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The Antibiosis Action and Rice-Induced Resistance, Mediated by a Lipopeptide from *Bacillus amyloliquefaciens* B014, in Controlling Rice Disease Caused by *Xanthomonas oryzae pv. oryzae*

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In the present study, a lipopeptide (named AXLP14) antagonistic to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) was obtained from the culture supernatant of *Bacillus amyloliquefaciens* B014. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis demonstrated that AXLP14 consisted of surfactin homologs. The minimum inhibition concentration and minimum bactericidal concentration of AXLP14 against *Xoo* were determined to be 1.25 and 2.50 mg/ml, respectively. At a concentration of 0.613 mg/ml, AXLP14 strongly inhibited the formation of *Xoo* biofilm. AXLP14 also inhibited the motility of *Xoo* in a concentration-dependent manner. Applying AXLP14 to rice seedlings significantly reduced the incidence and severity of disease caused by *Xoo*. In *Xoo*-infected rice seedlings, AXLP14 strongly and continuously up-regulated the expression of both *OsNPR1* and *OsWRKY45*. In addition, AXLP14 effectively inhibited the *Xoo*-induced up-regulation of the expression of the abscisic acid biosynthesis gene *OsNECD3* and the abscisic acid signaling-responsive gene *OsLip9*, indicating that AXLP14 may protect rice against *Xoo*-induced disease by enhancing salicylic acid defense and interfering with the abscisic acid response to virulence.

Keywords: Rice, *Xanthomonas oryzae* pv. *oryzae*, *Bacillus* lipopeptide, antibiosis action, induced resistance

Introduction

Rice (*Oryza sativa* L.) is one of the world's most important food crops, feeding about half of all people worldwide. Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most widespread and destructive rice diseases, resulting in an annual yield loss of up to 60% [25]. Currently, the rice disease is managed by the use of resistant cultivars and systemic bactericides. However, the lack of durable resistance, the existence of pathogenic variability, and concerns regarding chemical resistance have limited the potential of such strategies for the disease management [7, 15]. Thus, more efficient and environment-friendly biopesticides are urgently needed for control of the rice disease.

Many *Bacillus* species are considered antagonistic microorganisms owing to their potential to produce structurally diverse antimicrobial compounds [20]. Among the diverse biologically active molecules synthesized by *Bacillus*, lipopeptides classified into three different families (surfactins, iturins, and fengycins) are of particular interest in the context of biocontrol. *Bacillus* lipopeptides exhibit successful biocontrol effects not only by inhibiting pathogen growth, but also by re-inforcing the host resistance potential through the induction of plant defense responses [21].

The antimicrobial activity of *Bacillus* lipopeptides is more commonly described against fungal pathogens [6, 18, 21]. In contrast to the numerous studies of the antifungal action of *Bacillus* lipopeptides, few studies have reported their

antibacterial action, especially against *Xoo*. Furthermore, the majority of the studies on antibacterial effects of *Bacillus* lipopeptides focus on their activity spectrum, and the best-studied mechanism regarding antibacterial action of *Bacillus* lipopeptides is the disruption of membrane integrity [4, 19, 30]. The ability of *Bacillus* lipopeptides to induce plant defense responses upon pathogen attack has been extensively examined and confirmed for a wide variety of plants, including tomato [5], grapevine [11], perennial ryegrass [22], strawberry [28], citrus [24], and *Beta vulgaris* [10]. However, most such studies are conducted in plant-fungal pathogen systems.

Bacillus amyloliquefaciens B014 is a bacterial endophyte previously isolated from healthy *Anthurium* tissue by our group. Our previous studies have shown that *B. amyloliquefaciens* B014 has the potential to coproduce lipopeptide iturins and surfactins [17]. Considering the economic importance of rice bacterial leaf blight caused by *Xoo*, the goal of this study was to investigate the in vitro antibiosis action of a lipopeptide obtained from *B. amyloliquefaciens* B014 against *Xoo* and the ability of the *Bacillus* lipopeptide to trigger rice-induced resistance to *Xoo* infection and to evaluate and characterize its biocontrol potential and mechanisms.

Materials and Methods

Bacterial Strains and Culture Condition

Xoo 102, a virulent strain of *Xanthomonas oryzae* pv. *oryzae*, was originally isolated from rice (*Oryza sativa* L.) leaf exhibiting typical symptoms of bacterial blight by our laboratory. Purified *Xoo* 102 was subcultured on nutrient agar (NA) at 30°C for routine use and stored at −80°C for long-term storage. For the preparation of pathogen inoculums, *Xoo* 102 was inoculated into an NA slant and incubated at 30°C for 24 h. At that time, the cells on the surface of the slant were collected by washing with sterile ddH_2O and diluted to OD_{600} = 0.1 (approximately 10^8 CFU/ml). The antagonist bacterium *B. amyloliquefaciens* B014 was isolated from internal tissue of a healthy *Anthurium* plant [17]. The storage and cell maintenance conditions were the same as described for *Xoo* 102.

Extraction and Purification of Antibacterial Lipopeptides from *B. amyloliquefaciens* B014

Lipopeptides were extracted from the culture supernatant of *B. amyloliquefaciens* B014 according the methods described by Kim *et al*. [16] with some modification. In brief, strain B014 was incubated in nutrient broth (NB) broth with shaking (150 rpm) for 38 h at 30°C. The bacterial cells were removed from the lipopeptide-containing culture of strain B014 by centrifugation at 10,000 ×*g* for 20 min. The resulting supernatant was acidified to pH 2.0 with concentrated HCl and the suspension was incubated in a refrigerator overnight to facilitate precipitation of lipopeptides. The acid precipitate was collected by centrifugation at 10,000 ×*g* for 15 min at 25°C and was redissolved in ddH_2O to obtained crude water-soluble lipopeptide. After adjustment to pH 7.0 with 1.0 N NaOH, the water-soluble lipopeptide was heated in a water bath of 90°C for 30 min to remove the heat-unstable component in the lipopeptide extract.

To obtain anti-*Xoo* lipopeptides, the crude water-soluble lipopeptide extract was applied to a column (300 × 26 mm) of DEAE-cellulose (Sigma, USA) and eluted successively with 0.5 M NaCl solutions, at a flow rate of 1.0 ml/min. The products were monitored by absorbance at 215 nm. Fractions were pooled and concentrated with a rotary evaporator, and then antagonistic activity against *Xoo* 102 was tested according to previously described methods [17]. The fractions exhibiting anti-*Xoo* activity were merged and dialyzed overnight against deionized water and then concentrated under reduced pressure at 60°C. The final lipopeptide obtained was named AXLP14.

MALDI-TOF Mass Spectrometry Analysis of Lipopeptide AXLP14

The chemical composition of the obtained lipopeptide AXLP14 was analyzed using a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry performed using a Voyager DE-STR MALDI-TOF instrument (Applied Biosystems, USA) according the methods described by Kim *et al.* [16]. In brief, a 0.5 µl volume of AXLP14 (0.1 mg/ml) was mixed with an equal volume of 0.1% solution of α-cyano-4-hydroxycinnamic acid in acetonitrile-water-TFA (50:50:0.1 (v/v/v)) used as the matrix. The mixture was then spotted (1 µl) on the target plate and allowed to air dry. The instrument was operated in reflection-positive ion mode at an accelerating voltage of 28 kV. The N_2 laser was operated at the minimum threshold level required to generate a signal and minimize dissociation. A surfactin standard (Sigma, USA) composed of C13 to C15 surfactin homologues was also analyzed under the same conditions.

Evaluation of the Antibacterial Activity of AXLP14 against *Xoo* 102

The antagonistic activity against *Xoo* 102 growth was evaluated by the filter paper disc method as previously described [17]. The minimum inhibition concentration (MIC) and the minimum bactericidal concentration (MBC) of AXLP14 against *Xoo* 102 were determined by the 2-fold microbroth dilution method devised by Andrews [1]. The assay was performed at least in triplicates. To examine whether AXLP14 exhibits bacteriolytic activity against *Xoo* 102, a *Xoo* 102 cell suspension was mixed with AXLP14 at a final concentration higher than the MBC and then incubated at 30°C for 2 h. After incubation, a drop of *Xoo* 102 cell suspension was mounted on a glass cover slip and fixed with 2.5% glutaraldehyde for 24 h. After being serially dehydrated in a graded series of alcohol (50%, 70%, 80%, 90%, 95%, and 100% (v/v)), the samples were dried in a criticalpoint drier, coated with gold-

palladium, and viewed with a scanning electron microscope (SEM; Zeiss Gemini Ultra-55, Germany).

Evaluation of the Effect of AXLP14 on *Xoo* 102 Biofilm Formation

Xoo 102 biofilm was grown on glass coverslips as described by Banas *et al*. [2]. Briefly, a 10 µl volume of *Xoo* 102 cell suspension was inoculated into NB (1 ml) with the addition of AXLP14 at a final concentration of 0.613 mg/ml (1/2 MIC) or without (as control) in an 8-well microtiter plate. A sterile glass slide was placed into every well prior to the broth being added. Then, the final plate was incubated at 30°C. Five days after incubation, the glass coverslips with *Xoo* 102 cultures were washed three times with a phosphate buffer (pH 7.2) and *Xoo* 102 biofilm formation was observed by SEM as described above.

Evaluation of the Effect of AXLP14 on *Xoo* 102 Swimming

A 3 µl volume of *Xoo* 102 cell suspension was carefully placed onto the center of soft NA plates (0.5% agar) with the addition of AXLP14 at a final concentration less than the MIC (0.154, 0.307, and 0.613 mg/ml or 0 mg/ml). The final plates were then incubated at 30°C and were evaluated after 5 days by measuring the expanding diameter of *Xoo* 102 lawn [12]. The assay was performed at least in triplicates.

Evaluation of the Biocontrol Effect of AXLP14 on *Xoo* 102-Induced Rice Disease

The Japonica rice variety Zhonghua 11 was used in this study. The rice seeds were germinated for 3 days at room temperature. The seedlings were then planted in 15-cm-diameter pots containing sterile moist soil (clay: sand, 3:1) dipped in 1/2-diluted Hoagland micronutrients. The planted seedlings were kept in a greenhouse at 26 ± 2°C with a relative humidity of 80 ± 5% for another 15 days. After the 15 days, the seedlings were challenged by inoculation with freshly prepared cell suspensions of *Xoo* 102 using the standard leaf clipping method [14]. One day after inoculation, the seedlings were uniformly sprayed with a 1 ml volume of AXLP14 (10.0, 5.0, and 2.5 mg/ml) or sterile ddH_2O (as a pathogen inoculation control) on the leaf surface of every seedling. The seedlings were then returned to greenhouse conditions for disease development. At day 15 after pathogen challenge, the number of leaves exhibiting disease symptoms and the lesion size (lesion length/leaf length) were recorded. For each treatment, 30 seedlings were used, and the assay was repeated at least three times in independent experiments.

Detection of Expression of Rice Defense-Related Genes

Rice seedlings at the three-leaf stage were challenge inoculated with *Xoo* 102 and were then treated with AXLP14 (5 mg/ml) as described above or treated with sterile ddH_2O (as a only pathogen inoculated control). Rice seedlings inoculated with sterile ddH_2O and then treated with ddH_2O were included as a non-inoculated control. Every 24 h after inoculation, the expression of four well-characterized rice defense-related genes (*OsNDPR1*, *OsWRKY45*, *OsLip9*, and Os*NCED3*) in the seedlings were examined by RT-PCR using gene-specific primers as follows (5′-3′) [25]: CACGCC TAAGCCTCGGATTA (forward) and TCAGTGAGCAGCATC CTGACTAG (reverse) for *OsNPR1*; GGACGCAGCAATCGTCCG GG (forward) and CGGAAGTAGGCCTTTGGGTGC (reverse) for *OsWRKY45*; TACGGCTTCCACGGCACGTTC (forward) and TGCCAGATTGCCAGCCCGTC (reverse) for *OsNECD3*; and CGG CGGCCTCTTCGAGACAAC (forward) and CGGAAGTAGGCC TTTGGGTGC (reverse) for *OsLip9*. For RT-PCR analysis, total RNAs were extracted from the rice leaf samples next to the sites of bacterial infection using the RNeasy Plant Mini kit with DNAase (Qiagen) according to the manufacturer's instructions. The concentration and quality of total RNA were examined using a UVmini-1240 spectrophotometer (Shimadzu, Japan). cDNA was synthesized with a two-step RT-PCR kit (Takara) from 2 µg of extracted RNA according to the product manual. The resultant cDNA was used for PCR. Quantitative RT-PCR amplifications were conducted in optical 96-well plates using the Mx3005P real-time PCR detection system (Stratagene) with SYBR Green master mix to monitor dsDNA synthesis. The thermal profile used consisted of an initial denaturation step at 95°C for 8 min, followed by 35 cycles of 95°C for 30 sec, 59°C for 30 sec, and 72°C for 1 min. Control PCR amplifications involved the use of RNA as a template to verify the elimination of contaminated DNA. The specificity of each primer for the target gene was confirmed by sequencing of the PCR products. The amount of plant RNA in each sample was normalized using *OsActin* as an internal control. For each assay, RT-PCR was performed three times with three independently isolated batches of total RNA. The primer pair for *OsActin* PCR was 5′-TCCATCTTGGCATCTCTCAG-3′ (forward) and 5′-GTACCCGCATCAGGCATCTG-3′ (reverse) [13].

Results

MALDI-TOF Mass Spectrometry Analysis of Lipopeptide AXLP14

A semi-purified lipopeptide (named AXLP14) was obtained from the culture supernatant of *B. amyloliquefaciens* B014 by virtue of antagonistic activity against *Xoo* 102, a virulent isolate of *Xoo*. The MALDI-TOF mass spectrometry profile for AXLP14 revealed three main mass peaks at 1046.8, 1060.8, and 1074.6 (Fig. 1), which are specific for surfactin-like lipopeptides [24]. Those peaks detected in the MALDI-TOF profile of AXLP14 were also seen in the MALDI-TOF profile of a surfactin standard composed of C13 to C15 surfactin homologs (Fig. 1A). The results indicated that lipopeptide AXLP14 consists of surfactin homologs.

Evaluation of the Antibacterial Activity of AXLP14 against *Xoo* 102

Lipopeptide AXLP14 displayed a strong inhibition

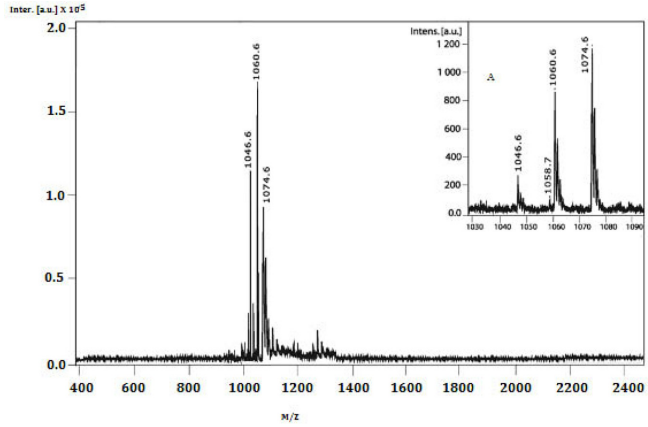

Fig. 1. MALDI-TOF mass spectra profiles of lipopeptide AXLP14 from *Bacillus amyloliquefaciens* B014.
The same surfactin-specific mass peaks at *m/z* = 1046.8, 1060.8, and 1074.6 detected in the mass spectra profiles of both AXLP14 and surfactin standard (A) indicate lipopeptide AXLP14 consisted of surfactin homologs.

against *Xoo* 102 growth at relative low concentrations, as revealed by the presence of close inhibition zones around the AXLP14-containing paper discs in a diffusion test (Fig. 2A). The diameters of the inhibition zones increased with increasing AXLP14 concentration, indicating the concentration-dependent inhibition of *Xoo* 102 growth by AXLP14. The MIC and MBC of AXLP14 against *Xoo* 102 were determined by the microbroth dilution method to be 1.25 and 2.5 mg/ml, respectively. At concentrations higher than the MBC, AXLP14 had bacteriolytic activity against *Xoo* 102 (Fig. 2B).

Evaluation of the Effect of AXLP14 on *Xoo* 102 Biofilm Formation

Xoo 102 cells grown on the glass coverslip with conventional NB were interconnected and encased in a scaffolding network composed of extracellular matrix, suggesting a three-dimensional architecture of biofilm formation (Fig. 3A). Under the same growth conditions but in the presence of AXLP14 at a concentration of less than the MIC (0.613 mg/ml), *Xoo* 102 cells were individually scattered over the surface rather than in any arrangement (Fig. 3B), indicating that AXLP14 strongly inhibits *Xoo* 102 biofilm formation.

Evaluation of the Effect of AXLP14 on *Xoo* 102 Swimming

The average lawn diameters of *Xoo* 102 incubated on an NA plate with AXLP14 at the final concentrations of 0.613,

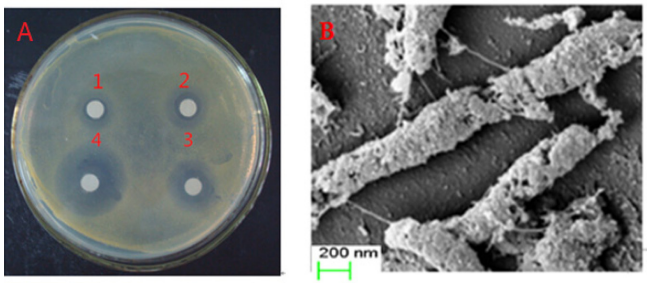

Fig. 2. The in vitro growth inhibition of lipopeptide AXLP14 (**A**) and bacteriolytic activity against *Xoo* 102 (**B**).
1, 2, 3, and 4 indicate a-10 µl volume of AXLP14 at the concentrations of 2.5, 5.0, 10.0, and 20.0 mg/ml, respectively, was added to the each paper disc. Bacteriolytic activity was observed 2 h after incubation in NB with AXLP14 (5.0 mg/ml) by scanning electron microscope.

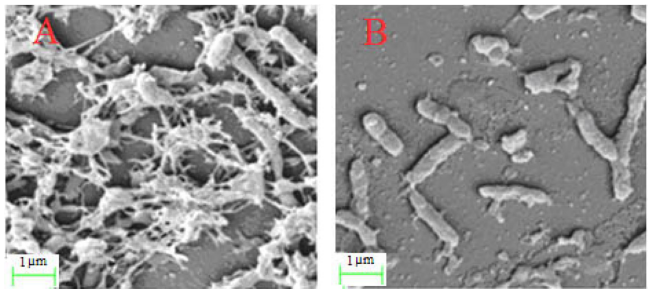

Fig. 3. Effect of AXLP14 on the biofilm formation of *Xoo* 102.
Biofilm formation was observed by scanning electron microscopy 5 days after incubation in NB with AXLP14 at the final concentration of 0.613 mg/ml (**B**), or without (**A**).

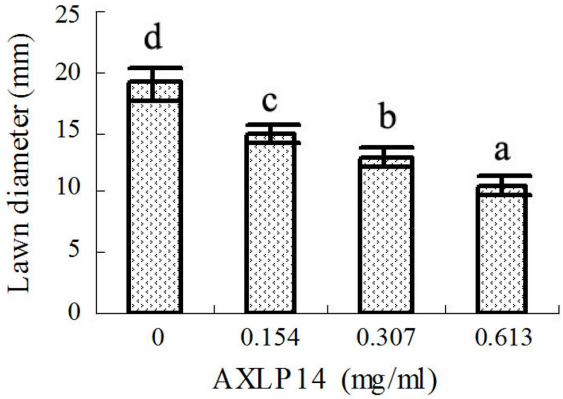

Fig. 4. Effect of AXLP14 on *Xoo* 102 swimming.
The lawn diameter of *Xoo* 102 was measured 5 days after incubation at 30°C on an NA plate with AXLP14 at the final concentrations of 0.613, 0.307, and 0.154 mg/ml, or without (0, as control), respectively. Data are the mean ± SD of three repeat tests for each test. Values in each bar with different letters mean significant difference ($p < 0.05$).

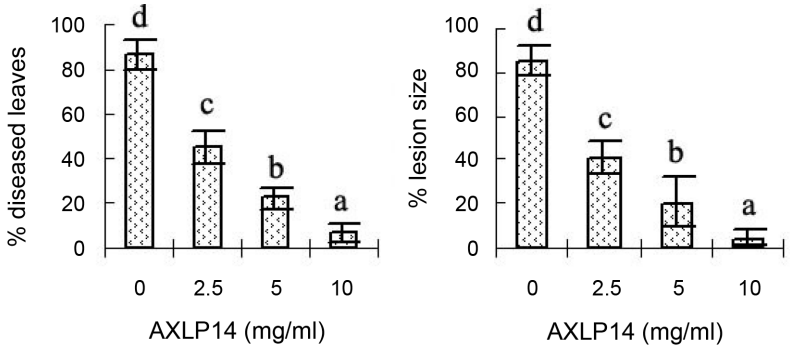

Fig. 5. AXLP14-induced suppression of rice disease caused by *Xoo* 102.
Data were recorded 15 days after inoculation with *Xoo* 102 and are reported as the mean ± SD of three repeated experiments. Each experiment was conducted with at least 30 seedlings in each group and the assay was repeated at least three times in independent experiments. Values in each band with different letters mean significant difference ($p < 0.05$).

0.307, and 0.154 mg/ml decreased by 44.5%, 31.9%, and 21.8%, respectively, compared with those incubated on a control plate without AXLP14 (Fig. 4). Thus, AXLP14 exerts concentration-dependent inhibition of *Xoo* 102 swimming.

Evaluation of AXLP14-Induced Suppression of Rice Disease Caused by *Xoo* 102

Under the conditions tested in this study, 15 days after challenge with the pathogen *Xoo* 102, >85% of rice seedling leaves inoculated with *Xoo* 102 developed foliar symptoms of rice bacterial blight disease, and the average lesion size was greater than 85%. The leaves of these rice seedlings were also obviously wilted and rolled, indicating that *Xoo* 102 is the virulent causal agent of rice bacterial blight disease. However, in rice seedlings treated with 2.0, 5.0, or 10.0 mg/ml of AXLP14, 15 days after inoculation with *Xoo* 102, only 45.11%, 22.10%, and 6.67%, respectively, of the leaves of inoculated seedlings developed the symptoms of rice bacterial blight disease (Fig. 5). The lesion sizes in the seedlings treated with 2.5, 5.0, or 10.0 mg/ml of AXLP14 were 40.93%, 20.56%, and 4.13%, respectively. These percentages were significantly lower than those of the control rice seedlings challenged with only the pathogen (Fig. 5). These results indicated that AXLP14 effectively protects against rice disease caused by *Xoo* 102 under the tested concentrations.

Detection of Expression of Rice Defense-Related Genes

Fig. 6 shows changes in the expression of four defense-related genes (*OsNDPR1*, *OsWRKY45*, Os*NCED3*, and *OsLip9*) in rice seedlings inoculated with *Xoo* 102 followed by treatment with AXLP14, non-inoculated control seedlings, and seedlings not treated with AXLP14. The expression of *OsNDPR1* in AXLP14-treated and untreated rice seedlings increased and peaked at day 2 after inoculation with *Xoo* 102. However, the peak expression of *OsNDPR1* in rice seedlings treated with AXLP14 was approximately 2.3 times higher than that of rice seedlings inoculated with only *Xoo* 102. After its peak, *OsNDPR1* remained at more than 86% of its peak expression level in AXLP14-treated rice seedlings. In rice seedlings inoculated with only *Xoo* 102, however, the expression of *OsNDPR1* decreased rapidly to a level lower than that detected in non-inoculated control seedlings. *OsWRKY45* showed a similar trend in expression in those seedlings, peaking in rice seedlings inoculated with *Xoo* 102 followed by treatment with AXLP14 at 4.2 times higher than the level observed in rice seedlings inoculated with only *Xoo* 102.

The changes in expression of genes *OsNCED3* and *OsLip9* in those rice seedlings also displayed the same tendency but were largely different from those of *OsNDPR1* and *OsWRKY45* (Fig. 6). In rice seedlings only inoculated with *Xoo* 102, the expression of *OsNCED3* and *OsLip9* remained static at early time points but increased steadily from day 2 after inoculation with *Xoo* 102 up to the end of the test period. At the time point, the expression of *OsNCED3* and *OsLip9* was approximately 18 and 12 times the levels found in non-inoculated control seedlings, respectively, indicating a strong induction of *Xoo* 102 on the expression of *OsNCED3* and *OsLip9* of rice seedlings. However, in rice seedlings treated with AXLP14 after inoculation with *Xoo* 102, the expression of both *OsNCED3* and *OsLip9* remained at the levels close to that detected in non-inoculated control seedlings at all tested time points (Fig. 6). Those results

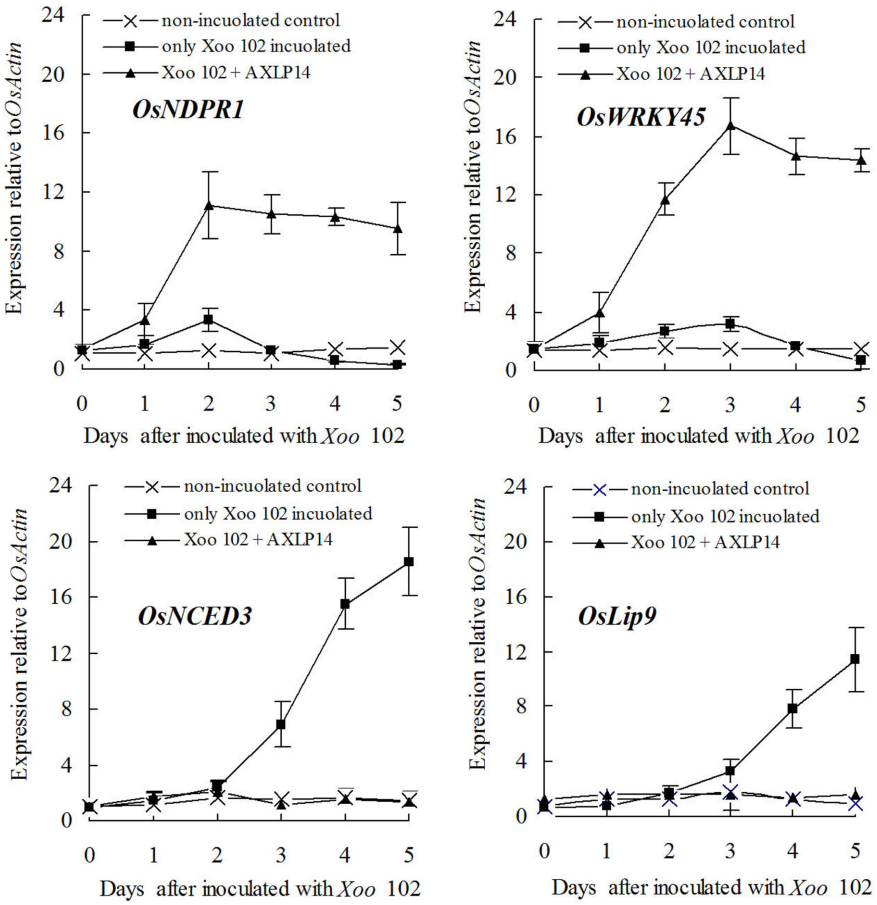

Fig. 6. Detection of expression of rice defense-related genes.
Gene expression was analyzed at days 0, 1, 2, 3, 4, and 5 after inoculation with *Xoo* 102. AXLP14 treatment was done by spraying a 1 ml volume of AXLP14 (5 mg/ml) onto the leaves of each seedling 12 h after inoculation with *Xoo* 102. Data are reported as the mean ± SD of three repeat tests.

indicated that AXLP14 can effectively inhibit the *Xoo* 102-induced expression of *OsNCED3* and *OsLip9* genes in rice seedlings.

Discussion

This direct antagonism of pathogen growth is the most powerful mechanism of a biocontrol agent as it usually provides the initial and the most rapid means of limiting the growth of a plant pathogen. In the present study, a lipopeptide named AXLP14 was obtained from the culture supernatant of *B. amyloliquefaciens* strain B014 and displayed concentration-dependent antibacterial, bactericidal, and bacteriolytic effects (Fig. 2). MALDI-TOF MS analysis demonstrated that AXLP14 is a surfactin homolog (Fig. 1), consistent with our previous investigation showing that *B. amyloliquefaciens* B014 produces surfactin-like lipopeptides [17]. Our present results were also consistent with several previous studies addressing surfactins as the major *Bacillus* metabolites involved in antagonism of bacterial pathogens [26, 31].

Swimming and biofilm formation play critical roles in facilitating adhesion of bacteria to the host surface during the initial stages of plant-pathogen interactions and disease development [27]. Our present results clearly show that the lipopeptide AXLP14 reduced swimming of pathogenic *Xoo* 102 in a concentration-dependent manner (Fig. 4), and also strongly inhibited *Xoo* 102 biofilm formation at a concentration less than the MIC (Fig. 3). The in situ production of lipopeptides by *Bacillus* sp. in soil- and plant-associated environments is very low [21]. The inhibitory effect of low-concentration AXLP14 on the virulence factors of *Xoo* is a notable trait for AXLP14 and its producer as a control agent to control rice disease caused by *Xoo*.

Plant-induced resistance has been recognized as the most attractive type of biocontrol agent for plant disease management in modern agriculture because it could provide plants with long-lasting protection from pathogens [5, 8, 29]. Furthermore, the ability of AXLP14 to trigger rice-induced resistance to *Xoo* attack was investigated by assessing the expression of several well-studied genes related to rice defense against *Xoo* in rice seedlings treated with AXLP14 during the progression of *Xoo* infection. The phytohormone salicylic acid (SA)-dependent pathway plays a vital role in rice resistance to *Xoo* [9, 23]. *OsNPR1* is a key positive regulator of SA-mediated rice immunity, and its ectopic expression in rice conferred high levels of resistance to *Xoo* [9]. *OsWRKY45* is a key transcription factor in the branched SA pathway. Similar to *OsNPR1*, rice plants overexpressing *OsWRKY45* are extremely resistant to *Xoo* [23]. Our present gene expression analysis showed that AXLP14 strongly and continuously up-regulated the expression of both *OsNPR1* and *OsWRKY45* in *Xoo*-infected rice seedlings (Fig. 6), suggesting AXLP14 may effectively trigger SA-mediated rice immunity and may therefore contribute to resistance to *Xoo*.

Abscisic acid (ABA) has been studied as an abiotic stress hormone. ABA has also recently emerged as a pivotal determinant in the outcome of plant-pathogen interactions. In some interactions, ABA enhances plant basal defense by priming for callose deposition and stomatal closure, and thereby positively influences disease outcomes [25, 27]. In most cases, however, ABA is reported to act as a pathogenic susceptibility-enhancing hormone or repressive hormones of plant immunity. In rice, application of exogenous ABA compromises rice resistance to *Xoo*, whereas the inhibition of ABA biosynthesis, degradation of ABA, and blocking of ABA signaling enhance rice resistance to *Magnaporthe oryzae* and *Xoo* [25, 27]. ABA also cross-communicates with bacterial QS systems, which could contribute to *Xoo* virulence in rice [27]. On the other hand, *Xoo* may dramatically increase the ABA biosynthesis and signaling of the host plant in order to cause disease [26]. *OsNCED3* encodes a key enzyme in the rice ABA biosynthesis pathway [3] and *OsLip9* is an ABA signaling-responsive gene of rice [25]. In the present study, consistent with the previous results [25], *Xoo* 102 induced a continuously high level of both *OsNCED3* and *OsLip9* expression in *Xoo* 102-infected rice seedlings. However, in *Xoo* 102-infected rice seedlings treated with AXLP14, the expression levels of both *OsNCED3* and *OsLip9* showed no large difference to those detected in non-inoculated control seedlings (Fig. 6). Such results indicate that AXLP14 may effectively inhibit the ABA biosynthesis and signaling of rice seedlings induced by *Xoo* 102, and consequently may reduce *Xoo* virulence to the rice plant and contribute to the resistance of rice against *Xoo*. The ability of *Bacillus* lipopeptides to trigger classical SA and JA/ET signal pathways to enhance plant defense upon pathogen attack has been extensively examined and confirmed in the plant-pathogen interaction [5, 10, 11, 22, 24, 28]. However, our present study, for the first time, addressed the antagonistic effect of *Bacillus* lipopeptide on repressive hormones involved in plant immunity.

In the present study, applying AXLP14 to rice seedlings significantly reduced the incidence and severity of disease caused by *Xoo* when compared with that of rice seedlings only challenged by the pathogen (Fig. 5). This result was consistent with the fact that AXLP14 possesses multi-layered biocontrol-related traits, as descried above. However, the present disease suppression test was performed only on a small scale and under indoor controlled conditions. Future studies will require a larger sample size and should be performed under field conditions to facilitate the application of AXLP14 as a biocontrol agent.

In conclusion, lipopeptide AXLP14 from *Bacillus amyloliquefaciens* B014 not only strongly inhibited the growth of *Xoo* but also negatively affected the virulence traits of *Xoo*, including motility and biofilm formation. In addition to its multilayered antibiosis actions against *Xoo*, AXLP14 was capable of protecting rice against *Xoo*-induced disease by re-inforcing SA defense signaling and by interfering with the ABA-based virulence of the pathogen. These results highlight the potential of AXLP14 and its producer as biopesticides to control rice diseases caused by *Xoo*. These results also improve our understanding of the biocontrol mechanism of *Bacillus* spp.

Acknowledgments

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