**Paenibacillus terrae** AY-38 resistance against *Botrytis cinerea* in *Solanum lycopersicum* L. plants through defence hormones regulation

Ah-Young Kim\(^a\), Raheem Shahzad\(^a\), Sang-Mo Kang\(^b\), Abdul Latif Khan\(^b\), Seok-min Lee\(^a\), Yeon-Gyeong Park\(^a\), Won-Hee Lee\(^a\) and In-Jung Lee\(^a\)

\(^a\)School of Applied Biosciences, Kyungpook National University, Daegu, Korea; \(^b\)UoN Chair of Oman’s Medicinal Plants & Marine Natural Products, University of Nizwa, Nizwa, Oman

**ABSTRACT**

This study aims to investigate the role of *Paenibacillus terrae* AY-38 to produce bioactive metabolites and to counterpathogenic infections caused by *B. cinerea*. The pure culture of *P. terrae* (AY-38) showed the secretion of significant amount of IAA (indole-3-acetic acid) (109.57 ± 3.2 µg mL\(^{-1}\)). The AY-38 strain also produced siderophore and glucanase, while in the in vitro test, it showed significant antagonism to *Botrytis cinerea*. In the in vivo plant experiment, the sole application of AY-38 significantly improved plant growth (plant height and leaf area), while in *B. cinerea* infected plants, AY-38 inoculation not only decreased the disease incidence on leaves and fruits but also reprogrammed the plants for higher growth. AY-38 treatments promoted and rescued plant growth by modulating the defence responses of endogenous hormones, such as jasmonic and salicylic acids. Our findings concluded that *P. terrae* possesses great potential as a possible biocontrol agent against *B. cinerea*-induced pathogenic infections.

**Introduction**

Plant diseases are the foremost constraint to sustainable agricultural productivity. Among different diseases, grey mold decay caused by *Botrytis cinerea* is a pervasive and destructive disease of many plants and vegetables, including tomatoes (Youssef & Roberto 2014). This pathogenic fungus either penetrates the plant parts directly or enters through wounds and causes infections (Lee et al. 2006). High humidity and free moisture on plant surfaces encourage infections caused by pathogenic *B. cinerea* (Lee et al. 2006). Currently, chemically synthetic fungicides are the primary source for the control of diseases caused by *B. cinerea* (Zhu et al. 2010; Zouari et al. 2016). However, because of environmental and human health risks, development of fungicide resistant fungal strains, and privation of most operative fungicides result in the need for alternative control measures that are safe, environment friendly, and effective (Janisiewicz & Korsten 2002; Droby et al. 2009; Sharifi & Ryu 2016).

Biological control offers ecofriendly alternatives to chemically synthesized fungicides, to control pests and overwhelm plant diseases with naturally occurring and environment-friendly microbes (Droby et al. 2009). The use of natural antagonistic microorganisms as biological control has been broadly studied. Some microbes have been shown to have significant antagonistic activity against grey mold decay (Salikarios et al. 2002; Utkhede & Mathur 2002), although on tomato plants, the biological control of grey mold decay is limited to few fungal and bacterial strains (Utkhede & Mathur 2002). According to a recent review, based on scientific and economic importance, *B. cinerea* is placed second among the top 10 pathogens (Dean et al. 2012).

Rhizospheric microorganisms live in symbiosis with plants and play a vital role in reprogramming the defence response by influencing the physiology of and modulating phytohormonal signaling in plants under pathogenic attack (Spence et al. 2014). Plants nurture valuable soil microorganisms by giving them a supporting environment in the rhizosphere, while, in return, the microbes provide numerous benefits to plants, such as increased phytohormone production (Kang et al. 2015), growth promotion (Vacheron et al. 2013), protection from phytopathogens (Lugtenberg & Kamalova 2009), and stimulation of physiohormonal signaling (Walker et al. 2003; Kang et al. 2015). Plant growth promoting rhizospheric bacteria (PGPR) can contribute favorably towards plant growth when it grows in association with plant roots via solubilizing nutrients, nitrogen fixing, nutrient acquisition, and phytohormone production (Zhou et al. 2016). In addition to the promotion of plant growth, PGPR also exhibit biological control potential including antibiotic secretion, nutrient competition, enzyme production, and induction of systemic resistance (Venturi & Keel 2016).

Among various plant growth promoting microbes, *Paenibacillus* sp. show significant biocontrol and plant growth promoting potential, because the metabolites that they produce not only encourage plant growth but also affect rhizospheric microflora, creating an antagonistic environment for the pathogen or triggering the host plant’s defence system (Ghazalibiglar et al. 2016). Plants initiate primary and secondary metabolism to overcome threats and in response to various biotic stresses. The immediate response of plant primary and secondary metabolites to various pathogenic attacks cannot be denied (Mason et al. 2016; Verma et al. 2016). Among several metabolites, plants recruit hormones from their...
arsenal for defence and growth (DeLucia et al. 2012). Hormones are small organic compounds usually present in very low concentrations at sites of origin or in distant tissues (Santner & Estelle 2009; Almeida Trapp et al. 2014). Plant hormones assume a vital role in plant improvement and response to biotic stresses. Salicylic acid (SA) and jasmonic acid (JA), in particular, have appeared in mediating stress reactions in plants (Verma et al. 2016). SA plays a crucial role in plant defence against various abiotic and biotic stresses. Particularly, in response to pathogens, SA is involved in multiple defence processes such as basal and resistance gene-mediated defence as well as systemic acquired resistance (Lu et al. 2016), while the induction of JA involved in the massive expression and activation of genes is responsible for direct and indirect defence systems. The activation of JA pathway long-distance signal mechanisms induces JA in many tissues other than the infected ones, in order to provide systemic protection against pathogenic attacks in the future (Ballaré 2014). This study was aimed to isolate and evaluate the in vitro and in vivo biocontrol efficiency of selected PGPRB strains against the tomato grey mold decay caused by *B. cinerea*. Moreover, this study evaluates the changes to physiohormonal levels after the inoculation of PGPRB and the grey mold decay causing *B. cinerea*.

**Materials and methods**

**Isolation of PGPRB and growth conditions**

The soil samples from agriculture fields of Geongbuk province, South Korea (37°North, 127°30′East), were collected and brought to the laboratory in sterilized zip bags. About 10 g of soil was transferred to an autoclave (autoclaved for 15 min at 121°C) sterilized flask containing sterile Amies solution (Amies 1967). About 0.1 mL aliquots of the serial diluted (10−4) suspension were plated on tryptic soy agar (TSA; Merck Co., Germany) and kept at 27°C for 5 days in incubator. The plates were monitored for bacterial growth on a daily basis and morphologically differentiated colonies were selected and re-streaked on fresh TSA plates.

**Screening for siderophore, glucanase, and IAA production**

Siderophore production was tested by growing AY-38 in the universal siderophore detection CAS (Chrome-Azurol-S agar) medium, while glucanase production was examined on the CMC (Carboxymethyl cellulose) medium. The plates were incubated for 5 days and the development of a clear zone around the colony was considered a positive result.

**IAA production**

The IAA producing potential of AY-38 was examined using gas chromatography–mass spectrometry (GC–MS) (6890N network GC system, and 5973 network mass selective detector) (Agilent Technologies, Palo Alto, CA, USA) according to the method described by Kang et al. (2015). Briefly, a 7-day old culture of AY-38 was centrifuged (10,000×g at 4°C) and the supernatant was filtered through a 0.45 μm cellulose acetate filter. The 50 mL culture filtrate was then extracted, dried, and methylated with diazomethane. GC–MS with selected ion monitoring (SIM) (6890N Network GC System and 5973 Network Mass Selective Detector; Agilent Technologies, Palo Alto, CA, USA) was used for analysis. The amounts of IAA produced by the bacterial isolate in broth were calculated from the peak areas of IAA and compared with the corresponding known standards.

**Genomic DNA extraction, PCR analysis, and sequencing for identification**

Among the screened bacterial isolates, the AY-38 strain possessed a strong potential to produce IAA and other PGPR traits. AY-38 was identified based on 16S rRNA gene sequences according to the protocol described by Shahzad et al. (2016). Briefly, the 16S rDNA was PCR (polymerase chain reaction) amplified using the 27F (5′-AGAGTTTTGATC (AC) TGGCTCAG-3′) and 1492R primers (5′-CGG (CT) TAGCTTGTTAGCAG-3′). The BLAST search program was used for the nucleotide sequence homology of this bacterial isolate. Closely related sequences with the highest homology, query coverage, and lowest E values were selected and aligned by ClustalW using MEGA version 6.0 software. The 16S rRNA gene sequence of isolate AY-38 was submitted to the NCBI GenBank under accession number KX129792.

**In vitro antifungal potential of AY-38**

The antagonistic activity of AY-38 against various pathogenic fungi was carried out in dual culture according to method described by Ji et al. (2014). Briefly, AY-38 was streaked on one side of the petri plates containing potato dextrose agar medium, while 0.5 cm diameter mycelial discs of *B. cinerea*, *Sclerotinia sclerotiorum*, *Colletotrichum truncatum*, and *Fusarium solani* were placed on the other side. The plates were incubated at 25°C for 3 week and the distance between AY-38 and the fungi was measured.

**Antagonism of AY-38 against Botrytis cinerea on tomato fruits**

The tomato fruits were surface-sterilized with 75% ethanol and then perforated with a sterilized toothpick. The AY-38 culture medium was then sprayed on them and then they were air-dried. After 3 h of complete drying, the 5 mm discs of *B. cinerea* were kept on the perforated area.

**Determination of effect of AY-38 on pathogenic Botrytis cinerea hyphal morphology**

The effect of AY-38 on the hyphal morphology of *B. cinerea* was examined using scanning electron microscopy (SEM) according to the method described by Pacioni et al. (2007). Briefly, the mycelia were taken from a pure culture as well as the interaction and distal parts of colonies in dual cultures. The collected samples were fixed in 3% glutaraldehyde and dehydrated in an ethanol series. After dehydration, the samples were subjected to critical point drying with carbon, coated with gold, and examined with SEM.

**PGPRB inoculation during pathogenic infection to tomato**

A complete randomized experiment was carried out to assess the plant growth promoter and biological control efficiency of
AY-38 against tomato grey mold decay causing *B. cinerea*. For plant experiments, a three-time autoclaved horticultural substrate (peat moss 10–15%, perlite 35–40%, coco peat 45–50%, and zeolite 6–8%, containing NH₄⁺ ∼0.09 mg g⁻¹, NO₃⁻ ∼0.205 mg g⁻¹, P₂O₅ ∼0.35 mg g⁻¹, and K₂O ∼0.1 mg g⁻¹) was used. The seeds were surface-sterilized and germinated. Equal sized germinated seeds were transferred to the germination tray for 1 week. The equal sized seedlings were then transferred to sterilized plastic pots. Before 5 days of pathogenic fungal inoculation, 2-week-old tomato seedlings were inoculated with AY-38 cells. The tomato leaves were perforated with a sterilized toothpick and the 5 mm discs of *B. cinerea* were attached to the perforated area with a squash tap. The control and infected plants were kept in dark conditions at 30°C for 3 days, to maximize the pathogenic effect. After pathogenic infection, the growth attributes were recorded daily and the plants were stored at −80°C for further analysis. Disease severity in tomato plants caused by *B. cinerea*, and the alleviative role of AY-38 in disease reduction was measured by recording the percentage of healthy and infected leaves after 7 days of inoculation.

**Endogenous phytohormonal quantification**

**JA extraction and quantification**

Endogenous JA was extracted and quantified according to the protocol described in previous studies (McCloud & Baldwin 1997; Shahzad et al. 2015). Briefly, the extracted plant samples from ground, freeze-dried plants were further analyzed using GC–MS. To expand the affectability of the method, the spectra were recorded in selected ion modes, i.e. in the JA determination case, we inspected the fragment ion at m/z = 83 amu, relating to the base peaks of JA and [9,10-²H₂]-9,10-dihydro-JA. Moreover, JA was calculated from the value of endo peak and compared with their respective standards.

**SA extraction and quantification**

SA was extracted and quantified from freeze-dried tomato samples according to the protocol described by Seskar et al. (1998). The extracted samples were subjected to high performance liquid chromatography (HPLC), which was performed using a Shimadzu device outfitted with a fluorescence indicator (Shinadzu RF-10AXL) with excitation at 305 nm and emission at 365 nm, and filled with a C18 reverse phase HPLC column (HP Hypersil ODS, particle size 5 µm, pore size 120 Å, Waters). Flow rates used were retained at 1.0 mL/min.

**Statistical analysis**

The triplicate data were collected from three independent experiments and subjected to Student *t*-tests using GraphPad Prism (v6.01) for statistical analysis. The graphs were drawn using GraphPad Prism (version 6.01, San Diego, California, USA).

**Results**

**IAA production by AY-38**

The amount of IAA produced by AY-38 in the pure culture was quantified by GC–MS/SIM (Figure 1(a, b)) using deuterated internal standards. The results indicated the presence of IAA in the culture filtrate of AY-38. The quantification results revealed that AY-38 produced significant amounts (109.57 ± 3.2 µg mL⁻¹) of IAA (Figure 1(a, b)).

**Siderophore and glucanase production by AY-38**

AY-38 was inoculated on media to determine its ability to produce glucanase and siderophore. The results indicated that AY-38 has the potential for siderophore and glucanase production. After 7 days of inoculation, AY-38 changed the color of the media to orange indicating the production of siderophore (Figure 1(c)), while on the glucanase producing media, AY-38 exhibited a clear zone (Figure 1(d)).

**Characterization and identification of isolated AY-38**

The identification of selected bacterial isolate AY-38 was carried out based on a 16S rDNA sequence. The sequence BLASTn results showed that AY-38 is closely related to members of the genus *Paenibacillus* and had the highest sequence identity (99%) with *P. terrae*. To further confirm this, a phylogenetic analysis was performed by constructing a maximum parsimony (MP) tree (Figure 2) and aligning it to the closely related sequences. The isolate AY-38 and 18 other strains from the same genus were used to generate the MP tree. The isolate AY-38 showed a high sequence homology and formed a distinct linkage in sub-clade same branch with type strain *P. terrae*, supported by 100% bootstrap value in the MP tree. Based on all these results, the isolate AY-38 was identified as *P. terrae* and its 16S rRNA gene sequence was deposited in the NCBI GenBank under accession number KX129792.

**Antagonism of *P. terrae* (AY-38) against pathogenic fungi**

The in vitro antagonistic activity of AY-38 against various fungi such as *B. cinerea*, *S. sclerotiorum*, *C. truncatum*, and *F. solani* were measured in a dual culture. The results showed that AY-38 exhibits strong antagonistic activity against the growth of *B. cinerea* (Figure 3(a)). To further confirm the antagonism of AY-38, *B. cinerea* was applied to the tomato fruits, followed by the foliar application of AY-38 cells. The results revealed that the tomato fruits treated with AY-38 inoculation strongly inhibited the growth of *B. cinerea* on tomato fruits unlike those inoculated with *B. cinerea* alone (Figure 3(b)).

**Determination of effect of *P. terrae* (AY-38) on pathogenic Botrytis cinerea hyphal morphology**

Under the SEM, the determination effect of AY-38 on pathogenic *B. cinerea* hyphal morphology was observed. The SEM analysis revealed the incompatibility of *B. cinerea* with AY-38. In the presence of AY-38, *B. cinerea* hyphal exhibited highly deformed and degenerative changes (Figure 3(c)).

**Ameliorative response of *P. terrae* (AY-38) to tomato growth against disease infection**

To assess the plant growth promoting and biological control efficiency of AY-38, a complete, randomized tomato plants experiment was carried out against tomato grey mold decay causing *B. cinerea*. The plants were pretreated with AY-38
cells and water to judge their growth promoting effects, and inoculated with *B. cinerea* in order to infect them. The disease symptoms were constantly increasing throughout the experiment and the plants were severely damaged after 7 days. Interestingly, the development of the plants treated with AY-38 cells improved, disease symptoms of *B. cinerea* dramatically decreased, and the survival of the plants was encouraged (Figure 4).

The results indicated that AY-38 significantly improved plant growth under non-pathogenic and pathogenic interactions (Figure 5). Under non-pathogenic interactions, the inoculation of AY-38 significantly increased plant height after the third (24.9%), fifth (25.05%), and seventh day (26.38%). A similar trend was found in diseased plants and the treatment of AY-38 significantly increased plant height after the third (48.08%), fifth (69.19%), and seventh day.

Figure 1. Bioactive traits of *Paenibacillus terrae* (AY-38). (a) The ability of *Paenibacillus terrae* (AY-38) to produce indole-3-acetic acid (IAA). (b) GC–MS/SIM spectrometry analysis of IAA produced by AY-38. *Paenibacillus* terrae (AY-38) was identified to produce high amounts of (c) glucanase and (d) siderophore. Columns with error bar represent mean ± SD from the data of three replicates.

Figure 2. MP tree constructed by aligning the sequence of AY-38 with homologous 16S rRNA sequences in related taxa for phylogenetic analysis MEGA (v7.01). Our isolated bacterial strain showed 100% homology as it formed a clade with *Paenibacillus* sp., and hence, was identified as *Paenibacillus terrae*. 
Furthermore, under non-pathogenic and pathogenic conditions there was no significant difference between leaf length and width except that leaf length increased after the seventh day (7.69%). However, under non-pathogenic conditions, AY-38 improved leaf length and width after the fifth (18.76% and 65.5%, respectively) and seventh day (29.45% and 76%, respectively) of application compared to plants inoculated with *B. cinerea* (Figure 5).

**Impact of *P. terrae* (AY-38) against disease severity**

The application of *P. terrae* significantly reduced the severity of the infection caused by *B. cinerea* compared to inoculations of *B. cinerea* alone (Figure 6). The healthy leaves were significantly reduced in sole *B. cinerea* application, whereas, in *P. terrae* (AY-38) inoculation has significantly increased the healthy leaves.

**Regulation of endogenous defence-related phytohormones**

**Modulation of JA during pathogenic infections**

Endogenous JA contents of tomato plants treated with *P. terrae* under non-pathogenic conditions and plants infected with *Botrytis cinerea* were significantly reduced in a time-dependent manner (Figure 7(a)). Before the inoculation of *P. terrae* and *B. cinerea* there was no significant difference found in the plants. Under non-pathogenic conditions, *P. terrae* treatment significantly decreased the JA contents after the second (14.30%), third (16.31%), fifth (9.74%), and seventh day (14.66%) compared to the control, and a similar trend was observed under pathogenic attacks. The endogenous JA contents of tomato plants infected with *B. cinerea* were significantly reduced in *P. terrae* (AY-38) treated plants after the second (2.83%), third (36.51%), fifth (53.85%), and seventh day (66.87%) compared to *B. cinerea* infected plants (Figure 7(a)).

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**Figure 3. Biocontrol potentials of *Paenibacillus terrae* (AY-38).** The images show (a) the antagonistic activity of AY-38 against *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Colletotrichum truncatum*, and *Fusarium solani*; (b) the pathogenic effects of *B. cinerea* on tomato fruits. The effects of AY-38 are ameliorative as seen here in comparison with water and *B. cinerea* infection alone. Scanning electron micrographs of *Botrytis cinerea* hyphae infection to tomato with or without *Paenibacillus terrae* (AY-38) inoculation. (a) Healthy hyphae development and (b) stunted growth in hyphae because of the presence of AY-38.

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Changes in endogenous SA under disease incidences

The treatment of *P. terrae* (AY-38) significantly increased the endogenous SA contents of tomato plants, both under non-pathogenic conditions and in plants infected with *B. cinerea*, in a time-dependent manner (Figure 7(b)). Prior to AY-38 and *B. cinerea* inoculation, no significant differences were recorded in the endogenous SA contents of the plants. Under control conditions, an increased endogenous SA content were recorded after the second (24.68%), third (24.98%), fifth (16.08%), and seventh day (114.47%) in plants treated with AY-38. A similar trend was under pathogenic attack by *B. cinerea*. AY-38 treatment significantly increased the endogenous SA contents of tomato plants after the second (17.42%), third (40.29%), fifth (16.71%), and seventh day (77.63%) in contrast with *B. cinerea* infected plants (Figure 7(b)).

Discussion

Plant rhizospheric bacteria have been extensively studied to discover the underlying mechanisms by which these organisms are involved in plant growth promotion and stress mediation (Babalola 2010; Pii et al. 2015; Vejan et al. 2016). Rhizospheric bacteria from different genera are identified for their plant growth promoting and stress mediating traits. However, the genus *Paenibacillus* has not been fully explored. Originally, *Paenibacillus* was included within the genus *Bacillus*, but was later reclassified as a separate genus 'Paenibacillus' (Ash et al. 1994). The *Paenibacillus* species are a widely distributed group of rhizobacteria in the soil and rhizosphere of different plants (Yoon et al. 2003). These species are recommended for their beneficial effects on plant growth and could be applied in fields because of their plant growth promoting potential and biocontrol properties (Kim et al. 2011).

The plant growth promoting and pathogen suppression potential of *Paenibacillus* is well documented (Phi et al. 2008; Mageeshwaran et al. 2012; Ren et al. 2012) and they are reported to produce various phytohormones, such as cytokinin and IAA. Therefore, this study investigated IAA production, and examined the antifungal potential of *P. terrae* in order to elucidate the mechanisms by which defence-related hormonal regulation under pathogenic attacks of *B. cinerea*, occur in tomato plants.

The AY-38 strain was isolated from the rhizospheric soil and identified as *P. terrae*. *P. terrae* has been never reported for IAA production and antagonistic activity against *B. cinerea*. This strain was found to produce significant amounts of IAA. The results of IAA production by AY-38 are in line with Phi et al. (2008) and Dama et al. (2008), who reported the production of IAA by *Paenibacillus*. IAA production is considered the major factor contributing to tomato plant growth promotion. Moreover, AY-38 produced significant amounts of siderophore and glucanase. Siderophore and glucanase producing PGPRB potentially function as biological control agents. The siderophore they produce binds to iron thereby preventing its utilization by pathogens, while the glucanase is known to be correlated with pathogen resistance as it degrades the components of fungal cell walls, resulting in pathogen suppression and a better crop growth and yield (Sayyed et al. 2005; Hong & Meng 2003). Similarly, in this study the SEM of co-inoculated AY-38 and pathogenic *B. cinerea* revealed the abnormal and degraded growth of the *B. cinerea* hyphal wall, which was correlated with the glucanase production ability of AY-38 (Hong & Meng 2003; Alt-Lahsen et al. 2001).

For biocontrol, the most substantial contributions of the *Paenibacillus* sp. are antibiotic production (Raza et al. 2008) and root colonization (Haggag & Timmusk 2008). In this study, AY-38 was examined for its antagonistic potential.

![Figure 4. Growth promoting effects and biocontrol potentials of *Paenibacillus terrae* (AY-38) in tomato plants under *Botrytis cinerea* infections. The foliar application of *B. cinerea* to the leaf became hazardous to tomato leaves; however, during *P. terrae* (AY-38) inoculation, the effects were comparatively reduced.](image-url)
against various pathogenic strains, and a strong antagonistic activity was found against \( B. \ cinerea \). The applicable biocontrol of \( B. \ cinerea \) is limited, compared to other pathogenic fungal strains, and the commercial biological control products are not yet available (Utkhede & Mathue 2002). This antagonism exhibited by \( Paenibacillus \) against pathogenic fungi highlighted its possible potential as a biological control agent.

In this study, the growth promoting potential of AY-38 was confirmed and the results are in line with the findings of Ahmad et al. (2016) and Lal and Tabacchioni (2009). They reported the growth promotion of many plants by

![Figure 5. Effects of Paenibacillus terrae (AY-38) on tomato growth characteristics with and without pathogenic infection of Botrytis cinerea. Columns with error bars represent mean ± SD from data of three replicates. Columns with different letters are significantly different at the .05 level of probability as identified by the least significant difference (LSD) test.](image-url)
P. polymyxa, P. dendritiformis, P. durus, and many other Paenibacillus sp. Three possible mechanisms of plant growth promotion under non-pathogenic and pathogenic condition have been suggested, namely antagonism of pathogens, promotion of host nutrients and growth, and stimulation of the plant defence system (Choudhary & Johri 2009).

Plants have an endogenous hormonal system that can be encouraged by the application of many plant growth promoting microbes (Khalid et al. 2006; Xin & He 2013). Encouraging plant resistance by the prior application of a biological inducer is viewed as a unique strategy for plant defence. In this study, after treatment with AY-38, the extensive modulation of endogenous defence hormones, such as JA and SA under normal and pathogenic conditions were recorded. These hormones can act as phytoalexins during pathogenic attacks and induce phytoresistance to pathogens (Halim et al. 2007; Schouten 2016). The hormonal signaling of JA and SA play an important role in the co-ordination of plant immune responses. The SA and JA hormonal cross talking is thought to optimize plant defence responses against pathogenic attacks that stimulate both signaling pathways (Pieterse et al. 2012). It is well reported that necrotrophic pathogenic infections up-regulate JA biosynthesis (Wasternack & Hause 2013), while SA is involved in multiple defence processes such as basal and resistance gene-mediated defence as well as systemic acquired resistance (Lu et al. 2016). Moreover, their antagonism co-ordinates plant immune responses (Pieterse et al. 2012). Similar results of enhanced SA and decreased JA following plant growth promoting microbes inoculation have been reported by many researchers (Kang et al. 2015; Waqas et al. 2015; Shahzad et al. 2016), suggesting the role of SA in induced systemic resistance (Pozo & Azcón-Aguilar 2007). Similarly, in solanaceous plants, Tjamos et al. (2004) reported the induced systemic resistance by P. alvei in the inhibition of pathogenic Verticillium dahliae, and in another study (Tjamos et al. 2005), they also confirmed the induced systemic resistance potential of this strain by using

**Figure 6.** Effect of Paenibacillus terrae (AY-38) on tomato plant during pathogenic infection of Botrytis cinerea as indicated by disease severity.

**Figure 7.** (a) Regulation of endogenous JA inoculation of AY-38 and pathogenic infection of Botrytis cinerea. (b) Regulation of endogenous SA inoculation of AY-38 and pathogenic infection of B. cinerea. Columns with error bars represent mean ± SD from data of three replicates. Columns with different letters are significantly different at the .05 level of probability as identified by the LSD test.
gene expression qRT-PCR in Arabidopsis thaliana. In an examination of the ethylene pathway using qRT-PCR, Lee et al. (2012) reported the production of the volatile compound ‘tridecane’ by Paenibacillus, which acts as a bacterial trigger to activate systemic resistance and induces the plant defence system by the SA/JA signaling pathway.

Our results demonstrated that the P. terrae (AY-38) isolate from the rhizospheric soil, is an ideal candidate for a broader application, as the strain did not only produce IAA, siderophore, and glucanase but also showed in vitro and in vivo antagonism against various phytopathogenic fungi. AY-38 not only improved tomato growth, but also conferred resistance against one of the most serious disease causing pathogens, B. cinerea. The endogenous hormonal modulation under normal and pathogenic attack may have activated the resistance against pathogenic fungi. PGPR isolates secrete a number of secondary metabolites, which can induce resistance to the plants against various biotic and abiotic stresses. Therefore, further studies are required to ascertain the secondary metabolites produced by rhizospheric bacterial isolates and determine their role in plant defence against various biotic stresses.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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