Research

Isolation and Characterization of *Bacillus velezensis* EB14, an Endophytic Bacterial Strain Antagonistic to Poplar Stem Canker Pathogen *Sphaerulina musiva* and Its Interactions with the Endophytic Fungal Microbiome in Poplar

Sachin Naik^{1,†} | Sylvester Palys¹ | Marcos Di Falco¹ | Adrian Tsang¹ | Pierre Périnet² | Uma Shaanker Ramanan³ | Selvadurai Dayanandan¹ |

¹ Centre for Structural and Functional Genomics, Department of Biology, Concordia University, Montreal, Quebec H4B 1R6, Canada

² Direction de la recherche forestière, ministère des Forêts, de la Faune et des Parcs, Québec, Québec G1P 3W8, Canada

³ Department of Crop Physiology and School of Ecology and Conservation, University of Agricultural Sciences, Bengaluru 560065, Karnataka, India

† Corresponding author: S. Naik; me.sach71@gmail.com

Accepted for publication 22 March 2021.

Abstract

Species of the genus *Populus* (commonly known as poplar) are one of the most widely used groups of forest trees in North America and Europe, and play a significant ecological role as a pioneer species in boreal forests and as a dominant species in the riparian forests that serve as wildlife habitats and watersheds. Natural and artificial hybrids of poplar are being extensively used in commercial plantations. However, many hybrid poplar trees are susceptible to *Sphaerulina musiva*, the pathogenic fungus that causes leaf spots and stem cankers and limits the utility of hybrid poplar as a plantation tree. We isolated an endophytic bacterial strain, *Bacillus velezensis* EB14, from a *Populus* hybrid which showed a strong antifungal activity against *S. musiva*. Through mass spectrometric analyses of cocultured *B. velezensis* EB14 and *S. musiva*, we identified five cyclic lipopeptides produced by *B. velezensis* EB14: iturin A1, iturin A2, iturin A9, subtilene A, and fengycin. In addition, *B. velezensis* EB14 produced four major unidentified compounds in cocultivation with *S. musiva*. The cyclopeptide production by *B. velezensis* EB14 was more pronounced (20- to 1,000-fold) in the cocultured plates due to elicitation by *S. musiva*. We also discovered that the native endophytic *B. velezensis* EB14 strain exhibited different levels of interactions against the endophytic fungal microbiomes of *Populus* sp. Overall, our results indicate that *B. velezensis* EB14 strain as a promising biocontrol agent that could be used against stem canker and leaf spot diseases caused by *S. musiva* in poplar plantations.

Keywords: antifungal, biocontrol, cyclopeptides elicitation, endophytic bacteria–endophytic fungal interaction, iturinic compounds, *Populus* microbiome, Septoria stem canker

Funding

Support was provided by a Natural Sciences and Engineering Research Council of Canada Discovery grant to S. Dayanandan.

The author(s) declare no conflict of interest

e-Xtra: Supplementary materials are available online.

Copyright © 2021 The Author(s). This is an open access article distributed under the CC BY-NC-ND 4.0 International license.

Sphaerulina musiva (Peck) Quaedvl., Verkley & Crous (syn. *Septoria musiva*; teleomorph = *Mycosphaerella populorum*) is a fungal pathogen which causes leaf spots and stem cankers in *Populus* spp. (Carroll 1988; Feau et al. 2010; Waterman 1946). *Sphaerulina musiva* is endemic to eastern North America and was first reported in eastern cottonwood trees (*Populus deltoides*) in 1954 (Waterman 1954). The pathogen is a major threat to hybrid poplar plantations (Bier 1939; Ostry and McNabb 1985; Waterman 1954) and affects poplar plantations of over 44,000 ha in Canada and 45,000 ha in the United States (Derbowka et al. 2012; FAO 2012; Sakalidis et al. 2016). The home range of *S. musiva* has been expanding worldwide (Sakalidis et al. 2016), with reports from Alberta (LeBoldus et al. 2009), British Columbia (Callan et al. 2007; Herath et al. 2016), Argentina (Sarasola 1944), Brazil (dos Santos et al. 2010), Asia (Maxwell et al. 1997), and Chile (Hamelin 2016). Not surprisingly, controlling stem cankers and leaf spots caused by *Sphaerulina* spp. has held center stage in poplar tree breeding programs (Newcombe and Ostry 2001; Ostry 1987; Ostry and McNabb 1985). Few canker-resistant clones (TD × D) were developed with *P. trichocarpa* (T) by backcross with *P. deltoides* (D) due to the recessive resistance imparted by *P. deltoides*. (Newcombe and Ostry 2001). In another study, the transgenic hybrid clone, *Populus* × *euramericana* ('Ogy') expressing a wheat oxalate oxidase gene demonstrated partial resistance to leaf spots caused by *S. musiva* (Liang et al. 2001). A study by Qin and LeBoldus (2014) indicated the importance of necrophylactic periderm layer formation in disease resistance occurring post penetration of *S. musiva* in resistant poplar trees.

Attempts have been made to explore the potential for biocontrol of *Sphaerulina* leaf spot. Yang et al. (1993) reported that the fungus *Phaeothea dimorphospora* inhibits the growth of *S. musiva* under laboratory and greenhouse conditions. However, later research showed that the *P. dimorphospora* promoted the colonization of another pathogen, *Heterobasidium annosum*, after a 2-month exposure and increased its pathogenicity. Shimizu (1994) used *Streptomyces* strains to inhibit the growth of *S. musiva* isolates in a leaf disk assay. Later, Gyenis and Ostry (2003) used two of these *Streptomyces* strains in a field assay to control *Sphaerulina* leaf spot diseases in hybrid poplar. Weekly spraying of the *Streptomyces* strains was necessary, suggesting its failure to colonize the *Populus* trees (Gyenys and Ostry 2003).

Due to limited knowledge of the interactions of biocontrol microbes with host plants and their native microbiome, most of the previous efforts to develop biocontrol agents were not effective (Gyenys and Ostry 2003; Roy et al. 2003). Understanding the effectiveness of endophytes as a biocontrol agent in plants is still in its infancy and the impacts on the native endophytic community of the host plant need to be explored before the introduction of biocontrol microbes (Whipps 2001). Introducing nonnative endophytes into the host plant may result in the loss of novel endophytes associated with the host and could adversely affect the host–microbial consortium (Herre et al. 1999; Pavlo et al. 2011; Rabiey et al. 2019; Zamioudis and Pieterse 2012). Therefore, isolation and characterization of endophytes native to host plants plays a crucial role in developing biocontrol agents.

In the present study, we investigated the interactions of an endophytic bacterium, *Bacillus velezensis*, that we isolated from a *Populus* × *jackii* tree against the fungal pathogen *S. musiva*. Through liquid chromatography–mass spectrometry (LC-MS) analyses, the antifungal metabolites produced during

the coculture were identified. We also investigated the interactions between *B. velezensis* and endophytic fungal microbiome isolated from various *Populus* spp. in the region. We discuss these results in the context of the management of *S. musiva* using endophytes of poplar.

MATERIALS AND METHODS

Source of the endophytic bacteria

A bacterial endophyte in the present study was discovered during the isolation of endophytic fungi from *Populus* × *jackii* (natural hybrid between *P. deltoides* and *P. balsamifera*) trees sampled from Mont Saint-Hilaire (45°33'8" N, 73°9'3" W) in the Montérégie region of the southern Quebec, Canada. Leaf explants were surface sterilized for 30 s with 70% ethanol, 3 min with 4% NaHOCl, and 30 s with 90% ethanol, with three to four rinses with sterile distilled water. To ensure surface sterilization efficacy, a few random explants were impressed onto potato dextrose agar (PDA) plates. In addition, the last rinse with sterile distilled water was spread on the PDA media. The Petri plates were incubated at 28°C and the explants were observed for microbial emergence after 5 days. The pure culture was obtained by culturing the bacterial colonies from the edge of the explant into Luria-Bertani (LB) agar media and further purified by a streak plate method. Isolated pure culture was maintained as a glycerol stock at −80°C.

Morphological and molecular identification of bacteria

Gram staining of the isolate was performed following the standard staining procedure (Bartholomew and Mittwer 2011). Morphological observation was made based on colony characteristics of the bacteria on LB agar media. For the molecular identification, genomic DNA was extracted following the protocol of MO BIO Qiagen Ultraclean Microbial DNA Isolation kit (catalog number 12224-50). PCR amplifications of 16S ribosomal RNA (rRNA) were performed using bacterial universal primers 27F and 1391R. PCR products were purified using a PCR product purification kit and sequenced at the Génome Québec Innovation Centre, Montreal, Canada. The resulting sequences were analyzed through the BLAST program available through GenBank of NCBI. The nucleotide sequence of 16S rRNA gene of the isolated bacterial isolate was deposited at GenBank. A neighbor-joining phylogenetic tree was constructed in MEGAX from the closest sequences available from the BLAST hits.

Dual-culture assay of bacterial strain EB14 with *S. musiva* (S9704)

The antibiosis assay was performed in PDA plates following two methods. In the first method, 200 µl of the *S. musiva* spores (1×10^6 spores/ml) was spread uniformly on a PDA Petri dish (100 by 15 mm) followed by the inoculation of 5 µl of bacterial culture EB14 (optical density [OD] of 0.01 to 0.02 at 600 nm) on a sterile paper disc (diameter = 0.25 cm) placed at the center of the Petri dish. PDA plates spread only with *S. musiva* spores (200 µl) or bacterial culture EB14 (5 µl) placed on a sterile paper disc were used as controls. All Petri dishes were incubated at room temperature for 7 days before photographs were taken. Growth area (in square millimeters) was measured using ImageJ software. Dual-culture assays along

with the controls were performed in triplicate. The antagonistic activity was measured as the inhibition of the mycelial growth of *S. musiva* in the presence of EB14 bacteria as compared with the control ($R1 - R2/R1 \times 100$, where R1 and R2 are the mycelial growth of the *S. musiva* in the control and in the presence of bacteria EB14, respectively). The experiment was repeated twice.

In the second method, 10 µl of the bacterial culture EB14 (OD of 0.01 to 0.02 at 600 nm) was streaked on one side of the PDA with a 2.5-cm distance from the *S. musiva* spore (50 µl) suspension (1×10^6 spores/ml) spread. PDA plates spread only with *S. musiva* spores (50 µl) or bacterial culture (10 µl) were used as controls. Dual-culture assays along with the controls were performed in triplicate. At the inhibition zone, agar slices of 1 cm² in size were cut and observed under the compound microscope for distortions in the fungal mycelium. Microscopic observations were all done in triplicate, along with the controls. The experiment was repeated twice.

Spore germination assay in the presence of bacteria

Approximately 50 µl of *S. musiva* spores (1×10^6 spores/ml) was resuspended in 5 ml of potato dextrose broth (PDB) inoculated with 2 µl of bacterial culture EB14 (OD of 0.01 to 0.02 at 600 nm). As a control, *S. musiva* spores were resuspended in 5 ml of PDB without the bacteria. After incubation at room temperature with shaking at 150 rpm for 24 h, 100 spores of *S. musiva* were observed under a compound microscope and the percentages of germinated spores of each suspension were calculated. The experiment was performed in triplicate.

Analyses of antifungal compounds produced by bacterial strain EB14

Briefly, 200 µl of *S. musiva* spores (10^6 spores/ml) were spread on the PDA plate followed by the inoculation of 5 µl of bacterial culture EB14 (OD of 0.01 to 0.02 at 600 nm) on a sterile paper disc (0.25 cm) placed at the center of the Petri dish and incubated at room temperature. Following a 72-h incubation period, agar plugs (300 mg) were sampled from the bacterial side and fungal sides of the growth inhibition zone. PDA plates spread only with *S. musiva* spores (200 µl) or bacterial culture EB14 (5 µl) placed on a sterile paper disc were used as controls. Agar plugs were processed by following the acetonitrile/water-based method. (Cawoy et al. 2015; Razafindralambo et al. 1993). Briefly, agar plugs were excised and placed in a glass vial. Acetonitrile and water (1:1 [wt/cvol], 800 µl) were added to the vial and the mix was sonicated for 2 min. The samples were then homogenized and centrifuged (14,000 rpm) for 20 min. Supernatant was syringe filtered (0.45 µm) and stored at -4°C. As a control, agar plugs were analyzed from the bacterial plates without *S. musiva* spores and also from *S. musiva* plates without bacteria. The experiment was repeated twice and one included a duplicate. The compounds present in these agar plug extracts were analyzed through MS using a Fourier Transform (FT) Ion Cyclotron Resonance Mass Spectrometer (Cao et al. 2018). Following identification of compounds, a time series analysis of fungal and bacterial cocultures at 0, 24, 48, and 72 h was performed to investigate the dynamics of compound production. Agar plugs for different time intervals were taken from a separate individual coculture of Petri plates and controls. The experiments were repeated twice, and one of the experiments had a p ESI mass cut of range between 100 and 1,400 m/z.

LC-MS analysis of metabolites

Analyses of samples through high-pressure LC-MS were performed using the 1260 Infinity II chromatography system (Agilent Technologies, Santa Clara, CA, U.S.A.) connected inline to a 7-Tesla Finnigan LTQ-FT mass spectrometer (Thermo Electron Corporation, San Jose, CA, U.S.A.). Molecular components in the sample were separated using a 2.1-by-150-mm, 5-µm Kinetex C18 reversed-phase column (Phenomenex, Torrance, CA, U.S.A.). The solvents used to generate the gradient during reversed-phase liquid chromatography separation were 0.1% formic acid in water for solvent A and 0.1% formic acid in acetonitrile for solvent B. The gradient method used was 3% B isocratic for 1 min, increased to 80% B over 10 min, increased to 95% B for 1 min, isocratic at 95% for 1 min, decreased to 3% B in 0.1 min, and held at 3% B for 5.9 min. Injection volume was 10 µl and flow rate was 250 µl/min. FT-MS scans were acquired in positive polarity from 100 to 2,000 m/z (experiment 1) and 100 to 1,400 m/z at 50,000 resolution at 200 m/z (experiment 2). Extracted ion chromatograms for the various theoretical iturin monoisotopic M+H m/z values were generated with the Xcalibur v.2.2 software (Thermo-Fisher, Waltham, MA, U.S.A.) using a 5-ppm extraction window.

Interaction of the endophytic bacteria with the poplar endophytic fungal microbiome

The interactions assay of bacterial strain EB14 against the collection of native endophytic fungal microbiomes (Table 1) of different *Populus* spp. available in our lab was investigated using a dual-culture assay. Representative strains of the endophytic fungal microbiome of *Populus* × *jackii*, *P. deltoides*, *P. grandidentata*, *P. tremuloides*, *P. alba*, and *P. balsamifera* were included in the dual-culture assay (Table 1). A fresh, actively growing mycelial plug was removed from the edges of the 7-day-old fungal culture plate and placed in a PDA Petri dish at a distance of 2.5 mm from the disc with 5 µl of bacterial strain EB14 (OD of 0.01 to 0.02 at 600 nm). The plates were incubated at 28°C for 7 days. The dual-culture assay was performed in triplicate.

Antagonistic activity was quantified measuring the mycelial growth compared with the control using the formula $R1 - R2/R1 \times 100$, where R1 and R2 are the mycelial growth of endophytic fungi in the control and in the presence of bacterial strain EB14, respectively.

RESULTS

Source and identification of bacteria

We observed the endophytic bacteria from the cut ends of a leaf explant forming a clear halo by inhibiting the nearby endophytic fungus (Supplementary Fig. S1). The colonies of endophytic bacteria were creamy white in color and usually with a smooth, raised border. The bacterial strain was Gram positive, rod shaped, motile, and endospore forming. 16S rRNA amplification resulted in the PCR product of 1,293 bp in length. The Blast search of the partial 16S rRNA showed 100% homology with *B. velezensis* and *B. amyloliquefaciens* strains CP007165.1 (*B. velezensis* NJN6), CP00560.2 (*B. velezensis* FZB42) and MT559810.1 (*B. amyloliquefaciens*). The neighbor-joining phylogenetic tree revealed that the bacterial strain EB14 is clustered with *B. velezensis* within the clade consisting of *B. velezensis* NNJ6 (Supplementary Fig. S2).

The sequence of the strain *B. velezensis* EB14 was deposited in GenBank under MW795349.1 and MW795348.1.

B. velezensis EB14 strain inhibits *S. musiva*

B. velezensis EB14 exhibited significant inhibition activity against *S. musiva* growth, as indicated by a visible inhibition zone (Fig. 1). *S. musiva* mycelial growth was 90% inhibited in the dual-culture assay type 1 and 77% inhibited in the dual-culture assay type 2 in the presence of the *B. velezensis* EB14. We also found that *S. musiva* hyphae exhibit hyphal swelling-like structures near the interaction zones, which resemble chlamydospores (Supplementary Fig. S3). After 24 h, the percentage of spore germination in *S. musiva* control was 88% whereas it was completely inhibited in the presence of bacteria (Supplementary Fig. S7). Similar results were obtained in the repeated experiments.

B. velezensis EB14 strain produces iturin and fengycins compounds

To identify the inhibitory metabolites during coculture of fungi and bacteria, the agar plug samples were sampled from both the bacterial side and the fungal side along with the controls. We also

investigated whether there were any metabolites elicited during the coculture. The comparison of compounds between fungal side samples in coculture to that of the fungal controls did not reveal detectable elicited compounds in response to the presence of the bacterium (Fig. 2) whereas comparison of compound production between bacterial side samples and controls showed production of elicited compounds in response to coculturing (Fig. 2). Five compounds with different masses were identified (Fig. 3; Supplementary Fig. S5). These included iturin A1 ($[M+H]^+ = 1,029.5345$), iturin A2 ($[M+H]^+ = 1,043.5498$), iturin A9 ($[M+H]^+ = 1,085.5981$) (Pathak and Keharia 2014), subtilene A ($[M+H]^+ = 1,057.5659$) (Thasana et al. 2010), and fengycin ($[M+H]^+ = 1,463.7988$) (Pathak et al. 2012). A purified standard compound was not available for the confirmation of isolated compounds, except for iturin A2 (Supplementary Fig. S4). Despite a lack of available standards, the masses identified in this study all correspond to compounds which are structurally similar to cyclic lipopeptide iturin A2. In addition to the identified compounds, four major peaks that were not corresponding to known compounds were also present: compound 1 ($[M+H]^+ = 1,015.5185$), compound 2 ($[M+H]^+ = 1,071.5780$), compound 3 ($[M+H]^+ = 1,431.7119$), and compound 4 ($[M+H]^+ = 1,491.8285$) (Fig. 3; Supplementary Figs. S5 and S8). In the case of fengycin and iturin A1, two peaks were identified for each

TABLE 1

Interactions between *Bacillus velezensis* EB14 and endophytic fungal operational taxonomic units isolated from different *Populus* spp.^a

Interaction types	Representative code	Host plant	Species name
Short-distance inhibition	PG74	<i>Populus grandidentata</i>	<i>Epicoccum nigrum</i>
Short-distance inhibition	PT29	<i>Populus tremuloides</i>	<i>Neofusicoccum</i> sp.
Short-distance inhibition	PT63	<i>Populus tremuloides</i>	<i>Diaporthe</i> sp.
Short-distance inhibition	PJ26	<i>Populus × jackii</i>	<i>Daldinia childiae</i>
Short-distance inhibition	PG25	<i>Populus grandidentata</i>	<i>Daldinia childiae</i>
Short-distance inhibition	PT69	<i>Populus tremuloides</i>	<i>Neonectria</i> sp.
Short-distance inhibition	PT3	<i>Populus tremuloides</i>	<i>Chaetomium</i> sp.
Short-distance inhibition	PA58	<i>Populus alba</i>	<i>Diaporthe caulivora</i>
Short-distance inhibition	PG41	<i>Populus grandidentata</i>	<i>Biscogniauxia mediterranea</i>
Long-distance inhibition	PG12	<i>Populus grandidentata</i>	<i>Chaetomium</i> sp.
Long-distance inhibition	PG75	<i>Populus grandidentata</i>	<i>Phomopsis</i> sp.
Long-distance inhibition	PT20	<i>Populus tremuloides</i>	<i>Xylaria</i> sp.
Long-distance inhibition	PJ1	<i>Populus × jackii</i>	<i>Alternaria</i> sp.
Long-distance inhibition	PD62	<i>Populus deltoides</i>	<i>Nectriaceae</i> sp.
Long-distance inhibition	PJ8	<i>Populus × jackii</i>	<i>Paraconiothyrium brasiliense</i>
Long-distance inhibition	PA07	<i>Populus alba</i>	<i>Paraconiothyrium brasiliense</i>
Long-distance inhibition	PD18	<i>Populus deltoides</i>	<i>Nectriaceae</i> sp.
Long-distance inhibition	PD2	<i>Populus deltoides</i>	<i>Epicoccum sorghinum</i>
Contact inhibition	PA65	<i>Populus alba</i>	<i>Diaporthe</i>
Contact inhibition	PD5	<i>Populus deltoides</i>	<i>Fusarium sporotrichioides</i>
Contact inhibition	PD54	<i>Populus deltoides</i>	<i>Fusarium sporotrichioides</i>
Contact inhibition	PG73	<i>Populus grandidentata</i>	<i>Valsaceae</i> sp.
Contact inhibition	PJ20	<i>Populus × jackii</i>	<i>Phomopsis vaccinii</i>
Contact inhibition	PJ24	<i>Populus × jackii</i>	<i>Daldinia childiae</i>
Contact inhibition	PJ55	<i>Populus × jackii</i>	<i>Phomopsis</i> sp.
Bacterial overgrowth	PA47	<i>Populus alba</i>	<i>Colletotrichum fioriniae</i>
Bacterial overgrowth	PA56	<i>Populus alba</i>	<i>Diaporthaceae</i> sp.
Bacterial overgrowth	PG15	<i>Populus grandidentata</i>	<i>Epicoccum</i> sp.
Bacterial overgrowth	PG23	<i>Populus grandidentata</i>	<i>Hypoxylon submonticulosum</i>
Bacterial overgrowth	PG79	<i>Populus grandidentata</i>	<i>Venturia</i> sp.
Bacterial overgrowth	PJ2	<i>Populus × jackii</i>	<i>Nothophoma quercina</i>
Bacterial overgrowth	PJ45	<i>Populus × jackii</i>	<i>Colletotrichum fioriniae</i>
Bacterial overgrowth	PJ6	<i>Populus × jackii</i>	<i>Phomopsis</i> sp.
Bacterial overgrowth	PT14	<i>Populus tremuloides</i>	<i>Epicoccum nigrum</i>
Bacterial overgrowth	PT31	<i>Populus tremuloides</i>	<i>Nemania diffusa</i>
Bacterial overgrowth	PT35	<i>Populus tremuloides</i>	<i>Nothophoma quercina</i>
Bacterial overgrowth	PT80	<i>Populus tremuloides</i>	<i>Paracamarosporium fagi</i>

^a Interaction types were defined by inhibition zone area and morphological interaction types. Inhibition zone areas below 400 mm² were assigned as short-distance inhibition (Supplementary Fig. S6).

compound, which may indicate potential isomers of these compounds (Supplementary Figs. S5 and S8). Similar results were obtained in the repeated experiment (Supplementary Fig. S8).

Coculturing *B. velezensis* EB14 with *S. musiva* increases the production of iturinic compounds

All iturinic and the unidentified major compounds increased over time in bacterial control and bacterial side of the agar on cocultures. Increases between 24 and 72 h of growth were more pronounced (20- to 1,000-fold) in the bacterial side of the

cocultured agar cores than in bacterial control cores (1.5- to 3-fold) (Fig. 4; Supplementary Fig. S9). However, in the second time interval experiment, we were not able to analyze the three compounds of *m/z* masses ranged above 1,400 because the FT-MS cut of mass scan range was set at 100 to 14,00 *m/z*.

B. velezensis exhibits different competitive ability against the endophytic fungal microbiome of genus *Populus*

The pairwise coculture of fungal species of the endophytic fungal microbiome of genus *Populus* exhibited different levels of competitive ability. The interaction types were grouped into

FIGURE 1 Antagonistic activity against *Sphaerulina musiva* in a dual-culture test on potato dextrose agar plates. **A**, The dual culture of plates from left to right: *Bacillus velezensis* EB14 control, *S. musiva* control, and coculture. **B**, *S. musiva* showing 1.5 cm of inhibition zone when streaked in the presence of *Bacillus* EB14 strain. **C**, The graph represents the mycelial growth area (in square millimeters) of *S. musiva* in a dual-culture assay type 1 and type 2 with *B. velezensis* EB14 strain. SM(Control)DC1 = *S. musiva* (control) dual-culture assay type 1, SM(*Bacillus*)DC1 = *S. musiva* (*Bacillus* coculture) dual-culture assay type 1, SM(Control)DC2 = *S. musiva* (control) dual-culture assay type 2, and SM(*Bacillus*)DC2 = *S. musiva* (*Bacillus* coculture) dual-culture assay type 2. Averages from three replicates are plotted in the graph and the error bars denote standard deviation. Similar results were obtained in two separate experiments for both DC1 and DC2 types.

FIGURE 2 Total ion chromatograms after 72 h of growth of **A**, fungal control; **B**, coculture fungal side inhibition zone; **C**, bacterial control; and **D**, coculture bacterial inhibition zone. A clear change in compound production is observed between the bacterial side sample (D) and the bacterial control (C) between 8 and 11 min.

four broad categories: short-distance inhibition, long-distance inhibition, contact inhibition, and bacterial overgrowth (Fig. 5; Table 1). *B. velezensis* EB14 inhibited most of the endophytic fungi from *Populus* spp. except for a few species of endophytic fungi which inhibited the *B. velezensis* EB14. We found that certain endophytes belonging to the same taxa isolated from different *Populus* spp. showed different levels of

competitive ability (Supplementary Fig. S6; Table 1). The endophyte *Epicoccum nigrum*—isolated from *P. tremuloides* and *P. grandidentata*—showed different types of inhibition such as bacterial overgrowth and short-distance inhibition, respectively. We also found that endophyte fungal species isolated from different explants within the species also possessed differing competitive ability against the *B. velezensis* EB14

FIGURE 3
Major peaks identified in bacterial side inhibition zone (72 h) samples.

FIGURE 4
Production of identified and unidentified compounds over 72 h of growth. BC = bacterial control; BIZ = bacterial side inhibition zone; FC = fungal control; FIZ = fungal side inhibition zone; and UNK = unknown compounds.

strain. The *Daldinia childiae* endophyte isolated from *Populus × jackii* leaves and bark showed differing types of inhibition: short-distance and contact inhibition, respectively. (Table 1) We also found that a large number of endophytes showed similar types of inhibition against the *B. velezensis* EB14 strain regardless of their isolation origin, such as *Nothophoma quercina* isolated from *Populus × jackii* and *P. tremuloides*, which both showed bacterial overgrowth; *Chaetomium* sp. isolated from *P. grandidentata* and *P. tremuloides*, which showed long-distance inhibition; and *Colletotrichum fiorinae* isolated from *P. alba* and *Populus × jackii*, which showed bacterial overgrowth (Table1).

DISCUSSION

Hybrids of poplar are being widely used in commercial plantations. However, many hybrid poplar trees are susceptible to *S. musiva*, which limits the utility of hybrid poplar as plantation trees. Research on application of biocontrol microbes as an effective strategy against stem canker diseases in *Populus* is in its infancy. Until now, there have been no alternative methods to control the disease except for controlled breeding of the resistant *Populus* varieties. However, the disease resistance may often be overcome by the pathogen. Among the biological control agents, *Bacillus* spp. are the most commercially successful, ecofriendly alternatives to chemical pesticides and remain as one of the most frequently isolated endophytic bacteria (Raaijmakers and Mazzola 2012). In this study, a native endophytic bacterium, *B. velezensis* EB14 strain, was isolated from leaves of *Populus × jackii*. This endophytic bacterium demonstrated a strong antifungal activity against *S. musiva*.

Many species in the *B. subtilis* species complex are known to produce iturins and closely related lipopeptides with antifungal activity (Blacutt et al. 2016; Fan et al. 2017; Romero et al. 2007; Torres et al. 2017; Yuan et al. 2017). It is

generally known that *B. velezensis* is potentially involved in the production of cyclic lipopeptides such as surfactin, fengycin, and, iturins (Coutte et al. 2017). In particular, fengycin and iturins are the most effective antifungal agents (DeFilippi et al. 2018). Over the past decade, various studies have shown that *Bacillus* spp. are effective as biological control agents in inhibiting plant-pathogenic fungi due to production of diverse cyclic lipopeptides (Arau et al. 2002; Arias et al. 1999; de Melo et al. 2009; Sari et al. 2007; Zerrouh et al. 2011). For example, *B. pumilus* strain JK-SX001 suppresses the fungal pathogens *Cytospora chrysosperma*, *Phomopsis macrospora*, and *Fusicoccum aesculi* that cause poplar cankers in China (Ren et al. 2013). Surovy et al. (2017) reported that plant probiotic bacteria such as *B. subtilis* BTS 3 (R2), *B. amyloliquefaciens* BTS 4 (R3), *Staphylococcus saprophyticus* BTS 5 (SL3), and *B. amyloliquefaciens* BTLK6A (K6A) inhibited the wheat blast fungus in vitro and also have potential in controlling the disease in vivo.

Cyclic lipopeptides are biosynthesized by the nonribosomal peptide synthetases encoded in soilborne or plant-associated bacteria of the genera *Actinomyces*, *Streptomyces*, *Bacillus*, and *Pseudomonas* (Schneider et al. 2014). These cyclic lipopeptides are amphiphilic molecules and primarily consist of various iturins, fengycin, and surfactins, which are all composed of a backbone lipid tail linked to a short oligopeptide which is cyclized to form a lactone or lactam ring, either between two amino acids in the peptide chain or between an amino acid and an amino- or hydroxyl-group bearing fatty acid moiety (Schneider et al. 2014). Iturins such as bacillomycin D/F/L/Lc; iturin A/C/D, mojavensin, and mycosubtilin; and fengycin (plipastatin) are the class of pore-forming lipopeptides that disrupt the cytoplasmic membranes and are reported to exhibit strong antifungal activity (Deleu et al. 2008). For instance, isomers of iturins and fengycin are reported to be produced from different species of genus *Bacillus* such as *B. subtilis*, *B. amyloliquefaciens*, *B. coagulans*, *B. cereus*, *B. licheniformis*, and *B. pumilus* (Koumoutsis et al.

FIGURE 5 Morphological interactions observed between the Bacteria (*B. velezensis* EB14) and endophytic fungal OTUs of *Populus* in a Petri dish. Colony morphologies of *B. velezensis* EB14 v/s Endophytic fungal co-cultures showing the four described 'interaction types': Short-distance inhibition, Long-distance inhibition, Contact inhibition and Bacterial overgrowth.

2004; Mukherjee and Das 2005; Ongena and Jacques 2008; Roongsawang et al. 2011). However, most of the studies on the antifungal activity of *Bacillus* spp. is limited to characterization of the lipopeptides. To date, no study has examined the biocontrol potential of *Bacillus* spp. against stem-canker-forming pathogen *S. musiva* in *Populus*, and no study has characterized the interaction of a biocontrol microbe *Bacillus* sp. with its native endophytic fungal microbiome. Understanding how the biocontrol microbe interacts with the plant fungal microbiome is important for the development of promising biocontrol microbes.

Lipopeptides are known to induce chlamydospore formation in fungi and are known to facilitate bacterial entry into fungal hyphae (Li et al. 2012; Spraker et al. 2016). Chlamydospores are formed in fungi to adapt and survive through unfavorable conditions (Li et al. 2012).

In this study, *B. velezensis* EB14 produced iturin A1, subtilin A, iturin A2, iturin A9, and fengycin. In addition to the above known compounds, there are four prominent unidentified compounds. Iturins are known to form many isomers with the same molecular mass (Pathak and Keharia 2014). A single strain of bacteria is known to produce many isomers of the same lipopeptide (Beltran-Gracia et al. 2017).

In addition to bacteria, a few fungal genera are also known to produce various forms of lipopeptides (Zhao et al. 2019). Furthermore, to confirm and understand the dynamics of lipopeptide production, a time series trial analysis of fungal and bacterial cocultures was performed. We observed, that lipopeptides were produced only in the bacterial side of the agar plugs and bacterial control plugs. However, it was clear from our results that the fungi induce the production of lipopeptides at significantly higher levels in the coculture plates as compared with the control.

The major bottleneck in lipopeptide commercialization is the yield and selection of hyperproducing strains (Beltran-Gracia et al. 2017). The strain *B. velezensis* EB14 is a suitable candidate to explore the potential for commercialization because the yield of lipopeptides can be increased in response to certain inducers from the fungus. Understanding the mechanism of induction of the lipopeptides gene clusters from the inducer will provide us a cost-effective production of lipopeptides. The native and endophytic nature of the isolated bacterium and its ability to produce the lipopeptides only in the presence of the pathogen makes it a potential biocontrol agent against the *Sphaerulina* cankers and leaf spots in *Populus* spp.

Understanding the effects of *Bacillus* spp. on the endophyte fungal microbiome of *Populus* spp. is crucial for the successful colonization of biocontrol microbes. The interaction investigated through dual-culture assay of the isolated *Bacillus* spp. against the endophytic fungal microbiome of the six *Populus* spp. revealed four types of interactions, and varied between fungal isolates.

The diversity of endophytic fungi harbored by poplar trees is known to influence the health of poplar trees (Cregger et al. 2018; Terhonen et al. 2019). Despite numerous studies reporting endophytic microbe associations with the plants, the functional roles of endophytes in host plants are still not well understood. Endophytic fungi in poplar are known to confer growth promotion (Khan et al. 2016), stress tolerance (Khan et al. 2016), phytoremediation (Doty et al. 2017; Lacercat-Didier et al. 2016; Schmidt et al. 2018), and protection from invaders. However, the functioning of endophytic microbes as a community within the host remains poorly understood and requires in-depth in vivo experimental investigation to further

our knowledge of the assembly and functioning of endophytic microbial communities within host plants.

The endophytic bacterial strain *B. velezensis* EB14 is inhibitory (70% antagonistic) to approximately 68% of fungal endophytes tested (Supplementary Fig. S6), and only a few of the endophytic fungi inhibited the growth of bacteria. Based on the dual-culture experiments, the long-distance inhibition showed that both the bacteria and fungi are inhibited to a greater extent (Table 1; Supplementary Fig. S6b). However, the results of antagonism imparted by bacteria when grown in the presence of a consortium of endophytic fungi may result in different interactions. Further in vivo studies are necessary to understand the nature of the interactions of the biocontrol bacteria against the native endophytic fungal community in the host plant.

In summary, these results suggest that the isolated *Bacillus* strain interacts differentially with the endophytic microbiome of the host plant and maintains an equilibrium in the microbiome within plants. Further in vivo studies of *Bacillus* spp., including colonization efficiency within *Populus* spp. against *Sphaerulina* canker in poplar and detailed knowledge of the interactions of *Bacillus* spp. with native microbiome of the host plant, are crucial for determining the efficacy of the isolated *B. velezensis* EB14 strain as a biocontrol agent in the field.

ACKNOWLEDGMENTS

We gratefully acknowledge the NSERC Discovery grant for financial support and David Maneli and administrative staff of Gault Nature Reserve, McGill University, for providing access to the field site. We thank the anonymous reviewers for detailed and constructive feedback for improving the manuscript.

LITERATURE CITED

- Arau, W. L., Marcon, J., Maccheroni, W., Van Elsas, J. D., and Van Vuurde, J. W. L. 2002. Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. *Appl. Environ. Microbiol.* 68:4906-4914.
- Arias, R. S., Sagardoy, M. A., and Van Vuurde, J. W. L. 1999. Spatio-temporal distribution of naturally occurring *Bacillus* spp. and other bacteria on the phylloplane of soybean under field conditions. *J. Basic Microbiol.* 39:283-292.
- Bartholomew, J. W., and Mittwer, T. 2011. The Gram stain. *MLO Med. Lab. Obs.* 43:38.
- Beltran-Gracia, E., Macedo-Raygoza, G., Villafañá-Rojas, J., Martínez-Rodríguez, A., Chavez-Castrillon, Y. Y., Espinosa-Escalante, F. M., Di Mascio, P., Ogura, T., and Beltran-Garcia, M. J. 2017. Production of lipopeptides by fermentation processes: Endophytic bacteria, fermentation strategies and easy methods for bacterial selection. Pages 199-222 in: *Fermentation Processes*. A. Faustino Jozala ed. InTech, London, U.K.
- Bier, J. E. 1939. Septoria canker of introduced and native hybrid poplars. *Can. J. Res.* 17c:195-204.
- Blacutt, A. A., Mitchell, T. R., Bacon, C. W., and Gold, S. E. 2016. *Bacillus mojavensis* RRC101 lipopeptides provoke physiological and metabolic changes during antagonism against *Fusarium verticillioides*. *Mol. Plant-Microbe Interact.* 29:713-723.
- Callan, B., Leal, I., Foord, B., Dennis, J. J., and van Oosten, C. 2007. *Septoria musiva* isolated from cankered stems in hybrid poplar stool beds, Fraser Valley, British Columbia. *Pac. Northwest Fungi* 2:1-9.
- Cao, Y., Pi, H., Chandransu, P., Li, Y., Wang, Y., Zhou, H., Xiong, H., Helmann, J. D., and Cai, Y. 2018. Antagonism of two plant-growth promoting *Bacillus velezensis* isolates against *Ralstonia solanacearum* and *Fusarium oxysporum*. *Sci. Rep.* 8:4360.
- Carroll, G. 1988. Fungal endophytes in stems and leaves: From latent pathogen to mutualistic symbiont. *Ecology* 69:2-9.

- Cawoy, H., Debois, D., Franzil, L., De Pauw, E., Thonart, P., and Ongena, M. 2015. Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis/amyloliquefaciens*. *Microb. Biotechnol.* 8:281-295.
- Coutte, F., Lecouturier, D., Dimitrov, K., Guez, J. S., Delvigne, F., Dhulster, P., and Jacques, P. 2017. Microbial lipopeptide production and purification bioprocesses, current progress and future challenges. *Biotechnol. J.* 12:1600566.
- Cregger, M. A., Veach, A. M., Yang, Z. K., Crouch, M. J., Vilgalys, R., Tuskan, G. A., and Schadt, C. W. 2018. The *Populus* holobiont: Dissecting the effects of plant niches and genotype on the microbiome. *Microbiome* 6:31.
- DeFilippi, S., Groulx, E., Megalla, M., Mohamed, R., and Avis, T. J. 2018. Fungal competitors affect production of antimicrobial lipopeptides in *Bacillus subtilis* Strain B9-5. *J. Chem. Ecol.* 44: 374-383.
- Deleu, M., Paquot, M., and Nylander, T. 2008. Effect of fengycin, a lipopeptide produced by *Bacillus subtilis*, on model biomembranes. *Biophys. J.* 94:2667-2679.
- de Melo, F. M. P., Fiore, M. F., de Moraes, L. A. B., Silva-Stenico, M. E., Scramin, S., Teixeira, M. de A., and de Melo, I. S. 2009. Antifungal compound produced by the cassava endophyte *Bacillus pumilus* MAIIM4a [Composto antifúngico produzido pelo endófito de mandioca *Bacillus pumilus* MAIIM4a]. *Sci. Agric.* 66:583-592.
- Derbowka, D. R., Andersen, S., Lee-Andersen, S., and Stenberg, C. 2012. Poplar and willow cultivation and utilization in Canada. 2008-2011 Canadian Country Progress Report. Canadian Report to the 24th IPC Session, Dehradun, India – International Poplar Commission for the period 2008-2011.
- dos Santos, Á. F., Machado, E. B., Stanosz, G. R., and Smith, D. R. 2010. First report of *Septoria musiva* in poplar in Brazil. *Trop. Plant Pathol.* 35:052-053.
- Doty, S. L., Freeman, J. L., Cohu, C. M., Burken, J. G., Firrincieli, A., Simon, A., Khan, Z., Isebrands, J. G., Lukas, J., and Blaylock, M. J. 2017. Enhanced degradation of TCE on a superfund site using endophyte-assisted poplar tree phytoremediation. *Environ. Sci. Technol.* 51:10050-10058.
- Fan, B., Blom, J., Klenk, H. P., and Borriess, R. 2017. *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* form an “operational group *B. amyloliquefaciens*” within the *B. subtilis* species complex. *Front. Microbiol.* 8:22.
- FAO. 2012. Improving lives with poplars and willows. Synthesis of Country Progress Reports. 24th Session of the International Poplar Commission, Dehradun, India, 30 Oct–2 Nov. Working Paper IPC/12. Forest Assessment, Management and Conservation Division, FAO, Rome, Italy.
- Feau, N., Mottet, M. J., Périnet, P., Hamelin, R. C., and Bernier, L. 2010. Recent advances related to poplar leaf spot and canker caused by *Septoria musiva*. *Can. J. Plant Pathol.* 32:122-134.
- Gyenis, L., and Ostry, M. E. 2003. Biological control of *Septoria* leaf spot disease of hybrid poplar in the field. *Plant Dis.* 87:809-813.
- Hamelin, R. 2016. #Chile's new #poplar #1 enemy septoria canker @nfeau @TAIGALab. Tweet.
- Herath, P., Beauseigle, S., Dhillon, B., Ojeda, D. I., Bilodeau, G., Isabel, N., Gros-Louis, M.-C., Kope, H., Zeglen, S., Hamelin, R. C., and Feau, N. 2016. Anthropogenic signature in the incidence and distribution of an emerging pathogen of poplars. *Biol. Invasions* 18:1147-1161.
- Herre, E. A., Knowlton, N., Mueller, U. G., and Rehner, S. A. 1999. Patterns of ecological transmission and evolutionary association. *Trends Ecol.* 14:49-53.
- Khan, Z., Rho, H., Firrincieli, A., Hung, S. H., Luna, V., Masciarelli, O., Kim, S.-H., and Doty, S. L. 2016. Growth enhancement and drought tolerance of hybrid poplar upon inoculation with endophyte consortia. *Curr. Plant Biol.* 6:38-47.
- Koumoutsis, A., Henne, A., Liesegang, H., Hitezeroth, G., Franke, P., Vater, J., and Borriess, R. 2004. Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive cyclic lipopeptides in *Bacillus amyloliquefaciens* strain FZB42. *J. Bacteriol.* 186:1084-1096.
- Lacercat-Didier, L., Berthelot, C., Foulon, J., Errard, A., Martino, E., Chalot, M., and Glaudez, D. 2016. New mutualistic fungal endophytes isolated from poplar roots display high metal tolerance. *Mycorrhiza* 26:657-671.
- LeBoldus, J. M., Blenis, P., Thomas, B. R., Feau, N., and Bernier, L. 2009. Susceptibility of *Populus balsamifera* to *Septoria musiva*: A field study and greenhouse experiment. *Plant Dis.* 93:1146-1150.
- Li, L., Ma, M., Huang, R., Qu, Q., Li, G., Zhou, J., Zhang, K., Lu, K., Niu, X., and Luo, J. 2012. Induction of chlamydospore formation in fusarium by cyclic lipopeptide antibiotics from *Bacillus subtilis* C2. *J. Chem. Ecol.* 38:966-974.
- Liang, H., Maynard, C. A., Allen, R. D., and Powell, W. A. 2001. Increased *Septoria musiva* resistance in transgenic hybrid poplar leaves expressing a wheat oxalate oxidase gene. *Plant Mol. Biol.* 45:619-629.
- Maxwell, D. L., Kruger, E. L., and Stanosz, G. R. 1997. Effects of water stress on colonization of poplar stems and excised leaf disks by *Septoria musiva*. *Phytopathology* 87:381-388.
- Mukherjee, A. K., and Das, K. 2005. Correlation between diverse cyclic lipopeptides production and regulation of growth and substrate utilization by *Bacillus subtilis* strains in a particular habitat. *FEMS Microbiol. Ecol.* 54:479-489.
- Newcombe, G., and Ostry, M. 2001. Recessive resistance to *Septoria* stem canker of hybrid poplar. *Phytopathology* 91:1081-1084.
- Ongena, M., and Jacques, P. 2008. *Bacillus* lipopeptides: Versatile weapons for plant disease biocontrol. *Trends Microbiol.* 16:115-125.
- Ostry, M. E. 1987. Biology of *Septoria musiva* and *Marssonina brunnea* in hybrid *Populus* plantations and control of *Septoria* canker in nurseries. *Eur. J. For. Pathol.* 17:158-165.
- Ostry, M. E., and McNabb, H. S., Jr. 1985. Susceptibility of *Populus* species and hybrids to disease in the north central United States. *Plant Dis.* 69:755-757.
- Pathak, K. V., and Keharia, H. 2014. Identification of surfactins and iturins produced by potent fungal antagonist, *Bacillus subtilis* K1 isolated from aerial roots of banyan (*Ficus benghalensis*) tree using mass spectrometry. *3 Biotech* 4:283-295.
- Pathak, K. V., Keharia, H., Gupta, K., Thakur, S. S., and Balam, P. 2012. Lipopeptides from the banyan endophyte, *Bacillus subtilis* K1: Mass spectrometric characterization of a library of fengycins. *J. Am. Soc. Mass Spectrom.* 23:1716-1728.
- Pavlo, A., Leonid, O., Iryna, Z., Natalia, K., and Maria, A. 2011. Endophytic bacteria enhancing growth and disease resistance of potato (*Solanum tuberosum* L.). *Biol. Control* 56:43-49.
- Qin, R., and LeBoldus, J. M. 2014. The infection biology of *Sphaerulina musiva*: Clues to understanding a forest pathogen. *PLoS One* 9:e103477.
- Raaijmakers, J. M., and Mazzola, M. 2012. Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. *Annu. Rev. Phytopathol.* 50:403-424.
- Rabiey, M., Hailey, L. E., Roy, S. R., Grenz, K., Al-Zadjali, M. A. S., Barrett, G. A., and Jackson, R. W. 2019. Endophytes vs tree pathogens and pests: Can they be used as biological control agents to improve tree health? *Eur. J. Plant Pathol.* 155:711-729.
- Razafindralambo, H., Paquot, M., Hbid, C., Jacques, P., Destain, J., and Thonart, P. 1993. Purification of antifungal lipopeptides by reversed-phase high-performance liquid chromatography. *J. Chromatogr. A.* 639:81-85.
- Ren, J.-H., Li, H., Wang, Y.-F., Ye, J.-R., Yan, A.-Q., and Wu, X.-Q. 2013. Biocontrol potential of an endophytic *Bacillus pumilus* JK-SX001 against poplar canker. *Biol. Control* 67:421-430.
- Romero, D., De Vicente, A., Rakotoaly, R. H., Dufour, S. E., Veening, J. W., Arrebola, E., Cazorla, F. M., Kuipers, O. P., Paquot, M., and Pérez-García, A. 2007. The iturin and fengycin families of lipopeptides are key factors in antagonism of *Bacillus subtilis* toward *Podosphaera fusca*. *Mol. Plant-Microbe Interact.* 20:430-440.
- Roongsawang, N., Washio, K., and Morikawa, M. 2011. Diversity of nonribosomal peptide synthetases involved in the biosynthesis of lipopeptide biosurfactants. *Int. J. Mol. Sci.* 12:141-172.
- Roy, G., Laflamme, G., Bussieres, G., and Dessureault, M. 2003. Field tests on biological control of *Heterobasidion annosum* by *Phaeothea dimorphospora* in comparison with *Phlebiopsis gigantea*. *For. Pathol.* 33:127-140.
- Sakalidis, M. L., Feau, N., Dhillon, B., and Hamelin, R. C. 2016. Genetic patterns reveal historical and contemporary dispersal of a tree pathogen. *Biol. Invasions* 18:1781-1799.

- Sarasola, A. A. 1944. Dos septorios is de las alamedas Argentinas. *Rev. Argent. Agron.* 11:20-43.
- Sari, E., Etebarian, H. R., and Aminian, H. 2007. The effects of *Bacillus pumilus*, isolated from wheat rhizosphere, on resistance in wheat seedling roots against the take-all fungus, *Gaeumannomyces graminis* var. *tritici*. *J. Phytopathol.* 155:720-727.
- Schmidt, C. S., Lovecká, P., Mrnka, L., Vychodilová, A., Střejček, M., Fenclová, M., and Demnerová, K. 2018. Distinct communities of poplar endophytes on an unpolluted and a risk element-polluted site and their plant growth-promoting potential in vitro. *Microb. Ecol.* 75:955-969.
- Schneider, T., Müller, A., Miess, H., and Gross, H. 2014. Cyclic lipopeptides as antibacterial agents—Potent antibiotic activity mediated by intriguing mode of actions. *Int. J. Med. Microbiol.* 304:37-43.
- Shimizu, K. 1994. Biological control of Septoria leaf spot on hybrid poplars using *Streptomyces* spp. M.S. thesis. University of Minnesota, St. Paul, MN.
- Spraker, J. E., Sanchez, L. M., Lowe, T. M., Dorrestein, P. C., and Keller, N. P. 2016. *Ralstonia solanacearum* lipopeptide induces chlamydospore development in fungi and facilitates bacterial entry into fungal tissues. *ISME J.* 10:2317-2330.
- Surovy, M. Z., Gupta, D. R., Chanclud, E., Win, J., Kamoun, S., and Islam, T. 2017. Plant probiotic bacteria suppress wheat blast fungus *Magnaporthe oryzae* Triticum pathotype. Figshare.
- Terhonen, E., Blumenstein, K., Kovalchuk, A., and Asiegbu, F. O. 2019. Forest tree microbiomes and associated fungal endophytes: Functional roles and impact on forest health. *Forests* 10:42.
- Thasana, N., Prapagdee, B., Rangkadilok, N., Sallabhan, R., Aye, S. L., Ruchirawat, S., and Loprasert, S. 2010. *Bacillus subtilis* SSE4 produces subtilene A, a new lipopeptide antibiotic possessing an unusual C15 unsaturated β -amino acid. *FEBS Lett.* 584:3209-3214.
- Torres, M. J., Pérez Brandan, C., Sabaté, D. C., Petroselli, G., Erra-Balsells, R., and Audisio, M. C. 2017. Biological activity of the lipopeptide-producing *Bacillus amyloliquefaciens* PGPBacCA1 on common bean *Phaseolus vulgaris* L. pathogens. *Biol. Control* 105:93-99.
- Waterman, A. M. 1946. Canker of hybrid poplar clones in the United States caused by *Septoria musiva*. *Phytopathology* 36:148-156.
- Waterman, A. M. 1954. Septoria canker of poplars in the United States. *U.S. Dep. Agric. Circ.* 947:1-22.
- Whipps, J. M. 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot.* 52:487-511.
- Yang, D., Plante, F., Bernier, L., Piche, Y., Dessureault, M., Laflamme, G., and Ouellete, G. B. 1993. Evaluation of a fungal antagonist, *Phaeotheca dimorphospora*, for biological control of tree diseases. *Can. J. Bot.* 71:426-433.
- Yuan, J., Zhao, M., Li, R., Huang, Q., Rensing, C., and Shen, Q. 2017. Lipopeptides produced by *B. amyloliquefaciens* NJN-6 altered the soil fungal community and non-ribosomal peptides genes harboring microbial community. *Appl. Soil Ecol.* 117-118:96-105.
- Zamioudis, C., and Pieterse, C. M. J. 2012. Modulation of host immunity by beneficial microbes. *Mol. Plant-Microbe Interact.* 25:139-150.
- Zerriouh, H., Romero, D., Garcia-Gutierrez, L., Cazorla, F. M., de Vicente, A., and Perez-Garcia, A. 2011. The iturin-like lipopeptides are essential components in the biological control arsenal of *Bacillus subtilis* against bacterial diseases of cucurbits. *Mol. Plant-Microbe Interact.* 24:1540-1552.
- Zhao, P., Xue, Y., Li, X., Li, J., Zhao, Z., Quan, C., Gao, W., Zu, X., Bai, X., and Feng, S. 2019. Fungi-derived lipopeptide antibiotics developed since 2000. *Peptides* 113:52-65.