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ORIGINAL ARTICLE

Identification and characterization of a cyclic lipopeptide iturin A from a marine-derived *Bacillus velezensis* 11-5 as a fungicidal agent to *Magnaporthe oryzae* in rice

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Abstract
Magnaporthe oryzae is an important rice pathogen globally. However, plant beneficial microbes and their secondary metabolites to control blast disease in rice are poorly understood. In the present study, a marine-derived *Bacillus velezensis* 11-5 has been characterized as an antagonist against an isolate of *Magnaporthe oryzae*, the causal agent of rice blast disease. A cyclic lipopeptide (CLP) iturin A has been identified from the fermentation broth of *B. velezensis* 11-5 by nuclear magnetic resonance spectroscopy and mass spectrometry. In addition, the in vitro and *in planta* biocontrol activities of CLP iturin A were evaluated in the further study, respectively. The results revealed that iturin A shows significant activity on the conidia germination and the relative appressoria formation rate of *M. oryzae* at the concentrations of 10 and 50 μM for 12 h and for 24 h, respectively. In addition, CLP iturin A shows the significant activity to control *M. oryzae* in rice plants when the concentration of the compound is higher than 10 μM. Taken all results together, this study shows that *B. velezensis* 11-5 has the possibility to be developed as a biopesticide to control rice blast disease in rice plants, and for the first time, this study shows a CLP iturin A produced by *B. velezensis* 11-5 is an agrochemical to control rice blast disease both in vitro and in rice. And therefore, the results provide information on the application of *B. velezensis* 11-5 and its CLP iturin A as potential biopesticides to control blast disease in rice for agricultural practice.

Keywords *Bacillus velezensis* · Iturin A · Nuclear magnetic resonance · Mass spectrometry · *Magnaporthe oryzae* · *Oryza sativa* L.

Introduction

Rice is an important staple food worldwide. However, rice blast disease is a devastating threat to the production of the rice in the region of the rice plantation, especially in tropical and subtropical areas. *Magnaporthe oryzae* (Anamorph: *Pyricularia oryzae*), a hemibiotroph pathogen, is the causal agent of blast disease in rice. A recent survey shows that *M. oryzae* ranks the first place among fungal plant pathogens (Dean et al. 2012). *M. oryzae* has the capacity to infect rice (Marcel et al. 2010). The infection of *M. oryzae* in rice mainly includes the germination of its conidia, the formation of appressoria on germ tubes of its hyphae, the penetration by appressoria on the surface of the rice tissues, the aggressive growth of *M. oryzae* in rice tissues and the formation of blast disease in rice (Wilson and Talbot 2009).

Due to the increasing needs for the public attention to developing the integrated pest management strategy to control plant diseases such as *M. oryzae*, developing plant

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beneficial microbes or their natural products as potent biopesticides is of utmost importance to deal with these public needs (Hofte and Altier 2010; Velivelli et al. 2014). *Bacillus* spp. can form spores, and these spores are relatively stable and are easy to be formulated and stored; thus, *Bacillus* spp. are notably candidates used for biocontrol research in the laboratory and for commercialized biopesticides in field trials and applications (Ongena and Jacques 2008; Velivelli et al. 2014). *Bacillus* spp. have the capacity to secrete biocontrol-related metabolites in situ, including those important metabolites CLPs. For instance, a recent study has confirmed the presence of iturin-, fengycin- and surfactin-type CLPs in the rhizosphere soil of lettuce (Chowdhury et al. 2015b). Moreover, it has been reported that CLPs produced by *Bacillus* spp. are the major antibiotic component displaying antagonistic activities to the fungal plant pathogens (Cawoy et al. 2015).

Previous reports have shown that certain *Bacillus* strains have biocontrol abilities to *M. oryzae* (Amruta et al. 2018; Sha et al. 2016; Yang et al. 2008; Zhang et al. 2014, 2015). It is worth mentioning that antifungal proteins and natural products (macrolactins and CLPs) characterized from *Bacillus* strains are determinants contributing to direct antifungal activity on *M. oryzae* (Cui et al. 2012; Ghasemi et al. 2011; Kouzai et al. 2012; Liao et al. 2016; Liu et al. 2007; Roh et al. 2009; Tendulkar et al. 2007; Xue et al. 2008). However, the detailed mechanisms of the interaction between *Bacillus* spp. and *M. oryzae* are poorly understood up to date, especially the roles of bacterial determinants produced by *Bacillus* spp. on the control of the *M. oryzae* in rice plants remain to be elucidated.

We recently isolated a strain *B. velezensis* 11-5 from a marine sediment sample, and in vitro assay displayed that *B. velezensis* 11-5 has excellent antibiotic activities against *M. oryzae*. The aim of this study is to investigate the bacterial strain by molecular phylogeny analysis, and to elucidate the chemical structure of active metabolites corresponding to control *M. oryzae* using multiple spectroscopic methods, and to evaluate the biological activities of the purified compound against *M. oryzae* under both in vitro and in rice, respectively.

Materials and methods

Strain isolation, media and cultural conditions

A marine sediment sample was collected from the South China Sea (118° 30.989′, 18° 5.255′) in the depth of 3928 m. The 0.5-g soil sample was suspended and homogenized well in 1 mL sterilized saline solution (0.85% sodium chloride, w/v), and the solution was diluted and plated on the agar plates for the strain isolation. Bacterial strains were routinely grown on Luria–Bertani (LB) (Sambrook et al. 1989) agar, and bacterial cells were suspended in 20% (v/v) glycerol solution and were stored at minus 80 °C condition. The gradient of the mineral salts medium (MSM) used for bacterial secondary metabolite production was shown previously (Ma et al. 2014). An isolate of *M. oryzae* (Lab stock) was routinely maintained on oatmeal agar (Thuan et al. 2006), and *M. oryzae* was cultured for 14 days for sporulation. Unless stated otherwise, microbes used in this study were incubated at 28 °C.

DNA manipulation, sequencing and phylogeny analysis

Bacterial strain was cultured on LB medium for 48 h. DNA was isolated by a kit (EZ-10 Spin Column Bacterial Genomic DNA Isolation Kit) from Sangon Biotech (Shanghai) Co., Ltd., China, following an instruction provided by the manufacturer. 16S rRNA sequence of the bacterial strain was used for phylogeny analysis. More specifically, universal primer pairs 8F (5′→3′: AGAGTTTGATCCTGG CTCAG) (Felske et al. 1997) and 1492R (5′→3′: TACCTT GTTACGACTT) (Lane 1991) were used in the polymerase chain reaction (PCR) for amplification of the 16S rRNA by applying the bacterial DNA as a template. 16S rRNA from the strain was detected for purity by gel electrophoresis (1% agarose, w/v), and the PCR product was directly sequenced by Sangon Biotech (Shanghai) Co., Ltd., China. Finally, the phylogeny tree of the bacterial strain was constructed by the Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 (Tamura et al. 2013).

Isolation and purification of CLPs

Bacterial strain was cultured on LB medium for 48 h, and a single colony was selected and inoculated into the 250-mL flask containing 50 mL of MSM medium and was maintained on a shaker (180 rpm) for 20 h. Then, bacterial cultures were transferred into 3-L flasks containing 1 L of MSM medium and maintained on a shaker (180 rpm) for 48 h, and the bacterial cultures were yielded subsequently. The bacterial supernatant was collected by centrifugation (4000 rpm for 20 min) of the bacterial cultures. The bacterial supernatant was adjusted to pH 2.0 by 6 N hydrochloric acid and kept overnight at 4 °C. Then, the precipitates were collected by centrifugation at 4000 rpm for 20 min and were extracted by methanol three times to ensure the maximum yield of the crude metabolite. Then, the organic layer was collected by centrifugation at 180 rpm for 10 min, the organic solvent was removed by a rotary evaporator under vacuum, and the crude extract was yielded. Then, the crude extract was dissolved in 70% (v/v) methanol/H_2O and the solution was loaded over a solid phase extraction (SPE) flash column (C18, 4 g,

20–45 µm, 100 Å, Agela technologies, Tianjin, China). And then the loaded SPE column was eluted by 80%, 90% and 100% (v/v) methanol/H_2O solutions and then three fractions were yielded. These fractions were further analyzed by a reversed-phase high-performance liquid chromatography (RP-HPLC) system (Dionex U3000, Sunnyvale, CA, USA) equipped with a C18 YMC-Pack ODS-A column (5 µm, φ 4.6 mm × 250 mm, YMC Co., Ltd., Japan), and a C18 YMC-Pack ODS-A column (5 µm, φ 10 mm × 250 mm, YMC Co., Ltd., Japan) was used for semi-preparative purification of the targeted compound; the details for analysis of the samples using RP-HPLC were shown previously (Ma and Hu 2018).

MS analysis

A TSQ Quantum Access Max Triple Quadrupole mass spectrometer (Thermo Scientific, San Jose, CA, USA) equipped with a RP-HPLC column (φ 2.1 × 150 mm, Waters Atlantis T3 Column, Waters Corporation, Dublin, Ireland) was used for liquid chromatography–mass spectrometry (LC–MS) and tandem mass spectrometry (MS/MS) analysis in this study. The wavelength of the ultraviolet (UV) detector was set up at 220 nm. The detection under mass spectrometer was set up either in positive mode or in negative mode.

NMR analysis

Purified compound (10 mg) was dissolved in 0.6 mL deuterated dimethyl sulfoxide (DMSO-d_6, $\delta_H = 2.49$ ppm, $\delta_C = 39.6$ ppm) and submitted for NMR spectroscopic analysis. The NMR spectroscopic data (^{1}H-NMR and ^{13}C-NMR) of the compound were recorded on a Bruker AV600 spectrometer (Leipzig, Germany, 600.13 MHz for ^{1}H-NMR, 150.90 MHz for ^{13}C NMR, respectively) at the temperature 297.9 K.

In vitro biocontrol assay

Stock solutions of the crude extract or purified compound from the bacterial strain were dissolved and homogenized well in DMSO, and then the stock solutions were diluted and applied to the desired concentrations for the further biological test. Unless stated otherwise, 50 µg/mL concentration of the crude extract and 1, 10 and 50 µM concentrations of the purified compound were used for bioassays in this study, respectively. And the same amount of DMSO was added to each corresponding control. Spores of *M. oryzae* were prepared by adding 10 mL sterilized H_2O in a two-week-old oatmeal plate, and the mycelia of the *M. oryzae* were scraped off from the surface of the plate and suspended carefully in the sterilized H_2O. The mycelia were removed by filtration, and the conidia suspension was prepared. Then, the conidia suspension was adjusted to approximately 1×10^5 CFU/mL. The conidia germination assay was conducted by following a protocol published previously (Ma and Hu 2018). Briefly, 10 µL volume of treated conidia suspension was dropped carefully on a microscopic slide and kept in a moistened condition for 12 h and 24 h, respectively. Unless stated otherwise, an Olympus TH4-200 Microscope (Olympus Corporation, Tokyo, Japan) was used for microscopic observation and data collection. The germination rate and the relative appressoria formation of the conidia of *M. oryzae* were calculated from at least 50 spores for each treatment, and the data were collected from three independent assays and were presented as means (± standard deviation, S.D.).

In planta biocontrol assay

Seeds of rice (*Oryza sativa* L. ZhenDao 99) were routinely used for plant assays in this study. The surface of the rice seeds was sterilized by 1% (w/v) sodium hypochlorite solution for 2 min, and then the seeds were rinsed with sterilized H_2O 5 times to remove extra chemicals. Rice seeds were germinated on the moistened (relative humidity ≥ 90%, 28 °C) filter paper in the round (Diameter = 9 cm) Petri dishes for 5 days. And then the germinated rice seedlings were transferred to soil (Pindstrup substrate for seeding, pH = 5.5, The Pindstrup Group) and then maintained in a light growth chamber (HPG-280BX, HDL Apparatus, Beijing Donglian Har Instrument Manufacture Co., Ltd., photoperiod with 12 h light/12 h dark, 25 °C). Four-week-old rice plants (five-leaf stage) were used for planta assays in this study. The second youngest leaves of the rice plants were detached and used for biocontrol assays. 10 µL volume of the treated spore suspension of *M. oryzae* was dropped carefully on the surface of detached leaves, and then the detached leaves were kept in a moistened (Relative humidity ≥ 90%, 25 ± 5 °C) condition on a laboratory bench for disease development. Disease assessment was conducted 7 days post-inoculation (dpi) from triplicate repeats, by calculating the mean area (mm^2 ± S.D.) of blast lesions with Assess 2.0 software (American Phytopathological Society).

Statistical analysis

The variance analysis of the data was conducted using the Statistical Package for the Social Sciences (SPSS 16.0; SPSS Inc., Chicago, USA). The mean values for germination rate and for relative appressoria formation rate among the different treatments were compared using the Turkey's test ($p = 0.05$), while the Dunnett's test was used for comparing the planta biological control data of different concentrations of iturin A treatments with control treatment ($p = 0.05$).

Results

Isolation and molecular characterization of the bacterial isolate

A *Bacillus* strain was isolated from the marine-derived sediment sample and was dubbed 11-5, and activity test of the bacterial supernatant from the MSM culture of the strain 11-5 on the conidia germination assay of *M. oryzae* suggests that the strain 11-5 shows excellent activity to reduce the conidia germination of the fungus (data not shown). Thus, the strain 11-5 displayed the potential as a biopesticide to control *M. oryzae*.

A 1.3 kb DNA fragment of 16S rRNA (GenBank accession number: MH348952) was amplified by PCR from the genomic DNA of the strain 11-5. The 16S rRNA sequences of the selected type strains of *Bacillus* spp. were retrieved from the GenBank database. The 16S rRNA sequences of *Bacillus* spp. were aligned by MUSCLE, and the tree was constructed by MEGA 6.0 using the neighbor-joining method (Bootstrap replications = 1000). Results from phylogeny analysis (Fig. 1) show that the 16S rRNA of the strain 11-5 has the highest similarity with the type strain *B. velezensis* LMG 22478^{T} (AB245422), indicating that the strain 11-5 belongs to the *B. velezensis* group.

Bioactivity-guided purification of the compound

To isolate and characterize the active secondary metabolite in the liquid MSM supernatant of *B. velezensis* 11-5 responsible for the suppression of the conidia germination of *M. oryzae*, a large-scale fermentation process has been conducted using the method mentioned in materials and methods part. Finally, 2.04 g of crude extract was obtained from 8 L bacterial supernatant of *B. velezensis* 11-5.

The crude extract was further fractionated on a SPE column and 80%, 90% and 100% (v/v) methanol/H_2O fractions were yielded, respectively. These fractions were dried, weighted, dissolved in DMSO and applied to the final concentration of 50 µg/mL in conidia suspension of *M. oryzae* to evaluate the conidia germination. The results showed that 80% and 90% SPE fractions display significant activities on the inhibition of the conidia germination of *M. oryzae* (data not shown). Further, LC–MS measurement of the two fractions showed that 80% SPE fraction (Fig. 2a) contained several compounds. While 90% SPE fraction contained a series of compounds different from the compounds from 80% SPE fraction, the mass-to-charge ratio (*m/z*) of them shows that these compounds could be the homologs (Ma Z., et al., unpublished data).

Therefore, we chose 80% SPE fraction of *B. velezensis* 11-5 to study the main compound responsible for the antibiotic activity against the sporulation of *M. oryzae*. The main compound (**1**) (Fig. 2b) in the 80% SPE fraction was purified by a semi-preparative RP-HPLC, the sufficient amount of the compound **1** was finally obtained for further structural identification and bioactivity tests.

MS and NMR characterization

LC–MS spectrum (Fig. 2c) of the 80% SPE fraction from the crude extracts of *B. velezensis* 11-5 shows a group of ion peaks (*m/z*, Table 1) for $[M+H]^{+}$) at 1043.35, 1057.33, 1057.37, 1057.40, 1071.38 and 1071.40, their corresponding ion peaks (*m/z*) for $[M+Na]^{+}$ at 1065.28, 1079.40, 1079.41, 1079.36, 1093.40, 1093.48, for ions ($[M+K]^{+}$) at *m/z* 1081.14, 1095.20, 1095.42, 1095.22, 1109.32, 1109.35, and their corresponding ion peaks (*m/z*) for $[M+2H]^{2+}$ at 522.16, 529.17, 529.15, 529.38, 536.27, 536.16, respectively. These LC–MS results indicate that the peaks from 80% SPE fraction of the crude extract of *B. velezensis*

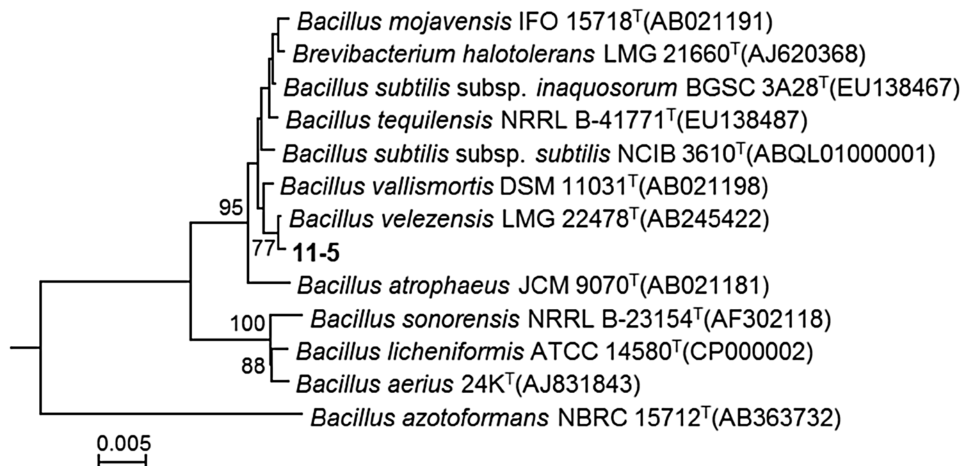

Fig. 1 16S rRNA sequence based phylogeny tree of *B. velezensis* 11-5 isolated and purified from a marine-sediment sample collected in the South China Sea. The 16S rRNA sequences of the selected type *Bacillus* strains were retrieved from NCBI database, and the accession numbers of these gene sequences in public databases were shown in round brackets of each corresponding strains. Scale bar = 0.005

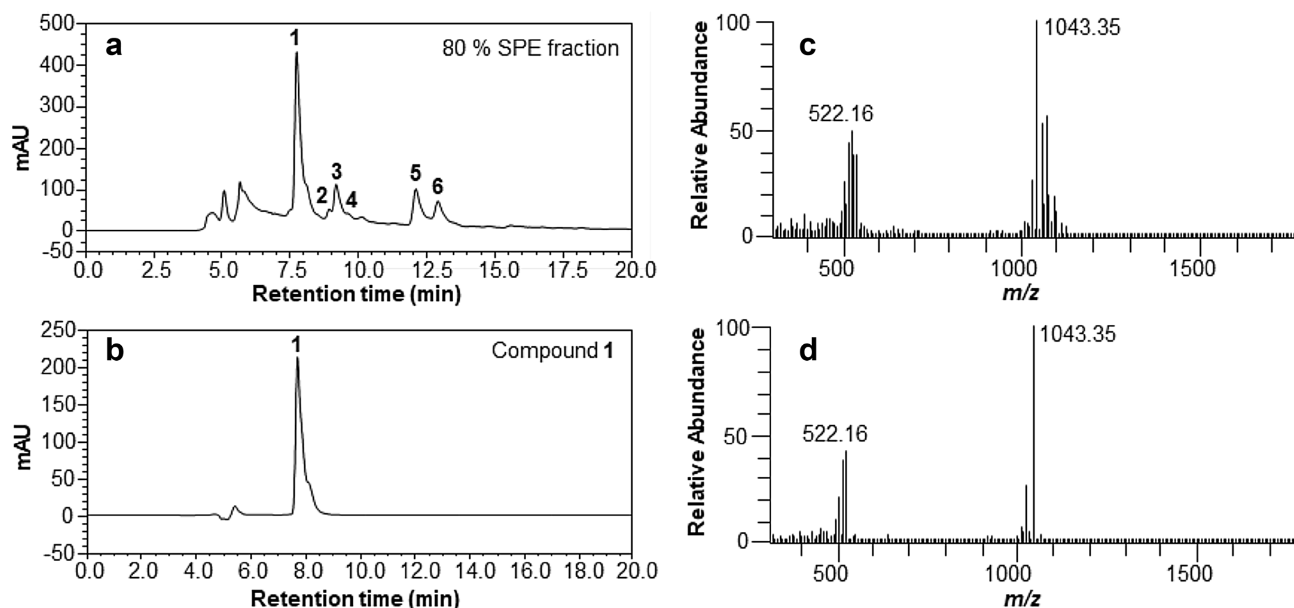

Fig. 2 RP-HPLC profiles of 80% SPE fraction from the crude extract of *B. velezensis* 11-5 (**a**) and purified compound **1** (**b**), respectively. Their corresponding LC-MS spectra were shown in (**c**) (for 80 % SPE fraction from the crude extract of *B. velezensis* 11-5) and (**d**) (for purified compound **1**), respectively. The homologues of the compound **1** detected from the 80% SPE fraction of the crude extract of *B. velezensis* 11-5 have been numbered in (**a**)

Table 1 LC-MS analysis of the 80% SPE fraction from the crude extract of CLPs extracted from the strain *B. velezensis* 11-5

Nos.	Ions			
	$[M+2H]^{2+}$	$[M+H]^+$	$[M+Na]^+$	$[M+K]^+$
1	522.16	1043.35	1065.28	1081.14
2	529.17	1057.33	1079.40	1095.20
3	529.15	1057.37	1079.41	1095.42
4	529.38	1057.40	1079.36	1095.22
5	536.27	1071.38	1093.40	1109.32
6	536.16	1071.40	1093.48	1109.35

The peak numbers shown in the table are labeled in Fig. 2a

11-5 contain compounds with the difference of a ‘$-CH_2-$’ ($m/z = 14$) group either for $[M+H]^+$ or for $[M+Na]^+$, respectively, showing the evidence that these compounds are probably the congeners.

LC–MS measurement of compound **1** was conducted under both positive mode and negative mode. Ion peak under positive mode of compound **1** shows $[M+H]^+$ at *m/z* 1043.35 (Fig. 2d), while ion peak under negative mode of the compound shows $[M\text{-}H]^+$ at *m/z* 1041.33 (Fig. S1), which confirmed that the exact mass of the compound **1** is 1042.3. The ion peak of $[M+H]^+$ was chosen as a mother ion for further MS/MS fragmentation. The MS/MS spectrum (Fig. 3a) of the compound **1** showed a series of the fragments derived from the mother ion, and these mass data provide ion peak fragments (y-type and b-type ions) that allow us to deduce the peptidic sequence of the compound **1** (Fig. 3b). The MS/MS data of the compound **1** produce a series of y-type ions, such as 211.47 (y_1), 298.47 (y_2), 523.77 (y_3), 637.64 (y_4), 801.00 (y_5), 914.74 (y_6) and 1043.17 (y_7), respectively. Moreover, the mass data also contain the corresponding b-type ions, such as 242.62 (b_2), 405.55 (b_3), 519.57 (b_4), 745.36 (b_5), 832.15 (b_6), 946.87 (b_7), respectively. These data indicate that there is an amino acid sequence of ‘Gln_4-Asn_3-Tyr_2-Asn_1-fatty acid-Asn_7-Gln_6-Pro_5’ in compound **1**.

Further NMR analysis results from ^{1}H-NMR (Fig. S2) and ^{13}C-NMR (Fig. S3) spectra of compound **1** confirm the presence of the peptidic sequence of this compound. More specifically, certain NMR signals show typical characteristics for peptide compound; for instance, signals (chemical shifts, δ) of 3.5–4.5 and 6.8–8.8 from the ^{1}H-NMR spectrum of the compound **1** show the signals for H_α of the amino acids, and the signals for ‘-NH-’ or ‘$-NH_2$’ groups, respectively, δ 40–65 and δ 170–175 from the ^{13}C-NMR spectrum indicate the signals for C_α of the amino acids, and ‘–(C=O)–’ groups, respectively. Taken the results from MS and NMR together, compound **1** contains a peptidic sequence of ‘Asn_1-Tyr_2-Asn_3-Gln_4-Pro_5-Gln_6-Asn_7’ linking to a saturated *normal*-C14 β-amino fatty acid residue. Therefore, the chemical structure of compound **1** has been determined as iturin A (Fig. 4). The assignment of the detailed NMR data of iturin A (**1**) is shown in Table S1.

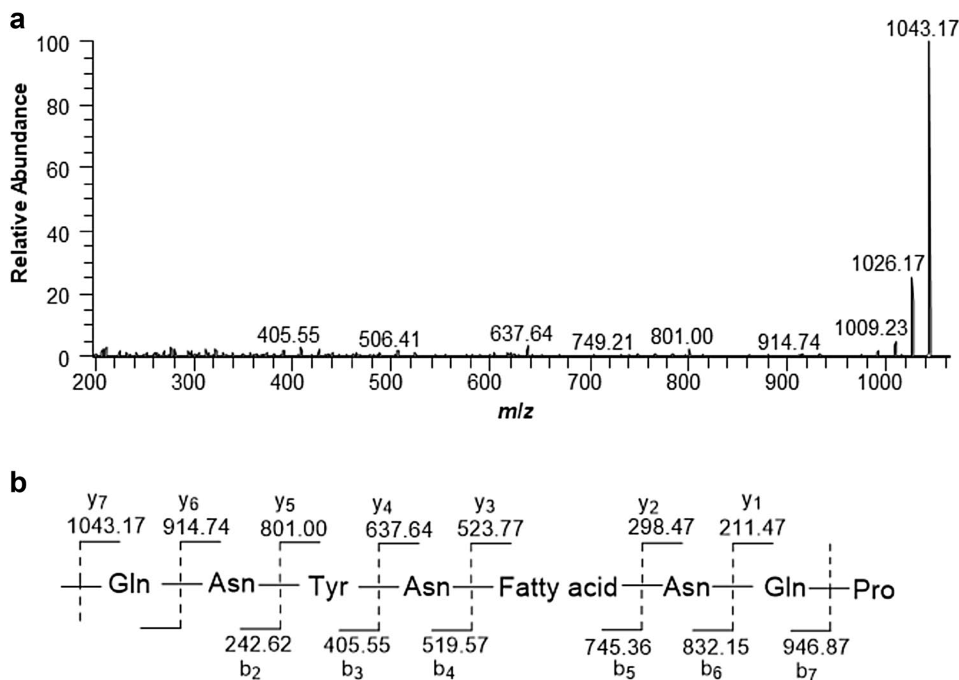

Fig. 3 MS/MS spectrum of the compound **1** produced by its mother ion $[M+H]^+$ (**a**), and data assignment of the different ions (b- and y-type, *m*/*z*) for compound **1** (**b**)

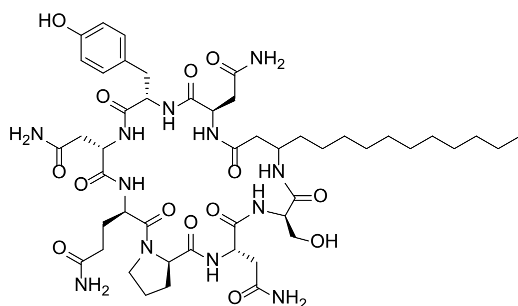

Fig. 4 Chemical structure of iturin A (**1**) isolated and characterized from the fermentation broth of *B. velezensis* 11-5

Biocontrol assays of iturin A (1) against *M. oryzae*

The germination rate of the conidia of *M. oryzae* displays that iturin A (**1**) can significantly suppress conidia germination and appressorium formation of the fungus (Fig. 5a), at the concentration of 10 and 50 µM after 12 h or 24 h incubation, compared to the control treatment at the same time point (Fig. 5b, c).

To further mimic the planta biocontrol effects of the compound to *M. oryzae*, a detached leaf assay was introduced in this study. Various concentrations of iturin A (**1**) were applied to conidia suspension of *M. oryzae*, and the mixture was dropped on the surface of the detached rice leaves, to test the *in planta* biocontrol effect of the compound. Data from disease assessment showed that iturin A (**1**) can successfully control *M. oryzae* at the concentration over than 10 µM, while the lower concentrations (0 or 1 µM) were ineffective (Fig. 6a–c).

Discussion

The CLP iturin family was originally assigned 'iturin' since the first producer of the compound was derived from a soil sample in the area of Ituri (Congo) (Delcambe 1950). CLP iturin family contains several subgroups, such as mycosubtilin, iturin A, bacillopeptin and mojavensin (Cochrane and Vederas 2016). These iturin-type CLPs generally compose by a peptidic sequence containing seven amino acids as a cyclic ring and a β-amino fatty acid residue. Data from organic synthesis and additional chemical analysis showed the evidence that iturin-type CLPs share the same amino acid configuration of 'LDDLLDL' for the peptide ring and a 'R'-type β-amino fatty acid residue (Besson et al. 2007; Bland 1996; Garbay-Jaureguiberry et al. 1978; Kajimura et al. 1995; Nagai et al. 1979; Nasir and Besson 2012). Remarkably, a partial sequence of 'L-Asn-D-Tyr-D-Asn' linking to the β-amino fatty acid moiety is the common structure of the peptidic ring of iturin-type CLPs. Moreover, it was confirmed that there are three types of terminal branches for the fatty acid residues in the iturinic CLPs, namely *anteiso*-type, *iso*-type and *normal*-type (Ongena and Jacques 2008). This study did not show any data on the stereochemistry survey of the iturin A produced by the strain *B. velezensis* 11-5; however, it is logical to assign the configuration of the amino acids in CLP iturin A as 'LDDLLDL' in this study due to the results

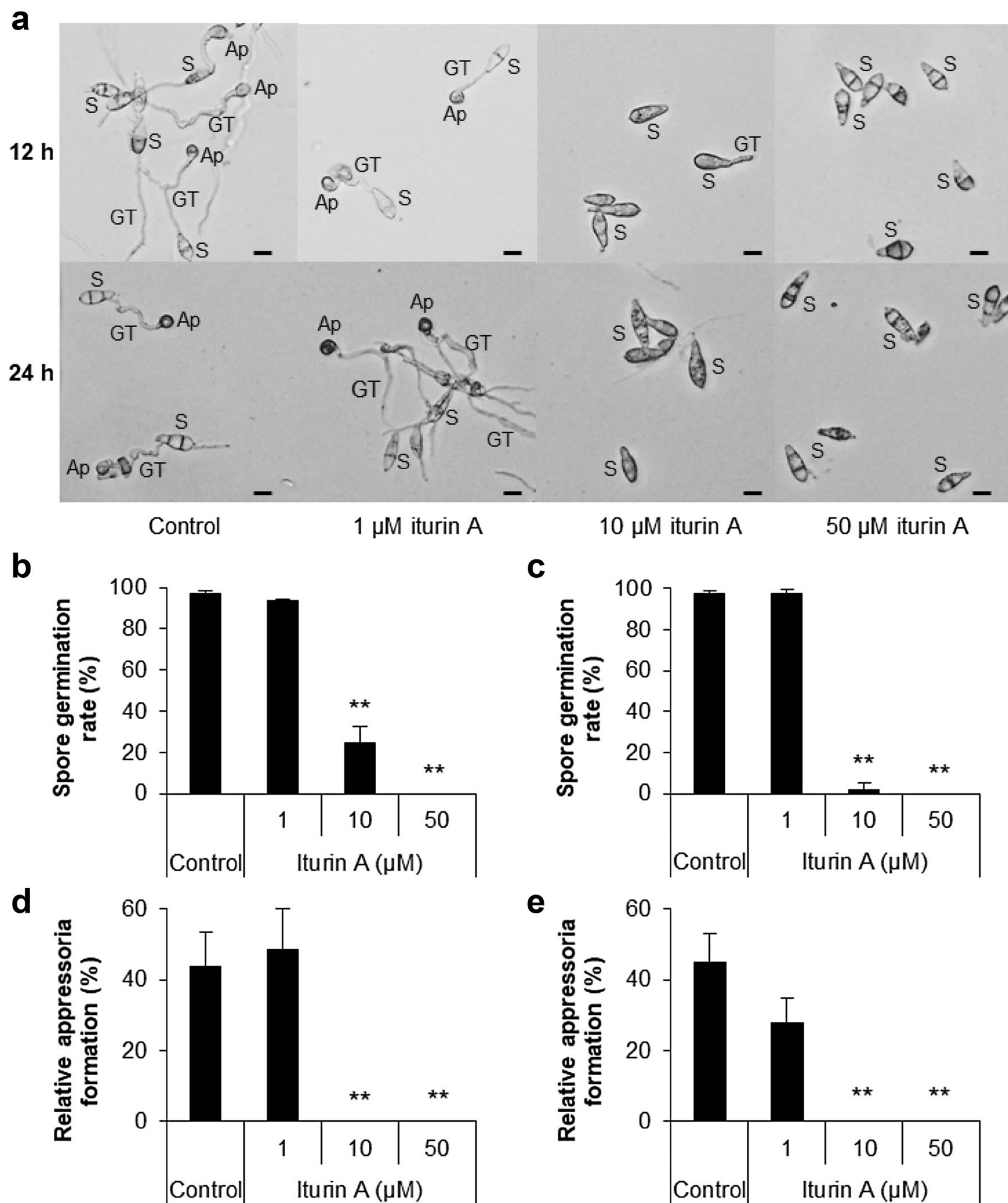

Fig. 5 In vitro biocontrol activity of the iturin A against *M. oryzae*. The conidia germination rate and appressorium formation rate were evaluated at 12 h and 24 h incubation, respectively, and the data were collected from triplicate experiments and were presented as means (± S.D.). The representative pictures of different treatments on the conidia of *M. oryzae* were shown in (**a**). S = spore, GT = germ tube, Ap = appressorium, scale bar = 20 μm. The spore germination rate of different treatments were shown in (**b**) (for 12 h incubation) and (**c**) (for 24 h incubation), and the relative appressorium formation rate of different treatments were shown in (**d**) (for 12 h incubation) and (**e**) (for 24 h incubation), respectively. Data marked with asterisks (**) show significant difference of the treatments compared to the control ($p < 0.01$)

from previous studies that iturin family CLPs share the same configuration on amino acid sequence (Fig. 4).

CLP iturins, as one of the most promising secondary metabolites produced by *Bacillus* strains, have grazed great attention as research topics for decades due to their excellent biological activities. The chemical structure of iturinic CLPs has both hydrophobic (fatty acid residue) and hydrophilic (peptidic sequence) traits, and thus they can interact

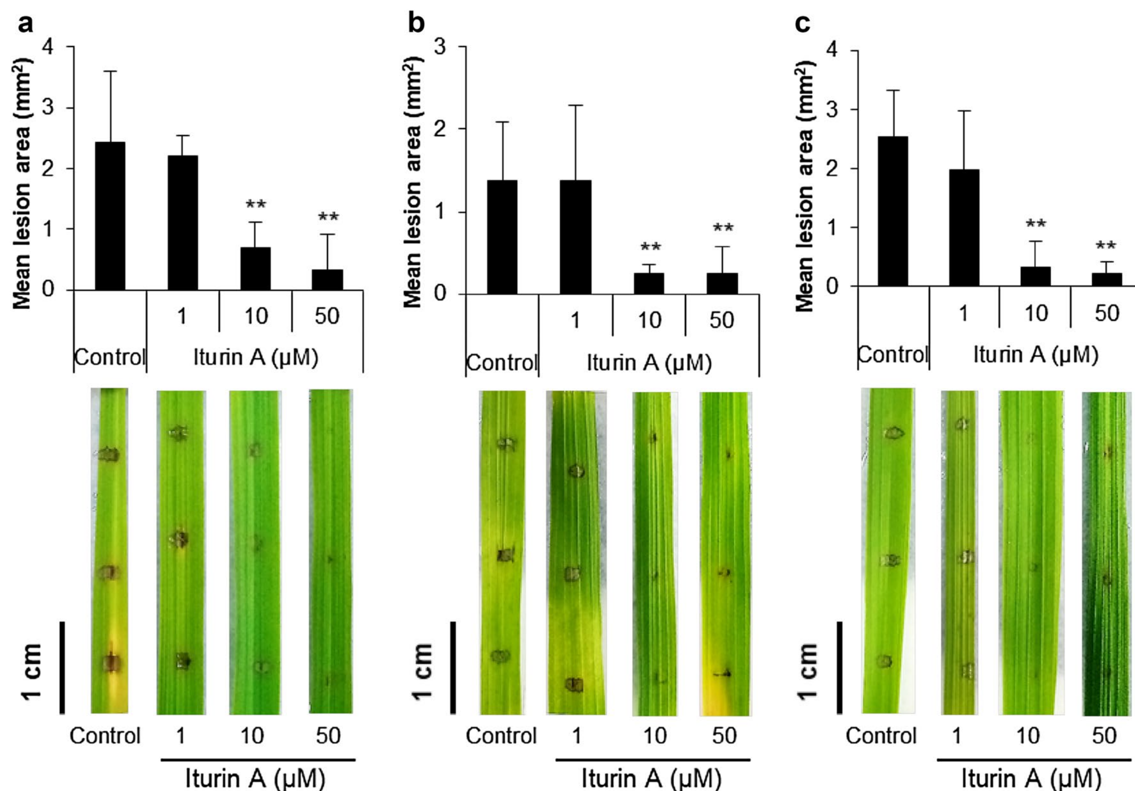

Fig. 6 *In planta* biocontrol efficacy of iturin A against *M. oryzae*, mean lesion area (mm^2 ± S.D.) of *M. oryzae* on the rice leaves are shown for three independent experiments (**a**, **b**, **c**, respectively) and a representative picture depicting the biological control effect of different treatments against *M. oryzae* is shown for each independent experiment. The biological control assays were conducted triplicates and each treatment contains at least 6 leaf segments, and three droplets of conidia suspension were carefully pipetted on each leaf segment. Data were collected 7 days post inoculation. Scale bar = 1 cm. Asterisks (**) indicate the significant difference of the treatment compared with control ($p < 0.01$)

with multiple cellular membranes of different organisms and subsequently trigger diverse biological responses on these organisms. Recent reports have shown that iturin A (or iturin A family CLPs) produced by various *Bacillus* strains have broad-spectrum activities against plant pathogens, such as *Phoma tracheiphila* (Leila et al. 2016), *Streptomyces scabies* (Lin et al. 2018), *Verticillium dahliae* (Han et al. 2015) and *Fusarium graminearum* (Kim et al. 2017). For the first time, this study shows that iturin A produced by *B. velezensis* 11-5 displays biocontrol activities against *M. oryzae* both in vitro and in rice leaves (Figs. 5, 6). More specifically, we show that this compound has the capacity to control the spore germination and appressorium formation of *M. oryzae* in vitro, and to control the disease development of *M. oryzae* in rice plants. Since the iturin family CLPs possess the similar chemical structures (Ongena and Jacques 2008), it is feasible for us to consider that iturin family CLPs have the direct antibiotic activity to suppress the conidia germination and appressorium formation of *M. oryzae* and thus show the protection of *M. oryzae* in rice plants. And the results from this study may also shed some clues for developing CLP iturins and their producing strains as biopesticides to control rice blast disease caused by *M. oryzae* in future agricultural practices.

It has been shown that either disturbing the growth of *M. oryzae* or inhibiting the appressoria formation during the infection cycle of *M. oryzae* will lead to the suppression of the disease incidence caused by *M. oryzae* (Rebollar and Lopez-Garcia 2013; Spence et al. 2014; Xu and Hamer 1996). This study shows that CLP iturin A has both the antibiotic activity to conidia and the suppression capacity of the appressorium formation of *M. oryzae* and can successfully control blast symptoms on the rice leaves at the concentration of 10 μM; however, a CLP orfamide produced by *Pseudomonas protegens* and related strains can suppress the rice blast disease on rice leaves at the concentration of 50 μM by blocking the appressoria formation of *M. oryzae* (Ma et al. 2016). These data provide that iturin A displays a better performance on controlling the blast disease in rice plants compared to *Pseudomonas protegens*-derived CLP

orfamide. Intriguingly, orfamide does not have any antibiotic activities neither to mycelia growth nor to spore germination of *M. oryzae*. We did not characterize the other metabolites produced by the strain *B. velezensis* 11-5 showing biological activity against *M. oryzae* in this study. However, to provide a better understanding of the interaction between *B. velezensis* 11-5 and *M. oryzae*, it is extremely interesting to elucidate the other secondary metabolites produced by the strain 11-5 and to study the mechanism on the interaction of the corresponding metabolite and the spores of *M. oryzae*, and to further compare their biological activities (minimal inhibitory concentration, mode of action, etc.) on the biological control of *M. oryzae*.

To sum up, we isolated and characterized an iturin A-producing strain *B. velezensis* 11-5, its secondary metabolite iturin A was purified by SPE and RP-HPLC methods and identified by MS and NMR techniques, and the in vitro and *in planta* biocontrol activities of the compound against *M. oryzae* were further studied. Data from biocontrol assays provided evidence that the cyclic lipopeptide iturin A is a promising agrichemical to control *M. oryzae* both in vitro and in rice plants. As a newly defined *Bacillus* group, *B. velezensis* contains sets of bacterial strains that have excellent activities on the control of various pests; for instance, the well-known model strain FZB42 has the great potential to be developed as a biopesticide (Chowdhury et al. 2015a; Dunlap et al. 2016; Ye et al. 2018). The strain 11-5 has also been characterized as *B. velezensis* based on the molecular evidence in this study (Fig. 1). Therefore, it is important to develop the biological activities of the strain 11-5 further to control diseases in different plants, and likewise to depth decipher the corresponding bacterial determinants produced by the strain and their mode of actions. To some extent, these theoretical research data will provide further implications for the future application of *B. velezensis* 11-5 as a sustainable biopesticide in agricultural production.

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Authors' contribution ZM conceived and designed the study, conducted the experiments and wrote the manuscript. ZM, SZ, KS and JH improved the manuscript and approved the final version for submission.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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