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ORIGINAL ARTICLE

Surfactin-producing *Bacillus velezensis* 1B-23 and *Bacillus* sp. 1D-12 protect tomato against bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganensis*

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Abstract
Bacillus strains are known to produce cyclic lipopeptides that are capable of providing protection against plant pathogens. Such abilities could be utilized to protect greenhouse tomatoes against diseases including bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*). In the present study, *Bacillus velezensis* strain 1B-23 and *Bacillus* sp. strain 1D-12 were assessed for their potential biocontrol abilities against *Cmm* strain 98–1 (*Cmm*98–1). Both *Bacillus* strains interfered with growth of *Cmm*98–1 *in vitro*, as determined by agar plate assays to screen for microbial antagonism. Inoculation of *Cmm*98–1 infected tomato plants with *B. velezenis* 1B-23 or *Bacillus* sp. 1D-12 lead to significantly reduced disease incidence in a greenhouse setting. Liquid Chromatography coupled to Mass Spectrometry (LC-MS) of 1B-23 and 1D-12 extracts identified [Leu7]surfactin C13 (often called surfactin A), [Leu7]surfactin C14 (often called surfactin B) and [Leu7]surfactin C15 (often called surfactin C) in fractions of extracts that inhibited growth of *Cmm*98–1.

Keywords Antibacterial · Bacterial antagonism · Biological control · Secondary metabolite

Introduction

Clavibacter michiganensis subsp. *michiganensis* (*Cmm*) causes bacterial canker of tomato. This aerobic Gram-positive bacterium infects host plants vertically via seed or laterally through wounds or natural openings. It proliferates in xylem vessels, allowing progression to the rest of the plant. Gradual degradation of vascular tissue leads to wilting, and sometimes to cankers due to epidermal necrosis. Fruit quality and yield are often reduced (Nandi et al. 2018; Sen et al. 2015; Eichenlaub and Gartemann 2011).

Controlling *Cmm* is accomplished mainly through seed testing and hygienic practices such as sterilization of equipment and tools (Nandi et al. 2018). While various chemical pesticides can contribute to disease management (Werner et al. 2002), their cost, potential to encourage resistance, and the possibility of residues left on food (Baysal and Tör 2014) has prompted calls for alternative means of controlling disease in agriculture. Newer developments in crop disease management include using beneficial microorganisms as biocontrol agents (Baysal and Tör 2014; Chen et al. 2017).

A few microbes have already been investigated for their potential to control *Cmm*. These include various pseudomonads (Amkraz et al. 2010) which can mediate their effects via direct antagonism (Deng et al. 2017), possibly involving the metabolites 2,4-diacetylphloroglucinol (DAPG) and hydrogen cyanide (Lanteigne et al. 2012), or via salicyclic acid (SA)-dependent induced systemic resistance (Takishita et al. 2018), which hypersensitizes the plant to pathogenic threats. Various strains of *Streptomyces* can directly antagonize *Cmm in vitro* (Zhang et al. 2010), while the fungus *Pseudozyma aphidis* mediates a SA-independent induced resistance in plants (Barda et al. 2015)

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Additionally, *Cmm* has been shown to be antagonised by strains of *Bacillus subtilis* both *in vitro* and *in vivo*. *B. subtilis* strain DJM-51, its culture supernatant, and its butanol-extracted compounds are capable of producing zones of inhibition on nutrient-broth yeast extract agar plates containing *Cmm* (Jung et al. 2014). Under greenhouse conditions, *Bacillus subtilis* strains GBO3 (Girish and Umesha 2007), Quadra 136, and Quadra 137 (Utkhede and Koch 2004) significantly reduce disease incidence of bacterial canker caused by *Cmm*.

Bacillus strains are known to produce biologically active compounds including cyclic lipopeptides (Dunlap et al. 2011; Li et al. 2012; Zhao et al. 2017), which can be further classified into fengycins, surfactins, and iturins (Ongena and Jacques 2007; Mora et al. 2015). Surfactins are a group of compounds typically containing the heptapeptide ELLVDLL linked via lactone bond to a beta-hydroxy fatty acid. The various surfactins can differ in the identity of amino acids at the second, fourth, and seventh positions in the peptide, in the number of carbon atoms in the fatty acid chain, or in structural conformation. These are broad-spectrum antibiotics that function to disrupt bacterial membranes, and their non-specific interactions may help to prevent the development of resistance (Zhao et al. 2017).

In this work, *B. velezensis* 1B-23 and *Bacillus* sp. D-12 were isolated from a remediated potato rhizosphere in Norfolk County, Ontario, Canada, and assessed for their biocontrol abilities against the common tomato plant pathogens, *Cmm* and *Pseudomonas syringae* DC3000, using antibiotic plate assays and pathogen challenge in a greenhouse setting. Liquid Chromatography coupled to Mass Spectrometry (LC-MS) was then used to identify antimicrobial compounds.

Materials and methods

Isolation and identification of strains

Soil samples were collected from Blizman potato fields in Norfolk County, Ontario, Canada in the summer of 2012. Over the previous 3 years, bio-organic fertilizer was added to the soil each spring in an effort toward natural remediation. In 2012 (the fourth year), 10.0 g of moist soil was collected, placed in 95 mL of sterile water, and shaken for 10 min. Then, 1.0 mL of this suspension was transferred for serial dilution up to 10^{-10}, and the dilutions were plated on tryptic soy agar (TSA) for 48 h at 28 °C to attain single colonies (Grady et al. 2019).

Isolates 1B-23 and 1D-12 were identified as *Bacillus* species via 16 s rRNA gene sequencing. The complete genome of strain 1B-23 was subsequently sequenced (unpublished data) and deposited to the National Center for Biotechnology Information (NCBI) as Accession CP033967. To identify strain 1B-23 at the species level, the genome sequence data was uploaded to the Type (Strain) Genome Server (TYGS), available at https://tygs.dsmz.de, for a whole genome-based taxonomic analysis (Meier-Kolthoff and Göker 2019). Briefly, closely related type strains were identified by 16S rRNA gene sequence comparison with each of the 10,087 type strains available in the TYGS database. The 50 best matches were used to calculate precise genome distances using the Genome BLAST Distance Phylogeny (GBDP) approach under the algorithm 'coverage' and distance formula d_5. Strain 1B-23 was thus identified as *Bacillus velezensis*.

In vitro assessment of antimicrobial activity

In order to determine the effectiveness of *B. velezensis* 1B-23 and *Bacillus* sp. 1D-12 against common tomato plant pathogens, their abilities to inhibit pathogens were initially determined *in vitro*. The pathogenic strains *Cmm*98–1 and *Ps*DC3000 (Cuppels 1986) were obtained from Dr. Diane Cuppels, whose laboratory isolated them from diseased tomatoes. *B. velezensis* 1B-23, *Bacillus* sp. 1D-12, *Cmm*98–1, and *Ps*DC3000 were suspended separately in 0.85% NaCl to an optical density (600 nm) of 1.0, as determined using a SmartSpec Plus Spectrophotometer (Bio-Rad Laboratories Inc., Hercules, California, U.S.A.). Fifty microliters of the *B. velezensis* 1B-23 or *Bacillus* sp. 1D-12 preparation were used to inoculate 5 mm discs of P8 Filter Paper (Thermo Fisher Scientific, Pittsburgh, PA, USA). These antimicrobial discs were placed on solid LB medium plated with 100 μL of either the *Cmm*98–1 or *Ps*DC3000 preparation. Plates were sealed with Parafilm M (Bemis Company Inc., Oshkosh, WI, USA) and incubated for 72 h at 28 °C, after which zones of clearance around antimicrobial discs were measured.

Hydroponic tank inoculation

To begin examining the effects of *B. velezensis* 1B-23 and *Bacillus* sp. 1D-12 on pathogen infection of tomato plants, plants were grown in hydroponic medium as previously described (Nathoo et al. 2017) with some modifications. Briefly, an autoclaved, 90 mm × 90 mm stainless steel mesh square (mesh count of 30 × 30, wire diameter of 0.012) with its corners bent at 90 degrees was placed into each of 18 Petri plates containing 20 mL of liquid Murashige and Skoog (MS) medium (Murashige and Skoog 1962), such that MS medium is touching the bulk of the steel mesh square. Sterilized Terero Beefsteak Tomato seeds were placed on the mesh squares with one seed in each corner and one in the center. The Petri dishes were sealed with porous surgical tape, wrapped in aluminum foil and placed in a 4 °C refrigerator for 24 h, after which the foil was removed and plates were incubated at 24 °C with a 16 h photo period using F54 T5 fluorescent bulbs (Koninklijke Philips N.V., Amsterdam, Netherlands) with a

light intensity 190umol. After 96 h, sterilized forceps were used to lift the steel mesh square with the seeds out of the MS medium and into a sterilized hydroponic cylindrical tank made with a 100 mm × 80 mm glass crystallizing dish and lid (Nathoo et al. 2017) containing 20 mL of MS liquid medium. Tanks were sealed with porous surgical tape and incubated for a further 72 h with gentle shaking at 50 rpm, allowing plants to adapt to the environment before inoculation.

For inoculation, *B. velezensis* 1B-23, *Bacillus* sp. 1D-12, or *Cmm*98–1 were grown on solid LB medium for 24 h and resuspended in 0.85% NaCl to an optical density (600 nm) of 1.0. One hundred and fifty microliters of *B. velezensis* 1B-23 or *Bacillus* sp. 1D-12, and/or *Cmm*98–1 were added to the 20 ml of MS medium in the hydroponic tanks. After seven additional days, the entire plant (including roots) was separated from the bacterial suspension of the hydroponic system by lifting the mesh plate.

Greenhouse trials

Torero Beefsteak Tomatoes were grown from seed (Paramount Seeds Inc., Stuart, FL, USA; Lot 102,683,913/0161510486 94% O2/17 s/c 170,525/lb. untreated seeds) under greenhouse conditions (18 h light period with 170 μmol/m^2 s^1, 26 °C day,18 °C night, 65% relative humidity). Germination occurred in 15 cm × 15 cm × 5 cm plastic potting trays filled with 2/3 ProMix BX Mycorrhizae (Premier Tech Ltd., Rivière-du-Loup, Québec, Canada) and 1/3 Fine Vermiculite (Therm-O-Rock East Inc., New Eagle, PA, USA), and covered with a plastic dome until sprouting. Tomato plants were then transferred to 4-in. plastic pots.

Plants were inoculated with bacteria using either protocol 1 or protocol 2. For protocol 1, 54 tomato plants were divided into 9 groups with 6 plants per group: one group contained plants that were not inoculated with any bacteria, while the remaining groups were exposed to *B. velezensis* 1B-23, *Bacillus* sp. 1D-12, *Cmm*98–1, *Ps*DC3000, or the combinations *B. velezensis* 1B-23 + *Cmm*98–1, *B. velezensis* 1B-23 + *Ps*DC3000, *Bacillus* sp. 1D-12 + *Cmm*98–1, and *Bacillus* sp. 1D-12 + *Ps*DC3000. Liquid cultures of each bacterial strain were grown in 4 mL of LB broth and placed on a TC-7 Tissue Culture Roller (New Brunswick Scientific Co. Inc., Enfield, CT, USA) at 28 °C incubator for 24 h. The 4 mL liquid cultures were then transferred into 250 mL of LB broth for 72 h. Liquid cultures were then centrifuged at 6000 rpm for 10 min, and the pellet was resuspended in 0.85% NaCl to an optical density (600 nm) of 1.0. Thirty-five day old tomato plants were inoculated with *B. velezensis* 1B-23 or *Bacillus* sp. 1D-12 as a 5 mL drench to the roots. After 5 days to allow for plant-microbe interaction, a 5 mL root drench of *Cmm*98–1 or *Ps*DC3000 was added to the appropriate plants. Two weeks after pathogen inoculation, disease incidence was determined for each plant as the percent of leaves with visible lesions.

Protocol 2 was similar to protocol 1 except that the 5 mL *Bacillus* root drench was applied at an OD_{600} of 0.7 to 32-day old tomato plants ($n = 7$ per group), pathogens were applied 3 days later, and observations were made 30 days after pathogen application.

Statistical analyses were performed using GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA).

Chemical separation of metabolites

To determine and isolate the active compounds in *B. velezensis* 1B-23 and *Bacillus* sp. 1D-12, the strains were separately cultured in LB broth for 72 h at 28 °C and washed twice with an equal volume of 100% ethyl acetate in a separatory funnel. The upper, organic phase was collected and dried using 99% anhydrous sodium sulfate. The liquid phase of the crude metabolite solution was boiled off in a round-bottom flask using an IKA® RV10™ rotary evaporator running at 960 mBar and 160 rpm. Using the same round bottom flask, the dried metabolite was resuspended using 5 mL of 100% ethyl acetate. Next, the resulting suspension was transferred to a 20 mL scintillation vial and dried under nitrogen gas to form a precipitate. The precipitate was resuspended in 300 ml 100% acetonitrile to create a crude biologically-active metabolite solution to test for antibacterial activity.

The crude solution was used to infuse antimicrobial discs to verify activity against *Cmm*98–1, while acetonitrile was used for control discs. The crude solution was also subject to reverse-phase High Performance Liquid Chromatography on a 1260 Infinity Series (Agilent Technologies, Santa Clara, CA, USA) with a C18 silica gel stationary phase column eluted using an acetonitrile-water gradient with 0.1% formic acid. Separated fractions were dried, weighed, and re-dissolved in methanol to a concentration of 100 mg/mL, then tested again for activity against *Cmm*98–1 using methanol as a negative control. The fractions displaying antibacterial activity were selected for further chemical analysis.

Identification of antibacterial compounds

Fractions from HPLC showing antibacterial activity were subjected to Ultraviolet-visible spectroscopy at 210 nm in order to identify active compounds. The separated peaks from UV-vis spectroscopy were then tested again against *Cmm*98–1 to confirm antibacterial activity. Isolated peaks displaying antibacterial activity were then subject to analysis using the 1260 Infinity Series (Agilent Technologies, Santa Clara, CA, USA) LC system coupled with a Q-Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (MS) run with ESI positive mode (Thermo Fisher Scientific, Waltham, MA, USA). The LC mobile phase consisted of acetonitrile water with 0.1% formic acid and was passed through a C18 silica gel

stationary phase column over a 6-min gradient period. Upon completion of LC-MS analysis using XCalibur software (Thermo Fisher Scientific, Waltham, MA, USA), specific compounds responsible for antibacterial activity were identified by comparing formulae against the AntiBase 2012 Natural Compound Identifier database (Laatsch 2012).

Following identification, surfactin B was isolated and used at various concentrations to inoculate 0.5 mm discs of P8 Filter Paper (Thermo Fisher Scientific, Pittsburgh, PA, USA), which were then placed on LB agar plated with 100 μL of a 0.1 OD suspension of either the *Cmm*98–1 or *Ps*DC3000. Plates were sealed with Parafilm M (Bemis Company Inc., Oshkosh, WI, USA) and incubated for 72 h at 28 °C.

Results

Antagonism toward *Cmm in vitro*

To determine the effectiveness of *B. velezensis* 1B-23 and *Bacillus* sp. 1D-12 against phytopathogens, filter discs inoculated with these strains were placed onto LB agar plates cultured with either *Cmm*98–1 or *Ps*DC3000 (Fig. 1a). Clear zones of inhibition were observed against *Cmm*98–1, with a larger zone for *B. velezensis* 1B-23 compared to *Bacillus* sp. 1D-12. The experiment was replicated using *B. velezensis* 1B-23 (Fig. 1b), showing zones of inhibition against *Cmm*. Neither *B. velezensis* 1B-23 nor *Bacillus* sp. 1D-12 inhibited *Ps*DC3000.

Protection against *Cmm*98–1 in a hydroponic tank

The effects of *B. velezensis* 1B-23 and *Bacillus* sp. 1D-12 on *Cmm*98–1 inoculated tomato plants were assessed qualitatively following co-cultivation in hydroponic tanks. Plants that were co-cultivated with *Cmm*98–1 and either *B. velezensis* 1B-23 or *Bacillus* sp. 1D-12, appeared healthier than plants co-cultivated with *Cmm*98–1 alone, similar to controls (Fig. 2).

Reduction of *Cmm*98–1 disease incidence in greenhouse tomatoes

Disease symptoms were assessed for tomato plants grown under greenhouse conditions using two protocols for inoculation with *B. velezensis* 1B-23 or *Bacillus* sp. 1D-12 and the phytopathogens *Cmm*98–1 and *Ps*DC3000. Using either protocol 1 or protocol 2, plants treated with both *Cmm*98–1 and *B. velezensis* 1B-23, or both *Cmm*98–1 and *Bacillus* sp. 1D-12, showed a significant decrease in disease incidence ($P < 0.01$ or $P < 0.001$) compared to *Cmm*98–1 alone (Fig. 3). The difference in disease incidence between plants

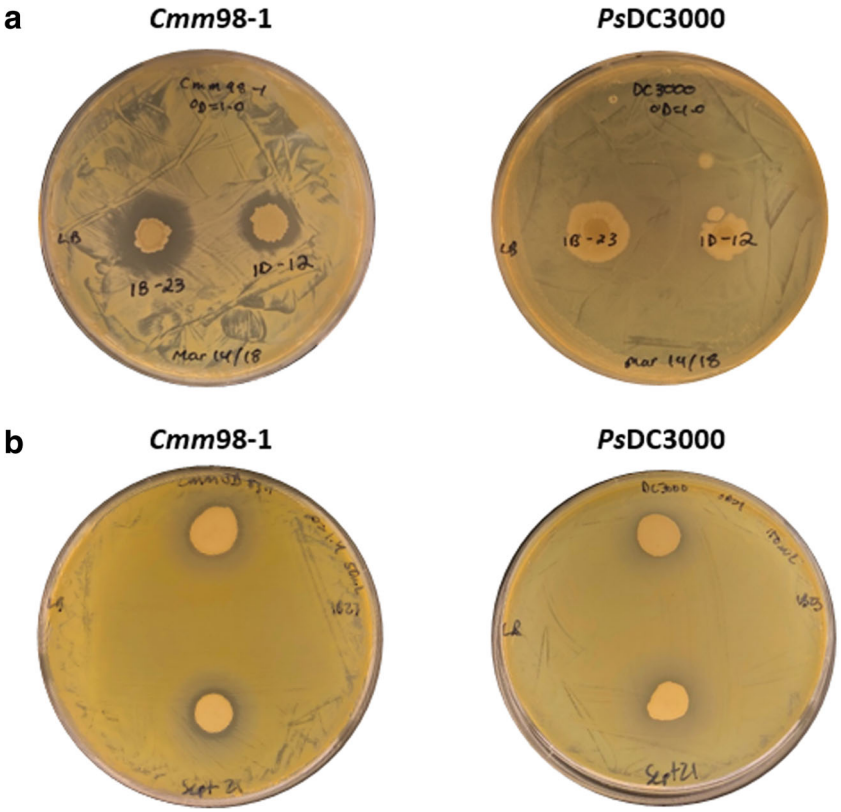

Fig. 1 ***Bacillus velezensis*** **1B-23 and** ***Bacillus*** **sp. 1D-12** ***in vitro*** **assessment against phytopathogens.** Pathogens were suspended in 0.85% NaCl and 100 μL was spread onto each LB plate. **a** Filter discs containing 50 μL of *B. velezensis* 1B-23 (left side of each plate) and *Bacillus* sp. 1D-12 (right side of each plate) were placed onto the pathogenic plates and incubated for 72 h. **b** Filter discs containing 50 μL of *B. velezensis* 1B-23 were placed onto the pathogenic plates and incubated for 72 h

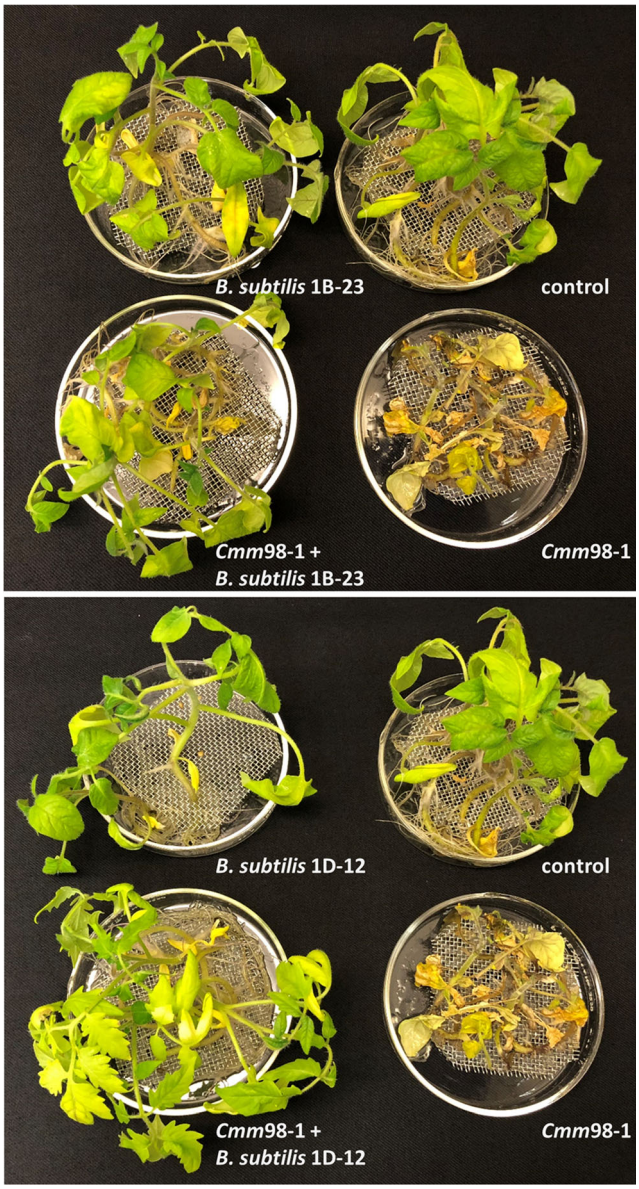

Fig. 2 Protection against *Cmm*98–1 in a hydroponic tank. Tomato plants were grown in hydroponic tanks with *B. velezensis* 1B-23, *Bacillus* sp. 1D-12, and *Cmm*98–1. Control plants were grown without added bacteria. Photographs were taken 7 days after pathogen inoculation

treated with both *Bacillus* and *Ps*DC3000 versus *Ps*DC3000 alone was not significant ($P > 0.05$; Fig. 3).

Production of antimicrobial compounds [Leu^7] surfactins C_{13}, C_{14} and C_{15}

Crude extracts from *B. velezensis* 1B-23 and *Bacillus* sp. 1D-12 were analyzed using LC-MS, revealing the presence of [Leu^7]surfactin C_{13} (often called surfactin A), [Leu^7]surfactin C_{14} (often called surfactin B) and [Leu^7]surfactin C_{15} (often called surfactin C) in the antibacterial fractions (Fig. 4). Surfactin B and surfactin C were present in greater quantities than surfactin A for both strains.

Filter discs inoculated with surfactin B produced zones of inhibition on *Cmm*98–1 plates at all tested concentrations, from 1 mg/mL. Zones of inhibition were not observed for *Ps*DC3000 at concentrations up to 10 mg/mL of surfactin B (Fig. 5).

Discussion

This study characterized *B. velezensis* 1B-23 and *Bacillus* sp. 1D-12 as potential biocontrol agents for tomato diseases. The *in vitro* assessment revealed clear zones of inhibition around *Cmm*98–1 in response to *B. velezensis* 1B-23 and *Bacillus* sp. 1D-12, indicating that these *Bacillus* strains secrete antibacterial compounds with activity against *Cmm*. This finding is consistent with a similar experiment using *Cmm*ATCC 7429 and *B. subtilis* DJM-51 (Jung et al. 2014). For *Cmm*98–1 inhibition by *B. velezensis* 1B-23, more prominent zones of clearing were observed on some plates compared to others. Because the replicate plates were run on different dates, this varying degree of inhibition could be due to some difference in the growth stage of either inoculant (1B-23 or *Cmm*98–1). In support of this explanation, a recent study found that the age of cultures of *B. subtilis* strain A18 and of *Heterobasidion* spp. (fungi) affected the ability of the former to inhibit the latter *in vitro* (Azeem et al. 2019).

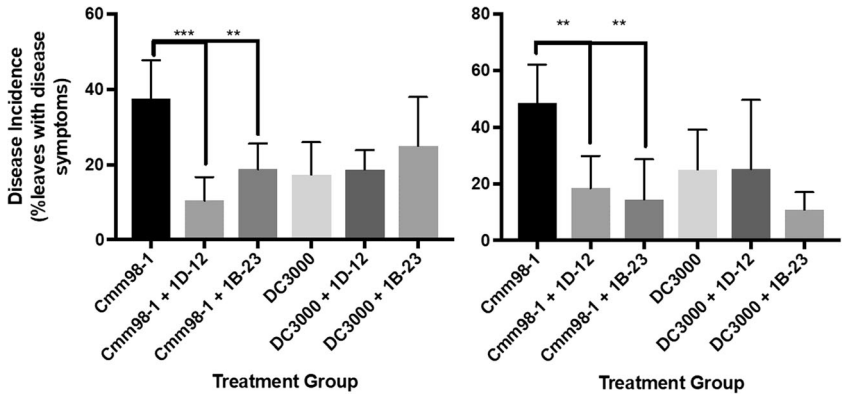

Fig. 3 Disease Incidence of tomato plants after pathogen inoculation. Mean disease incidence ± S.D. using Protocol 1, $n = 6$ (left panel). Mean disease incidence ± S.D. using Protocol 2, $n = 7$ (right panel). Data analysis involved one-way ANOVA followed by Tukey's multiple comparisons test (**, $P < 0.01$; ***, $P < 0.001$)

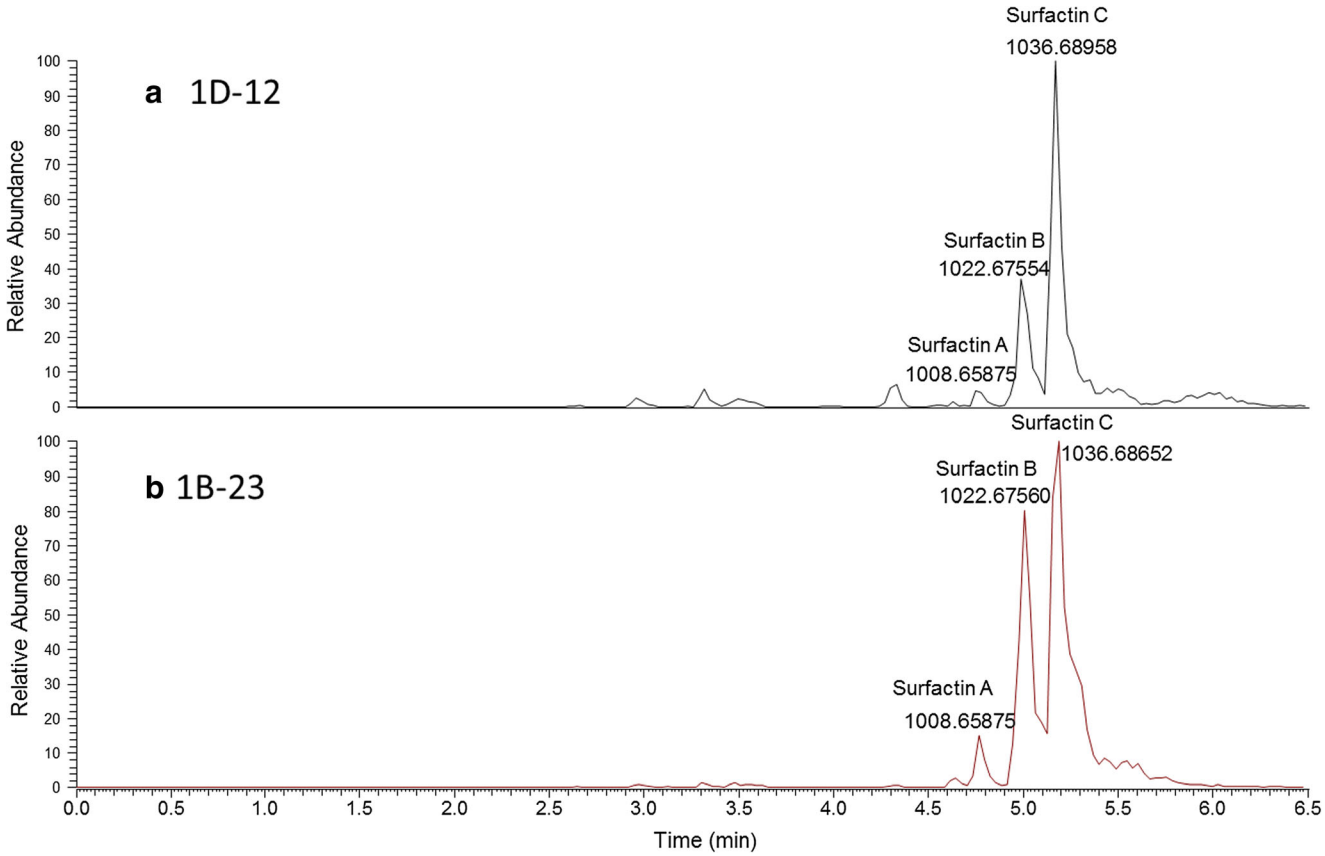

Fig. 4 Liquid chromatography coupled to mass spectrometry analysis of crude *B. velezensis* 1B-23 and *Bacillus* sp. 1D-12 surfactin abundance. Crude solutions of *B. velezensis* 1B-23 and *Bacillus* sp. 1D-12 were subject to liquid chromatography and mass spectrometry, indicating the presence of surfactin A, B, and C

To identify the potential antibacterial compounds, LC-MS analysis was performed on fractions of *B. velezensis* 1B-23 and *Bacillus* sp. 1D-12 extracts that inhibited growth of *Cmm*, revealing the presence of [Leu^7]surfactin C_{13} (often called surfactin A), [Leu^7]surfactin C_{14} (often called surfactin B) and [Leu^7]surfactin C_{15} (often called surfactin C). A previous study also implicates the presence of [Leu^7]surfactins C_{14} and C_{15} in inhibition of *Cmm* by *Bacillus velezensis* 9D-6 (Grady et al. 2019), suggesting that one or both of these surfactins may be relevant in a variety of *Bacillus* strains.

Despite the non-specific, broad-spectrum antibacterial properties of surfactin, neither *B. velezensis* 1B-23 (this study) nor *B. velezensis* 9D-6 (Grady et al. 2019) is able to appreciably inhibit *in vitro* growth of *Ps*DC3000. This may be due to the presence of resistance mechanisms in *Pseudomonas* that occur because this genus commonly produces its own biosurfactants (Raaijmakers et al. 2010), with at least one strain shown to produce surfactin itself (Xia et al. 2014). Still, some studies suggest that surfactin produced by *B. subtilis* can inhibit *Pseudomonas syringae* at relatively high

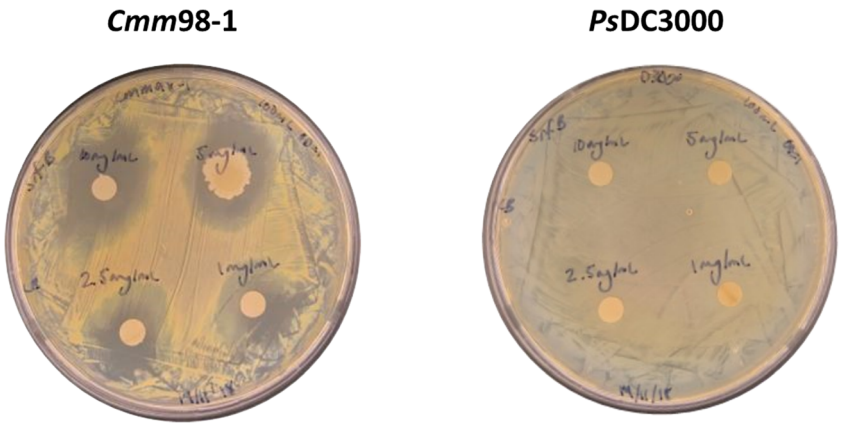

Fig. 5 Surfactin B inhibits *Cmm*98–1 *in vitro*. *Cmm*98–1 or *Ps*DC3000 was suspended in 0.85% NaCl to an OD_{600} = 1.0 and plated on LB plates. Surfactin B was used to inoculate filter discs at the indicated concentrations (clockwise from top left: 10 mg/mL, 5 mg/mL, 2.5 mg/mL, and 1 mg/mL), which were placed on the plates and incubated for 48 h at 28 °C

concentrations (25 μg on an antibacterial disc or 25 μg/mL in liquid) (Bais et al. 2004).

In addition to their antibiotic properties, various surfactins play a role in biofilm formation, which may in turn contribute to enhanced biocontrol *in vivo*. Surfactin-deficient *Bacillus* display reduced biofilm and reduced root colonization, which coincides with poorer biocontrol against phytopathogens (Bais et al. 2004; Zeriouh et al. 2014; Aleti et al. 2016).

The effects of *B. velezensis* 1B-23 and *Bacillus* sp. 1D-12 on *Cmm*-infected, greenhouse-grown tomatoes indicate that these strains can reduce disease symptoms *in vivo* and in a commercially relevant setting. Exposing tomato plants to *B. velezensis* 1B-23 or *Bacillus* sp. 1D-12 three to five days prior to *Cmm*98–1 exposure significantly reduced disease symptoms versus exposure to *Cmm*98–1 alone. Similarly, *Cmm* disease incidence in tomato is reduced by three bacilli, *B. subtilis* GBO3, *Bacillus amyloliquefaciens* IN937a, and *Brevibacillus brevis* IPC11 (Girish and Umesha 2007). While that study attributed the reduction to induced host resistance, it did not seek to characterize any antimicrobial compounds (Girish and Umesha 2007). In contrast, exposure to *B. velezensis* 1B-23 or *Bacillus* sp. 1D-12 did not reduce tomato disease symptoms due to *Ps*DC3000. It is worth noting that the potting mix used in our study contains mycorrhizal fungus, which may enhance the level of disease control of *Cmm* by bacilli through an unexplored synergistic mechanism.

Our results add to a limited knowledge base on biocontrol of the tomato pathogen *Cmm.* They suggest *B. velezensis* 1B-23, *Bacillus* sp. 1D-12, or other surfactin-producing microbes as potential biocontrol agents against this important crop pathogen.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Aleti G, Lehner S, Bacher M, Compant S, Nikolic B, Plesko M, Schuhmacher R, Sessitsch A, Brader G (2016) Surfactin variants mediate species-specific biofilm formation and root colonization in *Bacillus*. Environ Microbiol 18:2634–2645

Amkraz N, Boudyach EH, Boubaker H, Bouizgarne B, Ait Ben Aoumar A (2010) Screening for fluorescent pseudomonades, isolated from the rhizosphere of tomato, for antagonistic activity toward *Clavibacter michiganensis* subsp. *Michiganensis*. World J Microb Biot 26:1059–1065

Azeem M, Barba-Aliaga M, BorgKarlson AK, Terenius O, Broberg A, Rajarao GK (2019) Heterobasidion-growth inhibiting *Bacillus subtilis* A18 exhibits medium- and age-dependent production of lipopeptides. Microbiol Res 223-225:129–136

Bais HP, Fall R, Vivanco JM (2004) Biocontrol of *Bacillus subtilis* against infection of *Arabidopsis* roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. Plant Physiol 134:307–319

Barda O, Shalev O, Alster S, Buxdorf K, Gafni A, Levy M (2015) *Pseudozyma aphidis* induces salicylic-acid-independent resistance to *Clavibacter michiganensis* in tomato plants. Plant Dis 99:621–626

Baysal Ö, Tör M (2014) Smart biologics for crop protection in agricultural systems. Turk J Agric For 38:723–731

Chen J, Wu Q, Hua Y, Chen J, Zhang H, Wang H (2017) Potential applications of biosurfactant rhamnolipids in agriculture and biomedicine. Appl Microbiol Biotechnol 101:8309–8319

Cuppels DA (1986) Generation and characterization of Tn5 insertion mutations in *Pseudomonas syringae* pv. tomato. Appl Environ Microbiol 51:323–327

Deng Q, Wang W, Sun L, Wang Y, Liao J, Xu D, Liu Y, Ye R, Gooneratne R (2017) A sensitive method for simultaneous quantitative determination of surfactin and iturin by LC-MS/MS. Anal Bioanal Chem 409:179–191

Dunlap CA, Schisler DA, Price NP, Vaughn SF (2011) Cyclic lipopeptide profile of three *Bacillus subtilis* strains: antagonists of *Fusarium* head blight. J Microbiol 49:603–609

Eichenlaub R, Gartemann K (2011) The *Clavibacter michiganensis* subspecies: molecular investigation of gram-positive bacterial plant pathogens. Annu Rev Phytopathol 49:445–464

Girish N, Umesha S (2007) Effect of plant growth promoting rhizobacteria on bacterial canker of tomato. Arch Phytopath Plant Protec 5408:235–243

Grady EN, MacDonald J, Ho MT, Weselowski B, McDowell T, Solomon O, Renaud J, Yuan ZC (2019) Characterization and complete genome analysis of the surfactin-producing, plant-protecting bacterium *Bacillus velezensis* 9D-6. BMC Microbiol 19:5

Jung WJ, Mabood F, Souleimanov A, Whyte LG, Niederberger TD, Smith DL (2014) Microbial pathogenesis antibacterial activity of antagonistic bacterium *Bacillus subtilis* DJM-51 against phytopathogenic *Clavibacter michiganense* subsp. *michiganense* ATCC 7429 in vitro. Microb Pathog 77:13–16

Laatsch H (2012) AntiBase 2012 upgrade: the natural compound identifier. Wiley-VCH, Weinheim

Lanteigne C, Gadkar VJ, Wallon T, Novinscak A, Filion M (2012) Production of DAPG and HCN by *Pseudomonas* sp. LBUM300 contributes to the biological control of bacterial canker of tomato. Phytopathology 102:967–973

Li X, Mao Z, Wang Y, Wu Y, He Y (2012) ESI LC-MS and MS/MS characterization of antifungal cyclic lipopeptides produced by *Bacillus subtilis* XF-1. J Mol Microbiol Biotechnol 22:83–93

Meier-Kolthoff JP, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182

Mora I, Cabrefiga J, Montesinos E (2015) Cyclic lipopeptide biosynthetic genes and products, and inhibitory activity of plant-associated *Bacillus* against phytopathogenic bacteria. PLoS One 10:e0127738

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plantarum 15:473–497

Nandi M, MacDonald J, Liu P, Weselowski B, Yuan ZC (2018) *Clavibacter michiganensis* subsp. *michiganensis*: bacterial canker

of tomato, molecular interactions and disease management. Mol Plant Pathol 19:2036–2050

Nathoo N, Bernards MA, MacDonald J, Yuan ZC (2017) A hydroponic co-cultivation system for simultaneous and systematic analysis of plant/microbe molecular interactions and signaling. J Vis Exp 125: e55955

Ongena M, Jacques (2007) *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol 16:115–125

Raaijmakers JM, De Bruijn I, Nybroe O, Ongena M (2010) Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. FEMS Microbiol Rev 34:1037–1062

Sen Y, van der Wolf J, Visser RGF, van Heusden S (2015) Bacterial canker of tomato: current knowledge of detection, management, resistance, and interactions. Plant Dis 99:4–13

Takishita Y, Charron JB, Smith DL (2018) Biocontrol rhizobacterium *Pseudomonas* sp. 23S induces systemic resistance in tomato (*Solanum lycopersicum* L.) against bacterial canker *Clavibacter michiganensis* subsp. *michiganensis*. Front Microbiol 9:2119

Utkhede R, Koch C (2004) Biological treatments to control bacterial canker of greenhouse tomatoes. BioControl 49:305–313

Werner NA, Fulbright DW, Podolsky R, Bell J, Hausbeck MK (2002) Limiting populations and spread of *Clavibacter michiganensis* subsp. *michiganensis* on seedling tomatoes in the greenhouse. Plant Dis 86:535–542

Xia W, Du Z, Cui Q, Dong H, Wang F, He P, Tang Y (2014) Biosurfactant produced by novel *Pseudomonas* sp. WJ6 with biodegradation of n-alkanes and polycyclic aromatic hydrocarbons. J Hazard Mater 276: 489–498

Zeriouh H, de Vicente A, Pérez-García A, Romero D (2014) Surfactin triggers biofilm formation of *Bacillus subtilis* in melon phylloplane and contributes to the biocontrol activity. Environ Microbiol 16: 2196–2211

Zhang W, Yang W, Meng Q, Li Y, Liu D (2010) Screening and identification of antagonistic *Streptomyces* spp. against *Clavibacter michiganensis* subsp. *michiganensis* from tomato rhizosphere. Front Agric China 4:159–164

Zhao H, Shao D, Jiang C, Shi J, Li Q, Huang Q, Rajoka MSR, Yang H, Jin M (2017) Biological activity of lipopeptides from *Bacillus*. Appl Microbiol Biotechnol 101:5951–5960