Exploring elicitors of the beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 to induce plant systemic resistance and their interactions with plant signaling pathways

Running title: Exploring elicitors of plant systemic resistance

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Abstract: Beneficial rhizobacteria have been reported to produce various elicitors that induce plant systemic resistance, but there is little knowledge concerning the relative contribution of multiple elicitors from a single beneficial rhizobacterium on the induced systemic resistance in plants and the interactions of these elicitors with plant signaling pathways. In this study, nine mutants of the plant growth-promoting rhizobacterium *Bacillus amyloliquefaciens* SQR9 deficient in producing the extracellular compounds, including fengycin, bacillomycin D, surfactin, bacillaene, macrolactin, difficidin, bacilysin, 2,3-butandiol, and exopolysaccharides, were tested for the induction of systemic resistance against *Pseudomonas syringae* pv. *Tomato* DC3000 and *Botrytis cinerea* and the transcription of the salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) signaling pathways in *Arabidopsis*. Deficiency in producing any of these compounds in SQR9 significantly weakened the induced plant resistance against these phytopathogens. These SQR9-produced elicitors induced different plant defense genes. For instance, the enhancement of 1,3-glucanase (PR2) by SQR9 was impaired by a deficiency of macrolactin, but not surfactin. SQR9 mutants deficient in the lipopeptide and polyketide antibiotics remained only 20% functional for the induction of resistance-related gene transcription. Overall, these elicitors of SQR9 could act synergistically to induce plant systemic resistance against different phytopathogens through different signaling pathway genes, and the bacterial antibiotics are major contributors to the induction.

Keywords: Induced systemic resistance (ISR), elicitor, plant growth-promoting

rhizobacteria, phytopathogen, *Arabidopsis*, antibiotics

Introduction

Agricultural production is encountering great challenges from plant pathogens, which have caused worldwide significant yield decreases. Application of plant growth-promoting rhizobacteria (PGPRs) has been known to be an efficient way to suppress plant pathogens. One of the mechanisms of PGPRs in exerting their bio-control function is the induced systemic resistance (ISR) of plants against a broad spectrum of phytopathogens in aboveground plant tissues (Ryu et al. 2003; Glazebrook 2005; Hamid et al. 2005; Yi et al. 2013), which has been described as the "activation of the host plant's physical or chemical defenses by an inducing agent" (Kloepper 1993). Root colonized PGPRs induce systemic resistance by producing a range of secondary metabolites, which are called "elicitors". After the elicitors are sensed, the jasmonic acid (JA), ethylene (ET) and salicylic acid (SA) signaling pathways are activated to trigger plant resistance. Characterization of bacterial elicitors is meaningful for understanding the priming of plant defenses against phytopathogens and consequently guiding agricultural production.

So far, many elicitors produced by PGPRs have been identified and characterized. *Pseudomonas* elicitors, such as 2,4-diacetylphloroglucinol and N-acylated-l-homoserine lactones (AHLs), have been well characterized at the molecular level (Schuhegger et al. 2006; Iavicoli et al. 2003). Volatile organic

compounds (VOCs) produced by *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a have been reported to trigger the activation of ET-/JA-responsive gene *PDF1.2* (Ryu et al. 2004; Sharifi and Ryu 2016). Similarly, another volatile compound, dimethyl disulfide (DMDS), produced by *Bacillus cereus* C1L, plays an important role in inducing resistance to plant fungal diseases in tobacco and corn plants (Huang et al. 2012). Fengycin and surfactin, produced by *B. subtilis* strains, exhibit a significant ISR-mediated protective effect on bean plants and could activate the lipoxygenase pathway in tomato (Ongena et al. 2007). PeBA1 protein produced by *B. amyloliquefaciens* NC6 could induce systemic resistance against a broad spectrum of pathogens, including tobacco mosaic virus (TMV) and the fungal pathogen *B. cinerea*, since SA-responsive *PR1a*, *PR1b*, *PR5*, and *PAL*, as well as JA-responsive *PDF1.2* and *COI1*, were up-regulated upon treatment with PeBA1 (Wang et al. 2016). These studies indicated that SA, JA and ET signaling pathways are involved in corresponding elicitor processes.

However, most of the previous studies have focused on a single or few elicitors from rhizobacteria (Pieterse et al. 2014; Hélène et al. 2015). One bacterium is usually equipped with multiple potential elicitors to activate plant systemic resistance. For example, a plant beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 produced a range of secondary metabolites, such as the surfactin, fengycin, bacillomycin, bacillaene, macrolactin, difficidin, bacilysin, indole-3-acetic acid (IAA) and 2,3-butanediol (Shao et al. 2015; Li et al. 2014). Several of these compounds

have been reported to be elicitors of plant resistance, such as surfactin, fengycin and 2,3-butanediol (Ongena et al. 2007; Ryu et al. 2004). A comprehensive evaluation of these potential elicitors from one plant beneficial rhizobacterium to coordinate and contribute to the overall ISR response of the plant host is generally lacking. Moreover, SA, JA and ET signaling pathways are involved in plant ISR responses, and the interactions of these multiple elicitors and plant signaling pathways are in need of systemic exploration from a holistic view. Therefore, the objective of this study is to 1) systematically characterize and evaluate the relative contributions of multiple elicitors from a single rhizobacterium to the overall ISR and 2) explore their interactions with the plant signaling pathways.

To achieve these objectives, a well-studied plant beneficial rhizobacterium *B. amyloliquefaciens* SQR9 and the model plant *Arabidopsis thaliana* (L.) Columbia (Col-0) were used for this study. Strain SQR9 has been demonstrated for its efficient plant growth-promoting and bio-control activities (Li et al. 2014; Liu et al. 2016; Shao et al. 2015; Xu et al. 2013); SQR9 exerted its plant beneficial effects through sensing the root-secreted signals (Liu et al. 2014, 2017) and producing secondary metabolites to affect the plant host (Chen et al. 2016, 2017). In this study, we demonstrated that SQR9 produced secondary metabolites that acted as elicitors in inducing the systemic resistance of *Arabidopsis* against *P. syringae* pv. *Tomato* DC3000 (*Pst* DC3000) and *B. cinerea*, and the lipopeptides, polyketides and dipeptide antibiotics contributed the major roles for the ISR. Elicitors have specific effect on the

induction of plant defense pathways and against different phytopathogens.

RESULTS

Plant beneficial rhizobacterium B. amyloliquefaciens SQR9 induced plant systemic resistance

Infection of the phytopathogens *P. syringae* pv. *Tomato* DC3000 (*Pst* DC3000) and *B. cinerea* were used as indicators to test whether SQR9 induces resistance in *Arabidopsis*. The subsequent quantification of pathogens was based on plate counting and disease severity for *Pst* DC3000 and *B. cinerea*, respectively. The results showed that inoculation with SQR9 led to a significant decrease (190-fold at 4 days and 40-fold at 6 days post inoculation with *Pst* DC3000) of *Pst* DC3000 infection (Fig. 1A, Table S1). At 4 days and 6 days post inoculation with *Botrytis cinerea* (*B. cinerea*), the disease incidence decreased by 33.3% and 23.1%, respectively, and the area under disease progress curve (AUDPC) decreased by 25.8% and 28.4%, respectively, in plants treated with SQR9 compared with the control (Fig. 1B and 1C, Table S2).

SQR9 activated plant SA, JA and ET signaling pathways

To investigate whether the SA, JA or ET signaling pathways are involved in the ISR response activated by SQR9-produced elicitors, the contents of salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) in plants were measured. The plants without inoculation with SQR9 served as control (CK) plants. We observed that root contents of SA, JA and ET increased the most (1.4-fold, 2-fold and 1.4-fold of those

in CK, respectively) after inoculation with SQR9 for 1 day, after which the contents of SA and JA have quickly decreased, but the increase of ET content lasted longer; ET content in SQR9 inoculated root was still significantly higher than that in the CK root after 4 days (Fig. 2). However, the shoots showed a slower response than the roots did. The content of SA and JA in shoots gradually increased until reaching 1.3 fold higher than the CK at 4 days post-inoculation, while ET increased to 1.2-fold at 2 days post-inoculation (Fig. 2, Table S3). These results indicate that the accumulation of hormones in local tissues is faster than in those distal.

Furthermore, the transcription levels of genes involved in the SA, JA and ET signaling in leaves were evaluated. Generally, the tested genes involved in all the three signaling pathways were activated by SQR9 (Fig. 3, Table S4). For SA signaling, the NPR1 protein, which is a receptor of SA and a transcriptional co-regulator, increased to the highest value (8.4-fold) at 6 h post-inoculation (Fig. 3A). The transcription of the SA-inducible marker defense protein, PR1, increased and reached the highest value (3.7-fold) at nearly the same time as NPR1 (Fig. 3A). PR2 and PR5 responded to SQR9 inoculation faster but at a lower level than PR1. For JA signaling, the transcription of AOS, the key JA biosynthesis enzyme, increased to its highest level (5.7-fold) 1-h post inoculation (Fig. 3B), while the transcription of COI1 and the downstream transcription factor MYC2 reached their highest levels (7-fold and 4.7-fold) at 3 h and 12 h post inoculation, respectively (Fig. 3B). ERF1, a downstream regulator of the ET signaling pathway, was up-regulated to its highest level of 3.8-fold

3-6 h post inoculation; as a consequence, HEL (pathogenies-related protein 4), a defense gene under the regulation of the ET pathway, reached its highest level (4-fold) at the same time (Fig. 3C). Moreover, transcription of CHIB and PDF1.2 reached the highest levels (11.7-fold and 5.5-fold) at 12 h post inoculation (Fig. 3D). These results indicate that all three signaling pathways in *Arabidopsis* were activated by inoculation with SQR9.

SQR9 produced multiple elicitors to induce plant systemic resistance

To identify the SQR9-produced compounds that elicit a systemic resistance in *Arabidopsis*, SQR9 mutants deficient in the production of each potential elicitor (antibiotics, growth-promoting compounds and exopolysaccharides) were tested for their function in inducing systemic resistance. The descriptions of these SQR9 mutants are shown in Table 1.

In brief, the results showed that mutations in the production of lipopeptide and polyketide antibiotics (Δ*sfp* in the *sfp* gene, which is required for phosphopantetheine translocation and thus necessary for synthesis of all these antibiotics) caused a sharp decrease in the ability of SQR9 to induce plant resistance against *Pst* DC3000 and *B. cinerea* (Fig. 4). For *Pst* DC3000, the mutant Δ*sfp* showed only a quarter of the ability of the wild type strain to trigger the plant resistance against *Pst* DC3000 (Fig. 4A). Even more, induction of plant resistance against *B. cinerea* was completely blocked when *sfp* was knocked out in SQR9 (Fig. 4B). For single antibiotics, mutants deficient in surfactin, bacillomycin D, fengycin or macrolactin production showed half the

ability of SQR9 to induce plant resistance against *Pst* DC3000, while other antibiotics as bacillaene, difficidin and bacilysin did not show remarkable contributions (Fig. 4A). For inducing resistance against *B. cinerea*, mutations of each antibiotic production showed significant reductions compared to the wild type strain at 4 days post-infection (Fig. 4B); among them, bacillomycin D, surfactin, difficidin, bacillaene and bacilysin showed significantly higher contributions to plant resistance against *B. cinerea* than other antibiotics (Fig. 4B). These results indicate that all these antibiotics are elicitors of plant systemic resistance with specificity against different pathogens.

In addition to the antibiotics, SQR9 produced indole-3-acetic acid (IAA) contributed to plant ISR since an IAA deficient mutant (Δ*ysnE*) exhibits a significantly reduced ability to induce plant resistance against *B. cinerea*, but no significant effect on plant resistance against *Pst* DC3000 was observed (Fig. 4B). The SQR9 mutants Δ*alsD* and Δ*epsD*, deficient for 2,3-butanediol and exopolysaccharide production, respectively, showed significantly reduced abilities to induce plant resistance against both *Pst* DC3000 and *B. cinerea* (Fig. 4B).

Correspondence analysis of SQR9 elicitors and plant defense signaling pathways

To evaluate the contribution of SQR9-produced elicitors to these plant signaling pathways, the transcription of these signaling genes upon inoculation with SQR9 wild type and elicitor mutants was analyzed using a qRT-PCR approach at 6 h post-inoculation (Table S5). The contribution of each elicitor to the transcription of plant defense genes was calculated by dividing the reduced gene transcription of a

plant inoculated with the elicitor mutant to that of a plant inoculated with wild-type SQR9 (Fig. 5). The SQR9 mutant Δ*sfp*, deficient of all antibiotic production, showed a large decrease of 70% to 90% in the activation of transcription of the measured defense genes (Fig. 5). The lipopeptide antibiotic fengycin was effective in inducing both the SA- and JA-signaling pathways, especially in inducing the transcription of PR2 and COI1; a deficiency of fengycin caused more than a 70% decrease of upregulation of PR2 and COI1 by SQR9; bacillomycin D and bacillaene showed a broad range but weak contribution (no more than 50%) to all tested defense genes; surfactin showed a 60%-70% contribution to PR5, NPR1, AOS1, MYC2, HEL/PR4, CHIB, and PDF1.2. For polyketide antibiotics, macrolactin showed a 60%-70% contribution to PR2, PR5, HEL/PR4 and PDF1.2 and a 50% contribution to CHIB, ERF1 and AOS1; difficidin showed an 80% contribution to ERF1. The dipeptide bacilycin showed a great contribution to the ET-signaling pathway; a deficiency of bacilycin production caused more than an 80% reduction of the enhancement of HEL/PR4 and ERF1 by SQR9; moreover, bacilycin showed more than a 50% contribution to all the tested genes except for CHIB and COI1. For the non-antibiotic elicitors, the volatile compound 2,3-butanediol showed an 80% contribution to PR2 and HEL/PR4 and more than a 60% contribution to PDF1.2; reduced phytohormone IAA production (Δ*ysnE*) caused an 80% decrease of the induction of PR5 and 70% of AOS1; the exopolysaccharide showed more than a 70% contribution to COI1 and PR5 and an 80% contribution to PR2.

Discussion

In the present study, bacterial mutants were used to investigate which bacterial compound serves as the elicitor of plant ISR and which plant signaling pathway is activated by these elicitors. We elaborated the network of these elicitors in *B. amyloliquefaciens* SQR9 in inducing the systemic resistance of *Arabidopsis*. A conclusion is that the antibiotics, including lipopeptides (bacillomycin D, fengycin, surfactin), polyketides (bacillaene, macrolactin, difficidin) and the dipeptide bacilysin, play the most important role in triggering plant systemic resistance.

Bacillus spp.-produced surfactin, fengycin, cold shock protein, 2,3-butanediol, acetoin, 2-aminobenzoic acid, and dimethyl disulfide have been identified as elicitors of the plant defense response (Yang et al. 2011; Huang et al. 2012; Ongena et al. 2007; Yi et al. 2016). However, the effect of the lipopeptide bacillomycin D, the polyketide macrolactin (*mln*), difficidin, bacillaene, and the dipepetide bacilycin on plant systemic resistance has not been reported. We showed that macrolactin is a strong elicitor of plant resistance against *Pst* DC3000 (Fig. 4A). The dipeptide antibiotic bacilysin, which has shown antibacterial activity against *Xanthomonas oryzae* and *Erwinia amylovora* (Wu et al. 2015), showed active participation in regulating *Arabidopsis* resistance against *B. cinerea* through the ET and SA signaling pathways.

Furthermore, the overall correspondence of these elicitors and the transduction pathways in plants, which has not been studied previously, was comprehensively investigated in this study. Interestingly, we found that induction of the defense genes

by each elicitor is relatively specific. For example, bacilysin showed more than an 80% contribution to the ET signaling pathway but did not participate in the activation of CHIB (Fig. 5). Surfactin, macrolactin and bacillaene contributed to induction of CHIB. Some of the compounds showed similar effects as previously reported: surfactin has been reported to be important for bacteria to activate the SA signaling pathway and induce chitinase (CHIB) but showed little effect on 1,3-glucanase (PR2) (Farace et al. 2015). Accordingly, we found that deficiency of surfactin production reduced the enhancement of CHIB; however, the transcription enhancement by SQR9 was not completely blocked. One reason is there are two other elicitors (macrolactin and bacillaene) produced by this strain involved in the activation of the transcription of CHIB (Fig. 5). SQR9 mutant deficient in 2,3-butanediol, a kind of volatile organic compound, activated the transcription of PR2 at much lower levels than the wild type strain did, which is consistent with previous reports (Yi et al. 2016). Exopolysaccharides produced by *Burkholderia gladioli* IN26 enhanced the expression of PR1a in cucumber (Kyungseok *et al.*, 2008); however, in this study, the exopolysaccharides contributed greatly to the enhanced expression of PR2 and PR5, but not that of PR1. Exopolysaccharides synthesized by different bacteria vary greatly in their composition and hence in their chemical and physical properties (Flemming and Wingender 2010), which may affect their abilities to induce defense genes.

SQR9-produced macrolactin and fengycin induced stronger plant resistance against *Pst* DC3000 but not against *B. cinerea*, whereas bacilycin induced a stronger

resistance against *B. cinerea* but not against *Pst* DC3000 (Fig. 4). It is known that *Botrytis cinerea* is a kind of necrotrophic pathogen, and plant resistance against these pathogens generally depends on the JA/ET signaling pathways (Pieterse et al. 2009), while *Pst* DC3000 is a hemi-biotrophic pathogen, the plant resistance against it generally depends on the SA signaling pathway, although with exceptions (Pieterse et al. 2009). When comparing the effect of macrolactin, fengycin and bacilycin on plant resistance-related genes, bacilycin showed the strongest effect on the JA/ET signaling pathway genes compared to any other elicitors (Fig. 5), especially for the transcription factor ERF1 and HEL/PR4 (Fig. 5) (Fernández-Calvo et al. 2011; Mao et al. 2016). Macrolactin showed a stronger effect than bacilycin on the induction of CHIB, which is generally recognized to contribute to the plant defense through pathogen cell wall degradation (Pieterse et al. 2009). However, macrolactin was less effective than bacilycin in inducing plant resistance against *B. cinerea* (Fig. 4B). It indicated that up-regulation of CHIB is not necessary for induced systemic resistance against *B. cinerea*. However, it is still not clear whether CHIB is effective against different phytopathogens.

Interestingly, it was observed that phytohormone accumulation in distal plant tissue was slower than in local tissue after inoculation with SQR9 (Fig. 2). The transition of signal from local to distal tissue is achieved by a range of mobile chemicals. Methyl salicylate (MeSA) and ethylene could serve as media in the long distance signaling-transduction in plant (Dempsey and Klessig 2012; Shah and Zeier

2013). This indicated that the time-delay of phytohormones enhancement in distal tissue (shoot) could be caused by the signal translocation from root to shoot.

It is known that some of the secondary metabolites exert multiple functions in bacteria besides antagonistic activity and inducing plant resistance. For instance, surfactin has been reported to enhance the biofilm formation of *Bacillus subtilis* (Aleti et al. 2016). Experiments with pure surfactin showed consistent results with the experiment using an *srf* mutant (Fig. S1), which indicated that the strategy of this study to use a potential elicitor mutant to evaluate their contribution to plant ISR is reliable, but these results cannot be turned into the true effect of the chemically purified elicitor compound. Moreover, the correlation analysis between hormone accumulation and gene expression after inoculation with SQR9 and mutants confirmed the correspondence of bacterial genes and plant defense genes and the cross-talk between hormones and signaling pathways (Fig. S2).

In conclusion, plant beneficial rhizobacterium SQR9 produced multiple elicitors to induce systemic resistance in *Arabidopsis* against *Pst* DC3000 and *B. cinerea*, and these lipopeptides, polyketides, dipepetide antibiotics, 2,3-butandiol and exopolysaccharides played a major role to the ISR. Elicitors have specific effects on the induction of plant defense pathways and against different phytopathogens. Further investigation of the complex crosstalk between the multiple elicitors and the signaling pathways are needed to provide further insights into the interactions between beneficial rhizobacteria and plants.

Materials and methods

Growth conditions of plants and microbes

The *Arabidopsis thaliana* (L.) Columbia (Col-0) seeds were surface sterilized with 75% (v/v) ethanol and then with 2% (v/v) NaClO, after which they were placed in petri dishes containing 1/2 Murashige and Skoog (MS) medium with 2% (w/v) sucrose and 0.8% (w/v) agar. After vernalizing for 2 days at 4°C in darkness, plants were grown under 16 h: 8 h light-dark cycles at 22°C. Ten days later, seedlings were transferred to new petri dishes containing 1/2 MS medium with 2% (w/v) sucrose and 1.5% (w/v) agar for inoculation with SQR9 or its mutants. For assessing the resistance of *Arabidopsis* to *Pst* DC3000 and *B. cinerea*, ten-day-old seedlings were transferred to a growth chamber with a vermiculite-peat soil mixture and allowed to grow for 5 weeks.

Bacillus amyloliquefaciens SQR9 (China General Microbiology Culture Collection Center (CGMCC) accession No. 5808), including wild type and mutants (Table 1), was grown in Luria-Bertani (LB) liquid medium at 37°C and 170 rpm to an OD_{600} of 1.0. Subsequently, bacterial cells were pelleted by centrifugation and suspended to 5×10^8 CFU/mL for use. *Pst* DC3000 was grown in KB liquid medium containing 50 mg/L rifampicin at 28°C and 170 rpm for 18 h. Subsequently, bacterial cells were pelleted by centrifugation and suspended in 10 mM $MgCl_2$ to 10^6 CFU/mL. *Botrytis cinerea* was grown on petri dishes filled with PDA medium at 28°C for 10 days. Spores were collected by washing the colony with sterile water. The

316 concentration was evaluated under a microscope using a counter plate.

317 *Construction of SQR9 mutants*

318 To disrupt 2,3-butanediol synthesis in SQR9, the *alsD* gene was completely deleted
319 by double crossover (Yan *et al.*, 2008). The erythromycin-resistant cassette was
320 obtained from the plasmid pAX01. Two partial sequence fragments of the *alsD* gene
321 were amplified from SQR9 DNA. Then, the recombinant fragments were fused and
322 transformed into the SQR9 strain to generate the *ΔalsD* mutation. The transformants
323 were selected on LB agar plates containing erythromycin. After then, sequencing of
324 the transformants were performed to confirm that the gene was completely knocked
325 out. Mutant strains *Δbae*, *Δmln*, *Δdfn2*, *Δbac3*, *ΔepsD* and *ΔalsD* were constructed in
326 the same manner using chloramphenicol-resistance as the screening marker.

327 For all these mutants of polypeptides and polyketides, high performance liquid
328 Chromatography (HPLC) detection was performed to confirm that the syntheses of
329 the antibiotics were completely blocked (Xu *et al*., 2013; Li *et al*., 2014).

330 *Measurement of salicylic acid, jasmonic acid, ethylene contents in plant*

331 Ten-day-old seedlings of wild-type *Arabidopsis* were planted on new petri dishes
332 containing 1/2 MS medium with 2% (w/v) sucrose and 1.5% (w/v) agar. A 5-μL
333 SQR9 suspension (OD_{600}=1.0) was separately inoculated onto the petri dishes. At 1, 2,
334 4 and 6 days post-inoculation, plant tissues (shoots and roots) were collected and
335 ground in 1.5 mL of sodium phosphate buffer (pH 7.0), and then centrifuged at
336 12,000 rpm for 10 min, after which the supernatants were collected for the detection

of salicylic acid, jasmonic acid, and ethylene. Twelve biological replicates were included per treatment.

Measurements were performed using an enzyme linked immunosorbent assay (ELISA) (Lengton Bioscience Co., Ltd, Shanghai, China). Fifty microliters of supernatant and 50 μL of HRP-conjugate reagent were added to each well of the ELISA kit plate. The wells were incubated at 37°C for 60 min and then washed five times. Afterward, color reactions were performed for 15 min at 37°C in darkness. Absorbance at 450 nm was then measured, and the concentration was calculated based on the standard curve.

Extraction of RNA from plant tissue

Ten-day-old seedlings were transferred to new petri dishes containing 1/2 MS medium for two days. Afterward, 5 μL of suspension of SQR9 or its mutants was inoculated onto the 1/2 MS medium. After 6 h, RNA was extracted from the shoots of *Arabidopsis* in each treatment. The plant tissue was flash-frozen in liquid nitrogen and then ground. The extraction of RNA was performed using the Qiagen RNeasy Plant Mini Kit (Qiagen, Valencia, CA). The extracted RNA was evaluated on a 1% agarose gel, and the concentration and quality (A_{260}/A_{280}) were determined by a NanoDrop ND-2000 spectrophotometer (NanoDrop, Wilmington, DE).

Quantification of the transcription of defense-related genes

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed using a Prime Script RT Reagent Kit (Takara Biotechnology Co., Ltd,

Dalian, China) with an ABI7500 Cycler (Applied Biosystems, Foster City, CA). Their action solution was prepared with SYBR *Premix EXTaq*™ (Takara). The reaction system (20 μL) included 10 μL of SYBR® *Premix Ex Taq*™ (2×), 0.4 μL of PCR forward primer (10 μM), 0.4 μL of PCR reverse primer (10 μM), 0.4 μL of ROX reference dye (50×), 2 μL of DNA sample, and 6.8 μL of ddH_2O. Thermal conditions were as follows: 30 s at 95°C for initial denaturation and 40 cycles of 5 s at 95°C, followed by 34 s at 60°C. The transcription levels of *PR1* (encoding pathogenesis-related protein 1), *PR2* (encoding β-1,3-glucanase), *PR5* (encoding thaumatin-like proteins), *npr1* (encoding regulatory protein NPR1), *myc2* (encoding transcription factor MYC2), *coi1* (encoding coronatine-insensitive protein 1), *aos* (encoding allene oxide synthase), *hel* (encoding hevein-like protein), *erf1* (encoding ethylene-responsive transcription factor 1B), *etr1* (encoding ethylene receptor 1), *chiB* (encoding basic chitinase), and *pdf1.2* (encoding plant defensin) were measured. The *Arabidopsis actin* gene was used as an internal reference. For these genes, primers were listed in Table S6. Ct values (cycling threshold), which represent the relative expression, were used for further analysis.

To correlate the SQR9 metabolites with each plant defense gene, qRT-PCR was performed to determine how these mutant strains affect the transcription of defense genes. The RNA of the shoot tissue of plants inoculated with SQR9 or mutant strains was extracted. Using the effect of wild-type SQR9 on the gene transcription in *Arabidopsis* as 100% efficiency, the lost activity of each mutant strain (contribution

of the elicitor for enhancing the transcription of the defense gene against SQR9) was calculated for each plant defense gene using the following formula to show the contribution of each compound. Statistical analyses of these transcriptions were performed using ANOVA and shown in supplementary materials. The transcription levels of the defense gene in *Arabidopsis* inoculated with wild type SQR9, in *Arabidopsis* inoculated with an elicitor-deficient mutant of SQR9 and in un-inoculated *Arabidopsis* at 6 h post-inoculation were denoted with Q(SQR9WT), Q(SQR9mutant) and Q(CK), respectively.

$$\text{Contribution} = \frac{(Q(SQR9WT)\text{-}Q(CK))\text{-}(Q(SQR9mutant)\text{-}Q(CK))}{Q(SQR9WT)\text{-}Q(CK)}$$

Disease assays

Ten-day-old seedlings of wild-type *Arabidopsis* were transplanted into 200-mL pots filled with vermiculite-peat soil mixture and allowed to grow for five weeks. Seedlings were inoculated with 4 mL of SQR9 (OD_{600}=1.0) or its mutants.

Leaf injection of *Pst* DC3000 at 10^6 CFU/mL was performed 4 days after inoculation with SQR9 or its mutants. Ten millimolar $MgCl_2$ was injected as a mock treatment. The population of *Pst* DC3000 was measured after inoculation for 3 and 6 days. Each leaf sample was washed with sterile water, soaked in 75% (v/v) ethanol for 30 sec for surface sterilization, washed in sterile distilled water three times, and then extracted using grinding beads and 1 mL of $MgCl_2$ (10 mM) in a tissue grinder. Subsequently, appropriate dilutions were plated onto KB agar supplemented with 50 mg/L rifampicin and incubated at 28°C for 24 h. Afterward, rifampicin-resistant *Pst*

DC3000 colonies on plates were counted, and the *Pst* DC3000 density in the leaves was thus determined and expressed as CFU per gram of leaf fresh weight (FW). This experiment was repeated 12 times.

At 4 days post-inoculation with SQR9 or the mutants, five-week-old seedlings of *Arabidopsis* were sprayed with 5×10^5 spores/mL of *B. cinerea*. Water was included as a mock treatment. Symptoms were scored at 2, 4 and 6 days post inoculation with *B. cinerea*. The area under disease progress curve (AUDPC) of each leaf was measured, the disease incidence (DI) was calculated according to the incidence area based on previously described methods (Madden and Hughes 1999; Jeger and Viljanen-Rollinson 2001).

Supplementary data

Figure S1. Disease incidence and defense gene transcription in plant treated with pure surfactin.

Figure S2. Heatmap of the correlation between defense gene transcription and plant defense hormone accumulation at different time points.

Table S1. Infection of *Pst* DC3000 on leaf after inoculation with SQR9 and mutants.

Table S2. Infection of *B. cinerea* on leaf after inoculation with SQR9 and mutants.

Table S3. Phytohormone accumulation in the roots and shoots of *Arabidopsis* inoculated with SQR9.

Table S4. Expression pattern of defense-related genes at different times after

treatment of SQR9.

Table S5. Expression pattern of defense-related genes in response to the inoculation with SQR9 and mutants.

Table S6. Primers used in this study.

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G.W and Y.X performed the experiments, Y.L and G.W analyzed data and wrote the paper, G.Z, Q.S and R.Z designed the research.

Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

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562 **Figure captions**

563 **Figure 1.** Induction of systemic resistance by *Bacillus amyloliquefaciens* SQR9 in
564 *Arabidopsis*. Five-week-old seedlings were inoculated with SQR9. Four days later,
565 leaves were injected or sprayed with *Pst* DC3000 or *B. cinerea*, respectively. (A)
566 CFU of *Pst* DC3000 in the leaves of *Arabidopsis* plants inoculated by SQR9. (B)
567 Disease incidence (DI) of *Arabidopsis* caused by *Botrytis cinerea*. (C) The area under
568 the disease-progress curve (AUDPC) of *Arabidopsis* caused by *Botrytis cinerea*.
569 Symptoms were scored at 2, 4 and 6 days post-inoculation. Disease incidence and the
570 AUDPC were calculated following the method described previously (Madden and
571 Hughes 1999; Jeger and Viljanen-Rollinson 2001). The values are the means ± the
572 standard deviation of 12 replicates. An asterisk (*) indicates statistically significant
573 differences between plants inoculated with SQR9 and control plants without
574 inoculation ($P \leq 0.05$).

575

576 **Figure 2.** Plant hormone accumulation in *Arabidopsis* treated with SQR9.
577 Ten-day-old seedlings were treated with SQR9, and tissue sample of roots or shoots
578 were harvested at the indicated time points. (A) Salicylic acid content in *Arabidopsis*.
579 (B) Jasmonic acid content in *Arabidopsis*. (C) Ethylene content in *Arabidopsis*. The
580 values are the means ± standard deviation of 12 replicates. An asterisk (*) indicates a
581 statistically significant difference ($P \leq 0.05$).

582

583 **Figure 3.** Transcription of defense-related genes in *Arabidopsis* in response to SQR9
584 inoculation. Ten-day-old seedlings were treated with SQR9, and shoot samples were
585 harvested at the indicated time points for extracting total RNA. The results of
586 real-time quantitative polymerase chain reaction analysis of (A) SA-related, (B)
587 JA-related, (C) ET-related and (D) JA/ET-related gene transcript levels in response to
588 SQR9 at different times post-inoculation. The values are the means ± standard
589 deviation of 12 replicates. Different letters above the bars indicate significant
590 differences ($P \leq 0.05$).
591
592 **Figure 4.** Disease incidence of *Arabidopsis* after treated by SQR9 or its mutants.
593 Five-week-old seedlings were inoculated with SQR9 or its mutants. Four days later,
594 leaves were injected with *Pst* DC3000 or *B. cinerea*. (A) Growth curves of *Pst*
595 DC3000 in the leaves of *Arabidopsis* inoculated with SQR9 or mutant strains and the
596 statistical analysis. Different letters indicate significant differences between samples
597 ($P \leq 0.05$). (B) Disease severity of *Botrytis cinerea* in plants inoculated with SQR9 or
598 mutant strains. Symptoms were scored at 2, 4 and 6 days post-inoculation. Disease
599 incidence (DI) and the area under the disease-progress curve (AUDPC) were
600 calculated following the method described previously (Madden and Hughes 1999;
601 Jeger and Viljanen-Rollinson 2001). Different letters indicate significant difference
602 between samples ($P \leq 0.05$). The results are means of 12 independent experiments.
603

Figure 5. Contribution of each elicitor produced by *Bacillus amyloliquefaciens* SQR9 to the enhanced expression of plant defense genes. The shown values were calculated from the results of real-time quantitative polymerase chain reaction analysis of SA-, JA- and JA/ET-related gene transcript levels in the shoots of *Arabidopsis* in response to SQR9 and its mutants. Statistical analyses of these transcriptions were performed using ANOVA and shown in supplementary materials. *sfp*, SQR9 deficient in producing bacillomycin D, fengycin, surfactin, bacillaene, difficidin, macrolactin and bacilysin; *fen*, fengycin; *bam*, bacillomycin D; *srf*, surfactin; *bae*, bacillaene; *mln*, macrolactin; *dfn*, difficidin; *ysnE*, IAA; *alsD*, 2,3- butanediol; *bac*, bacilysin; *epsD*, exopolysaccharides.

Table 1. Bacterial and fungal strains used in this study

Strain	Description	Source
Bacillus amyloliquefaciens SQR9	A PGPR strain, isolated from cucumber rhizosphere	Cao et al. 2011
B. amyloliquefaciens SQR9*Δbam::Tc*^{r}	Deficient in producing bacillomycinD	Xu et al. 2013
B. amyloliquefaciens SQR9*Δfen::Tc*^{r}	Deficient in producing fengycin	Xu et al. 2013
B. amyloliquefaciens SQR9*ΔsrfA::Crm*^{r}	Deficient in producing surfactin	Li et al. 2014
B. amyloliquefaciens SQR9*Δbae::Crm*^{r}	Deficient in producing bacillaene	This study
B. amyloliquefaciens SQR9*Δdfn::Crm*^{r}	Deficient in producing difficidin	This study
B. amyloliquefaciens SQR9*Δmln2::Crm*^{r}	Deficient in producing macrolactin	This study
B. amyloliquefaciens SQR9*Δsfp::Erm*^{r}	Deficient in producing bacillomycin D, fengycin, surfactin, bacillaene, difficidin, macrolactin and bacilysin	Li et al. 2014
B. amyloliquefaciens SQR9Δbac*::Crm*^{r}	Deficient in producing bacilysin	This study
B. amyloliquefaciens SQR9*ΔalsD::Erm*^{r}	Deficient in producing 2,3-butanediol	This study
B. amyloliquefaciens SQR9*ΔepsD::Crm*^{r}	Deficient in producing extracellular polysaccharides	This study
B. amyloliquefaciens SQR9*ΔysnE::Crm*^{r}	Reduced IAA synthesis	Shao et al. 2015
Pseudomonas syringae pv. *Tomato* DC3000	A bacterial pathogen strain	
Botrytis cinerea	A fungal pathogen strain	

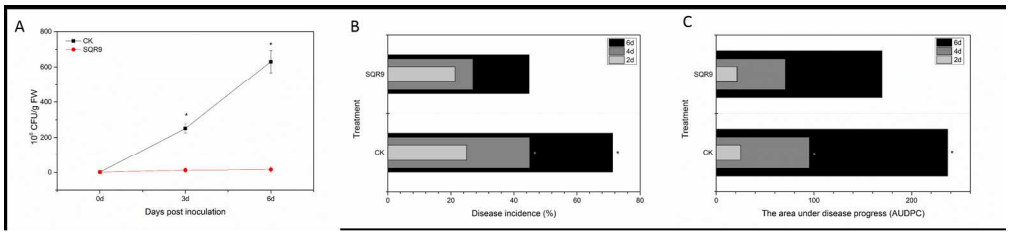

Figure 1. Induction of systemic resistance by Bacillus amyloliquefaciens SQR9 in Arabidopsis. Five-week-old seedlings were inoculated with SQR9. Four days later, leaves were injected or sprayed with Pst DC3000 or B. cinerea, respectively. (A) CFU of Pst DC3000 in the leaves of Arabidopsis plants inoculated by SQR9. (B) Disease incidence (DI) of Arabidopsis caused by Botrytis cinerea. (C) The area under the disease-progress curve (AUDPC) of Arabidopsis caused by Botrytis cinerea. Symptoms were scored at 2, 4 and 6 days post-inoculation. Disease incidence and the AUDPC were calculated following the method described previously (Madden and Hughes 1999; Jeger and Viljanen-Rollinson 2001). The values are the means ± the standard deviation of 12 replicates. An asterisk (*) indicates statistically significant differences between plants inoculated with SQR9 and control plants without inoculation ($P \leq 0.05$).

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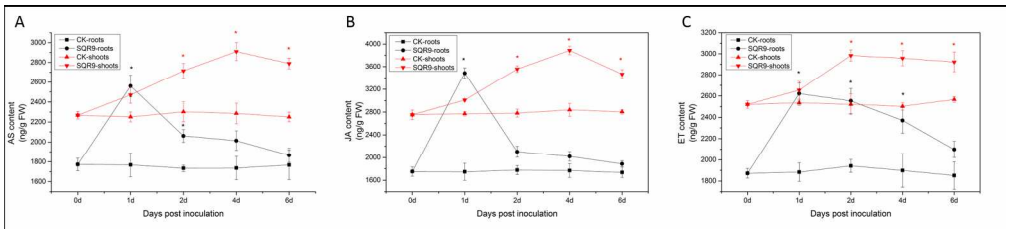

Figure 2. Plant hormone accumulation in Arabidopsis treated with SQR9. Ten-day-old seedlings were treated with SQR9, and tissue sample of roots or shoots were harvested at the indicated time points. (A) Salicylic acid content in Arabidopsis. (B) Jasmonic acid content in Arabidopsis. (C) Ethylene content in Arabidopsis. The values are the means ± standard deviation of 12 replicates. An asterisk (*) indicates a statistically significant difference ($P \leq 0.05$).

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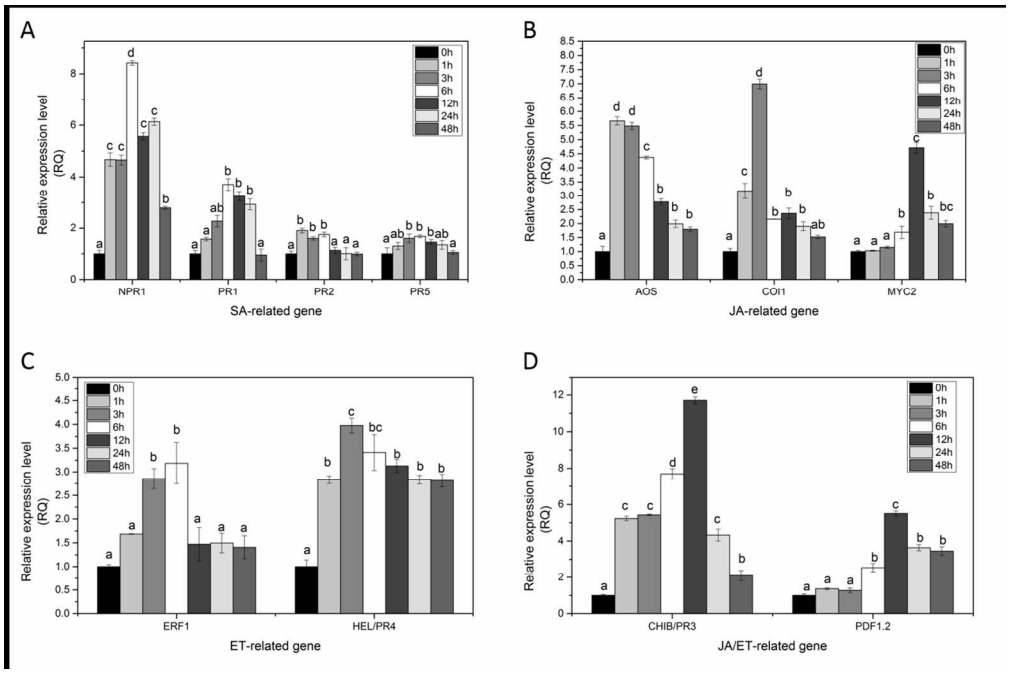

Figure 3. Transcription of defense-related genes in Arabidopsis in response to SQR9 inoculation. Ten-day-old seedlings were treated with SQR9, and shoot samples were harvested at the indicated time points for extracting total RNA. The results of real-time quantitative polymerase chain reaction analysis of (A) SA-related, (B) JA-related, (C) ET-related and (D) JA/ET-related gene transcript levels in response to SQR9 at different times post-inoculation. The values are the means ± standard deviation of 12 replicates. Different letters above the bars indicate significant differences ($P \leq 0.05$).

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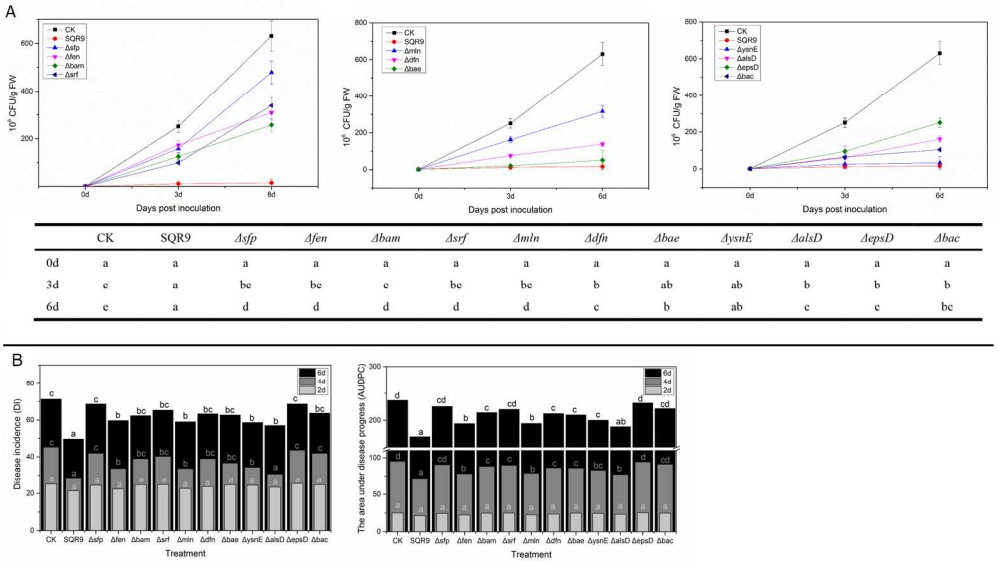

	CK	SQR9	*Δsfp*	*Δfen*	*Δbam*	*Δsrf*	*Δmln*	*Δdfn*	*Δbae*	*ΔysnE*	*ΔalsD*	*ΔepsD*	*Δbac*
0d	a	a	a	a	a	a	a	a	a	a	a	a	a
3d	c	a	bc	bc	c	bc	bc	b	ab	ab	b	b	b
6d	e	a	d	d	d	d	d	c	b	ab	c	c	bc

Figure 4. Disease incidence of Arabidopsis after treated by SQR9 or its mutants. Five-week-old seedlings were inoculated with SQR9 or its mutants. Four days later, leaves were injected with Pst DC3000 or B. cinerea. (A) Growth curves of Pst DC3000 in the leaves of Arabidopsis inoculated with SQR9 or mutant strains and the statistical analysis. Different letters indicate significant differences between samples ($P \leq 0.05$). (B) Disease severity of Botrytis cinerea in plants inoculated with SQR9 or mutant strains. Symptoms were scored at 2, 4 and 6 days post-inoculation. Disease incidence (DI) and the area under the disease-progress curve (AUDPC) were calculated following the method described previously (Madden and Hughes 1999; Jeger and Viljanen-Rollinson 2001). Different letters indicate significant difference between samples ($P \leq 0.05$). The results are means of 12 independent experiments.

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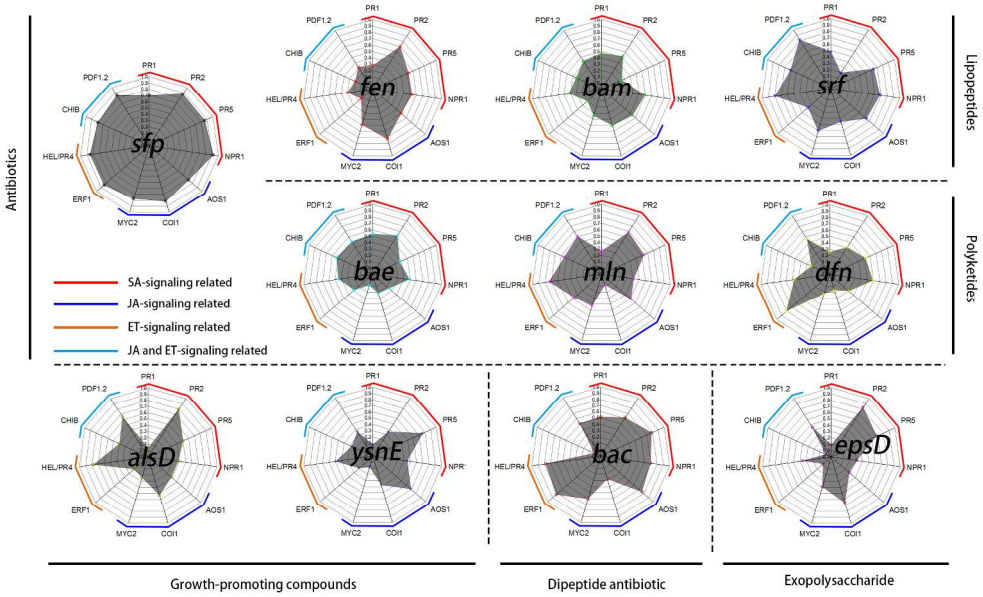

Figure 5. Contribution of each elicitor produced by Bacillus amyloliquefaciens SQR9 to the enhanced expression of plant defense genes. The shown values were calculated from the results of real-time quantitative polymerase chain reaction analysis of SA-, JA- and JA/ET-related gene transcript levels in the shoots of Arabidopsis in response to SQR9 and its mutants. sfp, SQR9 deficient in producing bacillomycin D, fengycin, surfactin, bacillaene, difficidin, macrolactin and bacilysin; fen, fengycin; bam, bacillomycin D; srf, surfactin; bae, bacillaene; mln, macrolactin; dfn, difficidin; ysnE, IAA; alsD, 2,3- butanediol; bac, bacilysin; epsD, exopolysaccharides.

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Supplementary data

Figure S1. Disease incidence and defense gene transcription in plant treated with pure surfactin.

Figure S2. Heatmap of the correlation between defense gene transcription and plant defense hormone accumulation at different time points.

Table S1. Infection of *Pst* DC3000 on leaf after inoculation with SQR9 and mutants.

Table S2. Infection of *B. cinerea* on leaf after inoculation with SQR9 and mutants.

Table S3. Phytohormone accumulation in the roots and shoots of *Arabidopsis* inoculated with SQR9.

Table S4. Expression pattern of defense-related genes at different times after treatment of SQR9.

Table S5. Expression pattern of defense-related genes in response to the inoculation with SQR9 and mutants.

Table S6. Primers used in this study.

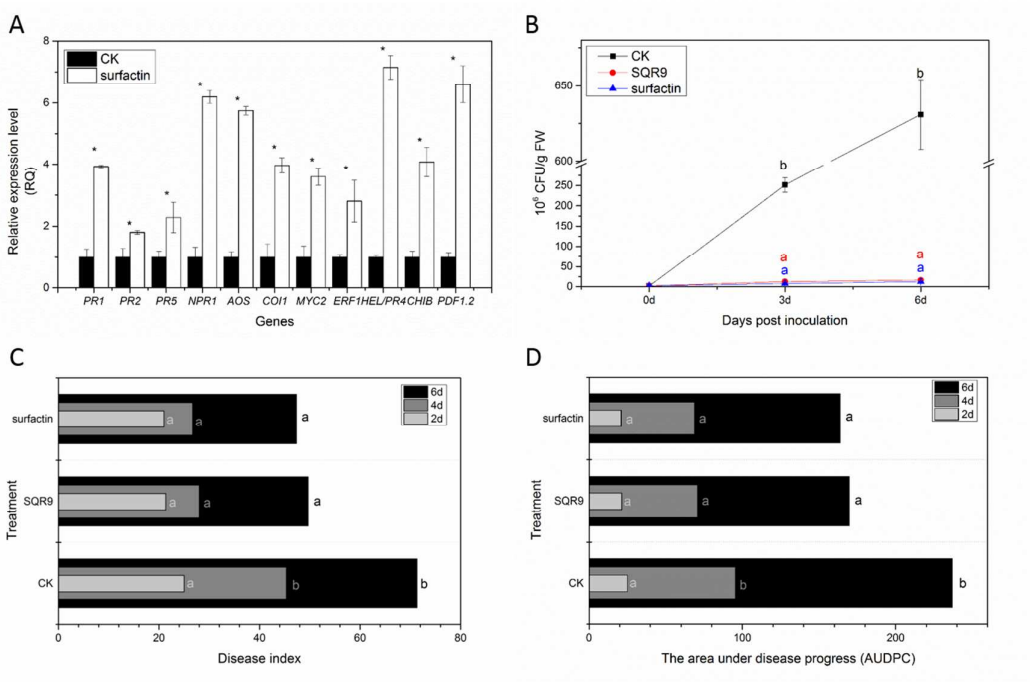

Figure S1. Disease and gene transcription in plant treated by pure surfactin. (A) Transcription of defense-related genes in Arabidopsis in response to SQR9 treatment. (B) Growth curves of *Pst* DC3000 in the leaves of *Arabidopsis* treated by surfactin. (C and D) Disease severity of *Botrytis cinerea* in plants inoculated with SQR9 or mutant strains. Symptoms were scored at 2, 4 and 6-day post-inoculation. Disease incidence (DI) and the area under the disease-progress curve (AUDPC) were calculated following the method described previously (Madden and Hughes 1999; Jeger and Viljanen-Rollinson 2001). Different letters indicate significant difference between samples ($P \leq 0.05$).

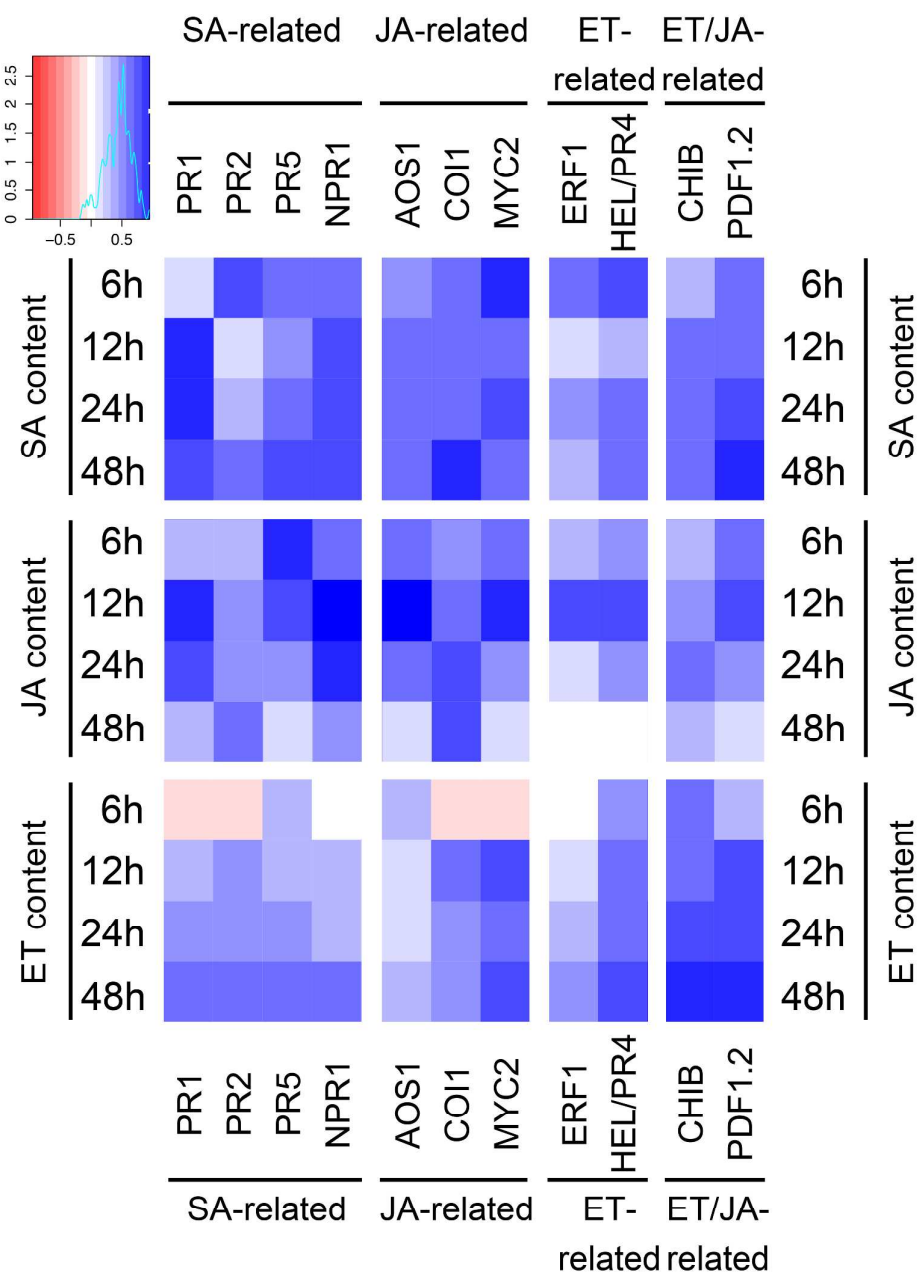

Figure S2. Correlation between defense gene expression and hormone accumulation at different time points. For each time points after inoculation of SQR9 and mutants, phytohormones were measured. All these data was correlated with the expression of defense-related genes at 6 h. The resulted R values were plotted. Blue color indicate a high correlation between the hormone content at this time and the gene expression at 6 h, while in opposite, red color indicate a low correlation.

Table S1. Infection of *Pst* DC3000 on leaf after inoculation with SQR9 and mutants.

	0d (10^6 CFU/g FW)	3d (10^6 CFU/g FW)	6d (10^6 CFU/g FW)
CK	1.58±0.017a	251.18±15.24c	630.98±60.10e
SQR9	2.04±0.022a	12.58±2.24a	16.22±3.54a
Δsfp	1.62±0.36a	158.48±10.27bc	478.63±12.18d
Δfen	2.51±0.32a	173.78±15.18bc	309.03±17.14d
Δbam	1.31±0.26a	125.89±5.14c	257.04±9.32d
Δsrf	1.65±0.25a	107.15±6.18bc	338.84±21.26d
Δmln	1.51±0.0018a	162.18±16.21bc	316.23±31.62d
Δdfn	1.77±0.017a	75.85±7.58b	138.04±10.62c
Δbae	1.54±0.017a	20.41±2.04ab	51.28±51.28b
ΔysnE	2.13±0.023a	26.91±4.28ab	33.11±5.15ab
ΔalsD	1.38±0.20a	61.66±7.15b	162.18±12.16c
ΔepsD	1.65±0.32a	95.50±6.12b	251.19±9.17c
Δbac	1.47±0.21a	64.56±3.21b	104.71±14.15bc

Different letters above the bars indicate significant differences ($P \leq 0.05$).

Table S2. Infection of *B. cinerea* on leaf after inoculation with SQR9 and mutants.

	DI[a]			AUDPC		
	2d	4d	6d	2d	4d	6d
CK	25.00a	45.33c	71.33c	25.00a	95.33d	237.00d
SQR9	21.33a	28.00a	49.67a	21.33a	70.667a	169.67a
Δsfp	24.33a	42.00c	68.67c	24.33a	90.67cd	225.67cd
Δfen	22.33a	33.67b	59.67b	22.33a	78.33b	194.00b
Δbam	24.67a	39.00bc	62.33bc	24.67a	88.33c	214.33c
Δsrf	24.74a	40.33bc	65.33bc	24.74a	89.82c	220.24cd
Δmln	22.67a	33.67b	59.00b	22.67a	79.00b	194.33b
Δdfn	23.67a	39.00bc	63.33bc	23.67a	86.33c	212.33c
Δbae	24.67a	36.67bc	62.67bc	24.67a	86.00c	210.00c
ΔysnE	24.33a	34.33b	58.64b	24.33a	83.00bc	200.31c
ΔalsD	23.33a	30.67a	57.00b	23.33a	77.33b	188.33ab
ΔepsD	25.33a	43.67c	68.67c	25.33a	94.33d	232.00d
Δbac	24.67a	42.00c	63.67bc	24.67a	91.33cd	221.67cd

a: Disease incidence (DI) and the area under the disease-progress curve (AUDPC) were calculated following the method described previously (Madden and Hughes 1999; Jeger and Viljanen-Rollinson 2001). Different letters indicate significant difference between samples ($P \leq 0.05$).

Table S3. Phytohormone accumulation in the roots and shoots of *Arabidopsis* inoculated with SQR9.

		CK-roots (ng/g FW)	SQR9-roots (ng/g FW)	CK-shoots (ng/g FW)	SQR9-shoots (ng/g FW)
	0d	1773.53±63.72	1773.91±72	2268.72±37.45	2268.72±37.45
	1d	1768.17±118.12	2559.51±107.17*	2251.54±49.48	2470.75±82.69
S	2d	1734.49±32.11	2061.42±64.27*	2302.28±99.01	2714.14±75.24*
A	4d	1736.97±117.79	2013.30±98.05	2285.57±102.41	2909.85±90.34*
	6d	1767.59±147.66	1865.89±70.05	2250.66±46.30	2788.95±54.82*
	0d	1752.16±82.46	1752.16±82.46	2752.62±82.58	2752.62±82.58
	1d	1749.15±150.81	3483.28±91.55*	2770.11±36.96	3008.57±19.29
JA	2d	1778.83±79.80	2103.72±96.12	2780.72±69.89	3555.50±57.16*
	4d	1769.36±121.38	2019.96±86.31	2837.05±112.01	3887.08±73.57*
	6d	1737.23±92.22	1886.56±56.55	2802.97±39.53	3465.38±79.68*
	0d	1872.25±45.37	1872.25±45.37	2521.72±38.74	2521.72±38.74
	1d	1883.35±87.51	2622.24±99.02*	2536.19±25.47	2654.28±82.61
	2d	1942.25±61.11	2553.86±116.61*	2521.87±97.39	2983.92±53.64*
ET	4d	1899.30±158.35	2370.93±121.10*	2503.21±34.71	2958.57±71.17*
	6d	1851.31±126.35	2098.76±76.73	2566.93±26.92	2922.48±93.86*

Asterisk (*) indicates statistically significant difference.

Table S4. Expression pattern of defense-related genes at different times after treatment of SQR9.

	0h	1h	3h	6h	12h	24h	48h
PR1	1.00±0.12[a]	1.57±0.067	2.27±0.22	3.71±0.22	3.25±0.18	2.93±0.21	0.96±0.22
PR2	1.00±0.11	1.91±0.088	1,60±0.064	1.75±0.091	1.14±0.12	1.01±0.23	0.99±0.070
PR5	1.00±0.23	1.30±0.13	1.61±0.16	1.68±0.055	1.48±0.095	1.35±0.17	1.06±0.068
NPR1	1.00±0.12	4.67±0.25	4.65±0.19	8.42±0.088	5.57±0.13	6.13±0.14	2.79±0.055
AOS	1.00±0.18	5.66±0.14	5.48±0.12	4.37±0.047	2.78±0.11	1.99±0.13	1.80±0.080
COI1	1.00±0.10	3.18±0.27	6.99±0.17	2.16±0.0081	2.37±0.19	1.90±0.15	1.53±0.054
MYC2	1.00±0.044	1.03±0.017	1.15±0.041	1.69±0.21	4.71±0.18	2.38±0.23	1.99±0.12
HEL/PR4	1.00±0.14	2.83±0.085	3.98±0.15	3.41±0.37	3.13±0.13	2.83±0.093	2.81±0.13
ERF1	1.00±0.046	1.68±0.0078	2.86±0.21	3.18±0.43	1.47±0.35	1.50±0.20	1.41±0.24
CHIB/PR3	1.00±0.051	5.23±0.12	5.43±0.045	7.69±0.29	11.71±0.18	4.32±0.32	2.09±0.28
PDF1.2	1.00±0.079	1.36±0.056	1.28±0.12	2.54±0.22	5.50±0.14	3.64±0.17	3.45±0.22

a Numbers in table indicate the expression level in relation with that at 0 h post-inoculation.

Table S5. Expression pattern of defense-related genes in response to the inoculation with SQR9 and mutants.

	PR1[a]	*PR2*	*PR5*	*NPR1*	*AOS1*	*COI1*	*MYC2*	*ERF1*	*HEL/PR4*	*CHIB/PR3*	*PDF1.2*
CK	1.00±0.012a	1.00±0.035a	1.00±0.028a	1.00±0.082a	1.00±0.085a	1.00±0.026a	1.00±0.022a	1.00±0.044a	1.00±0.030a	1.00±0.073a	1.00±0.10a
SQR9	2.67±0.053c	1.53±0.0057c	1.69±0.016d	1.99±0.11c	4.06±0.060d	3.45±0.067d	3.41±0.10c	2.16±0.013c	2.04±0.031c	2.24±0.053c	3.45±0.0069d
Δsfp	1.51±0.12ab	1.07±0.22a	1.10±0.035ab	1.08±0.073a	1.85±0.12b	1.44±0.11ab	1.54±0.12ab	1.18±0.020a	1.16±0.0031a	1.26±0.095a	1.38±0.054ab
Δfen	2.22±0.065bc	1.16±0.026ab	1.33±0.011bc	1.46±0.11b	2.54±0.12bc	1.66±0.14ab	2.22±0.027b	2.00±0.011c	1.73±0.019b	1.99±0.014bc	2.68±0.012c
Δbam	1.89±0.069b	1.25±0.0041b	1.51±0.042c	1.41±0.12b	2.39±0.046bc	2.21±0.050bc	2.58±0.11bc	1.93±0.10c	1.60±0.084b	1.82±0.090b	2.38±0.062bc
Δsrf	1.87±0.12b	1.42±0.050c	1.24±0.019b	1.31±0.072b	2.10±0.12b	2.33±0.099bc	1.95±0.10b	1.72±0.010b	1.22±0.091ab	1.50±0.087ab	1.47±0.085ab
Δbae	1.78±0.090b	1.20±0.038ab	1.43±0.030c	1.50±0.11b	3.31±0.12c	2.92±0.13c	3.21±0.13c	1.83±0.041bc	1.58±0.058b	1.58±0.017ab	2.21±0.060bc
Δmln	2.23±0.11bc	1.17±0.0041ab	1.25±0.042b	1.58±0.12b	2.49±0.083bc	3.26±0.15c	2.36±0.12bc	1.61±0.011b	1.27±0.014ab	1.55±0.090ab	1.97±0.062b
Δdfn	2.25±0.078bc	1.31±0.050bc	1.33±0.038bc	1.40±0.012b	3.12±0.042c	3.06±0.20c	2.87±0.12bc	1.23±0.020ab	1.56±0.018b	1.96±0.087bc	2.06±0.086b
Δbac	1.81±0.015b	1.20±0.036ab	1.15±0.060ab	1.30±0.033b	1.73±0.017b	2.78±0.16c	1.95±0.11b	1.20±0.041ab	1.23±0.013ab	2.23±0.024c	2.21±0.037bc
ΔalsD	2.61±0.021c	1.11±0.028ab	1.33±0.062bc	1.61±0.032bc	2.88±0.12c	2.14±0.019bc	2.83±0.089bc	1.93±0.015c	1.34±0.0097ab	1.74±0.034b	1.86±0.017b
ΔysnE	2.54±0.027c	1.34±0.012bc	1.16±.035ab	1.55±0.018b	1.95±0.084b	2.54±0.12c	3.22±0.16c	1.94±0.10bc	1.53±0.029b	1.91±0.044bc	2.59±0.026c
ΔepsD	2.53±0.073c	1.09±0.027a	1.21±0.086b	1.68±0.022bc	3.21±0.015c	1.83±0.035b	2.45±0.083bc	2.08±0.035c	1.67±0.012b	2.24±0.030c	2.35±0.044bc

a Numbers in table indicate the expression level in relation with that in CK

Table S6. Primers used in this study

Name	Sequence(5'to3')	Target gene
ACTIN2-F	CCTGCCATGTATGTTGCCATT	
ACTIN2-R	AATCGAGCACAATACCGGTTGT	Internal reference
PR1-F	AGGTGCTCTTGTTCTTCCCT	
PR1-R	ACCCCAGGCTAAGTTTTCCC	Detection of expression of PR1
PR2-F	TGGTGTCAGATTCCGGTACA	
PR2-R	TCATCCCTGAACCTTCCTTG	Detection of expression of PR2
PR5-F	GGAACAATTGCCCTACCACC	
PR5-R	GCCGTTACATCTTAGACCGC	Detection of expression of PR5
NPR1-F	ACCGATAACACCGACTCCTC	
NPR1-R	GCACCGGTGGAAAGAAACTT	Detection of expression of NPR1
AOS-F	TGAGTTTGTGCCGGAGAGAT	
AOS-R	ATCACAAACAACCTCGCCAC	Detection of expression of AOS
COI1-F	TCAAATCGGTGCACTTCCGA	
COI1-R	ACCTCAAAAGCATCGAGCCA	Detection of expression of COI1
MYC2-F	ATAAATCTCCAGCTCCGCCG	
MYC2-R	AAGCGTTTGCAACGGGTAAC	Detection of expression of MYC2
ERF1-F	AGGATGGTTGTTCTCCGGTT	
ERF1-R	AGACCCCAAAAGCTCCTCAA	Detection of expression of ERF1
HEL-F	ATCTGCTGCAGTCAGTACGG	
HEL-R	TGAGCTCATTGCCACAGTCG	Detection of expression of HEL
CHIB-F	GCTTCAGACTACTGTGAACC	
CHIB-R	TCCACCGTTAATGATGTTCG	Detection of expression of CHIB
PDF1.2-F	CACCCTTATCTTCGCTGCTC	
PDF1.2-R	GCACAACTTCTGTGCTTCCA	Detection of expression of PDF1.2

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