSR) disease of *Elaeis guineensis*. In *Trichoderma: The most widely used fungicide* (Shah M.M., Sharif U., Buhari R.T., ed), IntechOpen, London, United Kingdom, 1–12. DOI: 10.5772/intechopen.84469

O'Donnell K., Kistlerr H.C., Cigelnik E., Ploetz R.C., 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences of the United States of America* 95(5): 2044–2049. DOI: 10.1073/pnas.95.5.2044

Pikovskaya R.I., 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiology* 17: 362–370.

Pimentel M.F., Arnao E., Warner A.J., Subedi A., Rocha L.F., … Fakhoury, A., 2020. *Trichoderma* isolates inhibit *Fusarium virguliforme* growth, reduce root rot, and induce defense-related genes on soybean seedlings. *Plant Disease* 104(7): 1949–1959. DOI: 10.1094/PDIS-08-19-1676-RE

Poudel M., Mendes R., Costa L.A.S., Bueno C.G., Meng Y., … Martins S.J., 2021. The role of plant-associated bacteria, fungi, and viruses in drought stress mitigation. *Frontiers in Microbiology* 12: 744512. DOI: 10.3389/fmicb.2021.743512

Rangel Montoya, E.A. 2021. *Characterization, evaluation of the colonization process and search for biological control strategies of fungi of the Botryosphaeriaceae family associated with grapevines in Mexico*. Doctoral thesis. Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California. 103 pp.

Rangel-Montoya E.A., Paolinelli M., Rolshausen P.E., Valenzuela-Solano C., Hernandez-Martinez R., 2021. Characterization of *Lasiodiplodia* species associated with grapevines in Mexico. *Phytopathologia Mediterranea* 60 (2): 237–251. DOI: 10.36253/phyto-12576

Rangel-Montoya E.A., Delgado-Ramírez C.S. Sepulveda E., Hérnandez-Martínez R., 2022. Biocontrol of *Macrophomina phaseolina* using *Bacillus amyloliquefaciens* strains in cowpea (*Vigna unguiculata* L.). *Agronomy* 12 (3): 676. DOI: 10.3390/agronomy12030676

Reyes-Figueroa O., Ortiz-García C.F., Torres-de la Cruz M., Lagunes-Espinoza L.D.C., Valdovinos-Ponce G., 2016. *Trichoderma* species from the cacao agroecosystem with biocontrol potential of *Moniliophthora roreri*. *Revista Chapingo serie ciencias forestales y del ambiente* 22 (2): 149-163. DOI: 10.5154/r.rchscfa.2015.08.036

Riseh R.S., Ebrahimi-Zarandi M., Gholizadeh-Vazvani M., Skorik Y.A. 2021. Reducing drought stress in plants by encapsulating plant growth-promoting bacteria with polysaccharides. *International Journal of Molecular Sciences* 22(23): 12979. DOI: 10.3390/ijms222312979

Rocha-Ramirez V., Omero C., Chet I., Horwitz B.A., Herrera-Estrella A., 2002. *Trichoderma atroviride* G-protein alpha-subunit gene tga1 is involved in mycoparasitic coiling and conidiation. *Eukaryotic Cell* 1(14): 594-605. DOI: 10.1128/EC.14.594-605.2002

Salvatierra-Martinez R., Arancibia W., Araya M., Aguilera S., Olalde V., … Stoll A., 2018. Colonization ability as an indicator of enhanced biocontrol capacity an example using two *Bacillus amyloliquefaciens* strains and *Botrytis cinerea* infection of tomatoes. *Journal of Phytopathology* 166(9): 601–612. DOI: 10.1111/JPH.12718

Schwyn B., Neilands J.B., 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, 160(1): 47–56. DOI: 10.1016/003-2697(87)90612-9

SEFOA, 2011. Estudio estadístico sobre producción de uva en Baja California. Available at: https://www.nacionmulticultural.unam.mx/empresasindigenas/docs/1873.pdf. Accessed January 15, 2021.

Silva-Valderrama I., Toapanta D., Miccono M. de los A., Lolas M., Díaz G.A., … Castro A., 2021. Biocontrol potential of grapevine endophytic and rhizospheric fungi against trunk pathogens. *Frontiers in Microbiology* 11: 614620. DOI: 10.3389/fmicb.2020.614620

Spagnolo A., Magnin-Robert M., Alayi T.D., Cilindre C., Schaeffer-Reiss C., … Fontaine F., 2014. Differential responses of three grapevine cultivars to Botryosphaeria dieback. *Phytopathology* 104(10): 1021–1035. DOI: 10.1094/PHYTO-01-14-0007-R

Sood M., Kapoor D., Kumar V., Sheteiwy M.S., Ramakrishnan M., … Sharma A., 2020. *Trichoderma*: The "secrets" of a multitalented biocontrol agent. *Plants* 18(6): 762. DOI: 10.3390/plants9060762

Úrbez-Torres J.R., Gubler W.D., 2009. Pathogenicity of Botryosphaeriaceae species isolated from grapevine cankers in California. *Plant Disease* 93(6): 584–592. DOI: 10.1094/PDIS-93-6-0584

Úrbez-Torres J.R., Peduto F., Striegler R.K., Urrea-Romero K.E., Rupe J.C., … Gubler W.D., 2012. Characteriza-

tion of fungal pathogens associated with grapevine trunk diseases in Arkansas and Missouri. *Fungal Diversity* 52: 169–189. DOI: 10.1007/s13225-11-0110-4

Úrbez-Torres J.R., Tomaselli E., Pollard-Flamand J., Boulé J., Gerin D., Pollastro M., 2020. Characterization of *Trichoderma* isolates from southern Italy. And their potential biocontrol activity against grapevine trunk disease fungi. *Phytopathologia Mediterranea* 59(3): 425–439. DOI: 10.14601/Phyto-11273

Vasiliki E.G., Despoina M., Konstantinos M., Christos D., Aikaterini G., … Xanthi T., 2021. First case of pneumonia-parapneumonic effusion due to *Trichoderma longibrachiatum*. *IDCases* 25: e01239. DOI: 10.1016/j.idcr.2021.e01239.

Wagner D.B., Furnier G.R., Saghai-Maroof M.A., Williams S.M., Dancik B.P., Allard R.W., 1987. Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. *Proceedings of the National Academy of Sciences of the United States of America* 84(7): 2097–2100. DOI: 10.1073/pnas.84.7.2097

Walker M.A., Heinitz C., Riaz S., Uretsky J., 2019. Grape taxonomy and germplasm. In: *Compendium of Plant Genomes* (D. Cantu M. A. Walker ed.), Springer, Cham, California, United States of American, 25–38. DOI: 10.1007/978-3-030-19601-2

Zehra A., Raytekar N.A., Meena M., Swapnil P., 2021. Efficiency of microbial bio-agents as elicitors in plant defense mechanism under biotic stress*:* A review. *Current Research in Microbial Sciences* 2: 100054. DOI: 10.1016/j.crmicr.2021.100054

Zhang W., Groenewald J.Z., Lombard L., Schumacher R.K., Phillips A.J.L., Crous P.W., 2021. Evaluating species in *Botryosphaeriales*. *Persoonia*. 46: 63–115. DOI: 10.3767/persoonia.2021.46.03

Zhao X., Zhou J., Tian R., Liu Y., 2022. Microbial volatile organic compounds: Antifungal mechanisms, applications, and challenges. *Frontiers in Microbiology* 13: 922450. DOI: 10.3389/fmicb.2022.922450

Zheng B.X., Ibrahim M., Zhang D.P., Bi Q.F., Li H.Z., … Yang X.R., 2018. Identification and characterization of inorganic-phosphate-solubilizing bacteria from agricultural fields with a rapid isolation method. *AMB Express* 8(1): 47. DOI: 10.1186/s13568-018-0575-6