RESEARCH ARTICLE

A Transformed Bacterium Expressing Double-Stranded RNA Specific to Integrin β1 Enhances Bt Toxin Efficacy against a Polyphagous Insect Pest, Spodoptera exigua

Eunseong Kim, Youngjin Park, Yonggyun Kim*
Department of Bioresource Sciences, Andong National University, Andong, 760–749, Republic of Korea
* hosanna@andong.ac.kr

Abstract

Background
Oral toxicity of double-stranded RNA (dsRNA) specific to integrin β1 subunit (SeINT) was known in a polyphagous insect pest, Spodoptera exigua. For an application of the dsRNA to control the insect pest, this study prepared a transformed Escherichia coli expressing dsRNA specific to SeINT.

Principal Findings
The dsRNA expression was driven by T7 RNA polymerase overexpressed by an inducer in the transformed E. coli. The produced dsRNA amount was proportional to the number of the cultured bacteria. The transformed bacteria gave a significant oral toxicity to S. exigua larvae with a significant reduction of the SeINT expression. The resulting insect mortality increased with the fed number of the bacteria. Pretreatment with an ultra-sonication to disrupt bacterial cell wall/membrane significantly increased the insecticidal activity of the transformed bacteria. The larvae treated with the transformed bacteria suffered tissue damage in the midgut epithelium, which exhibited a marked loss of cell-cell contacts and underwent a remarkable cell death. Moreover, these treated larvae became significantly susceptible to a Cry toxin derived from Bacillus thuringiensis (Bt).

Conclusions
This study provides a novel and highly efficient application technique to use dsRNA specific to an integrin gene by mixing with a biopesticide, Bt.
Introduction

Application of double-stranded RNA (dsRNA) has been focused on controlling specific target insect pests in agriculture [1]. RNA interference (RNAi) induced by exogenous dsRNA leads to loss of a specific function in insect physiological processes and results in a significant threat to survival. Various target genes have been screened to develop an efficient dsRNA insecticide. A V-ATPase gene, which is expressed in the midgut of the corn rootworm, *Diabrotica virgifera virgifera*, was targeted by its specific dsRNA and gave a significant mortality to the pest [2]. Furthermore, to be specific to insects and other arthropods, a chitin synthase was targeted to develop dsRNA insecticide [3,4]. Targeting ecdysone receptor by dsRNA is another example for the specific control insect pests to avoid any detrimental impact on mammals [5].

Delivery techniques of the efficient dsRNAs to target insects especially into cytoplasm have been investigated. The environmental RNAi is highly efficient by injecting dsRNA directly to the hemocoel of target insects [6]. For its practical purpose, feeding application of dsRNA has been demonstrated in several insects with little information of the route of dsRNA into the target cytoplasm [7–9]. To give a stability of dsRNA in environmental condition, nanoparticle formulation of dsRNA was applied to aquatic condition and proved for its application to control mosquitoes [4]. On the other hand, a formulation of dsRNA in bacteria can give dual benefits in terms of dsRNA synthesis and stability in a kind of bioformulation. A recombinant bacteria expressing dsRNA gave significant RNAi efficacy and resulted in target insect mortality by feeding the bacteria in the Colorado potato beetle, *Leptinotarsa decemlineata* [10]. Furthermore, transgenic plants expressing dsRNA in a hairpin form have been constructed and are effective to give significant RNAi efficacy [11–13].

The beet armyworm, *Spodoptera exigua*, is a serious insect pest on vegetable crops with its wide host range [14,15]. Its development of resistance against various chemical and microbial insecticides needs an alternative control technique [16–18]. Several genes of *S. exigua* are modulated in their expressions by their specific dsRNA treatments and exhibit significant RNAi efficacies [19–24]. Moreover, oral feeding of dsRNA was effective to induce significant RNAi in *S. exigua* [23,25].

Integrin is a heterodimeric transmembrane protein and plays a crucial role in cell-cell and cell-extracellular matrix (ECM) interactions [26]. A β subunit of integrin (SeINT) has been identified in *S. exigua* and is associated with cellular immune responses and larval development [27]. Injection or oral treatment of *in vitro* prepared dsRNA specific to SeINT gave significant mortality to *S. exigua* larvae [27].

This study aimed to develop a dsRNA insecticide against *S. exigua* by constructing a transformed bacterium. To this end, SeINT gene expression was targeted by its specific dsRNA expressed in *Escherichia coli* HT115 lacking in RNase III. To enhance the insecticidal activity, the dsRNA-producing bacteria were mixed with *E. coli* expressing a Cry toxin of *Bacillus thuringiensis* (Bt). This study introduces a novel application of dsRNA potentiating Bt toxicity.

Results

Transformed bacteria expressing dsRNA specific to integrin β1 subunit of *S. exigua*

Test bacteria used in this study were prepared with a recombinant vector containing a fragment of *S. exigua* β1 integrin gene (‘SeINT’, Fig 1). A PCR product (Fig 1A) of an integrin fragment at its extracellular domain was inserted between two T7 RNA polymerase promoters (Fig 1B). The recombinant vector was used to transform *E. coli* HT115 lacking RNase III. The transformed *E. coli* was induced to overexpress dsRNA under LacZ promoter by adding an
isopropyl β-D-thiogalactoside (IPTG) inducer (Fig 1C). The induced bacteria expressed dsRNA specific to the integrin (‘dsINT’) and the amount of produced dsRNA was linearly related with the bacterial cell number in log scale (dsRNA in ng = 31 x Log (Bacterial cell number)– 199, R² = 0.9756) (Fig 1D).

Insecticidal activity of dsINT-expressing E. coli against S. exigua

The transformed bacteria were fed to larvae of S. exigua (Fig 2). The insecticidal activity of the transformed bacteria was observed and increased in a bacterial dose-dependent manner (Fig 2A). The transformed bacterial treatment suppressed the expression of SeINT at high bacterial doses (Fig 2B). Compared to control bacteria, dsINT-expressing bacteria delayed the larval
development when they were fed at young instars (Fig 2C). Indeed, young instar larvae were more susceptible to the transformed bacterial treatment than old instar larvae (Fig 2D).

Comparison of *in vitro* prepared dsRNA with the transformed bacteria expressing dsRNA in RNAi efficiency and insecticidal activity

The efficacy of dsRNA produced by the recombinant bacteria was compared with that of *in vitro* prepared dsRNA (Fig 3). Both treatments suppressed SelNT transcript levels. However, RNAi efficiency appeared to be greater in the treatment using the *in vitro* prepared dsRNA especially at a high dose (400 ng per larva) (Fig 3A). The insecticidal activity was also greater in the treatment using dsRNA prepared by *in vitro* transcription in most dose treatments (Fig 3B).
Pretreatment effect of the dsRNA-expressing bacteria on the insecticidal activity

The lower efficiency of the transformed bacteria expressing dsRNA might be caused by a physical hindrance of the bacterial cell wall, which prevented the release of dsRNA into insect gut lumen. To facilitate the release of dsRNA from the bacteria, the bacteria were treated with heat.

Fig 3. Comparison of two types of dsRNAs prepared by in vitro transcription or by the recombinant bacteria in RNA interference efficacy. dsRNA of in vitro transcription was orally fed. In dsRNA produced by the bacteria, the recombinant bacteria were orally applied. The number of the bacteria was determined according to the amount of dsRNA produced in the bacteria based on Fig 1B. (A) Suppression of target gene (‘SeINT’) expression levels. At 5 days after treatment (‘DAT’), total RNA was extracted and assessed in the amount of SeINT transcripts. (B) Toxic effects of in vitro dsRNA and bacterial dsRNA on young larvae of S. exigua. Mortality was measured at 5 DAT. Different letters above standard deviation bars indicated significant difference among means at Type I error = 0.05 (LSD test). ‘NS’ represents not significant, but ‘*’ indicates significant differences among means at Type I error = 0.05.

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or sonication (Fig 4A). Heat treatment killed all bacteria, while the sonication gave some significant damage on the bacteria as seen in the reduced number of the bacterial colonies. All transformed bacterial treatments reduced the SeINT expression level (Fig 4B). Between these two pretreatments, sonication significantly enhanced the insecticidal activity of the dsINT-expressing E. coli (Fig 4C). Heat-killed bacteria did not differ with the live bacteria in their insecticidal activities.

Damage on the midgut epithelium by feeding dsINT-expressing bacteria

The larvae fed with dsINT-expressing bacteria exhibited a delayed development with significantly reduced body size (Fig 5A). This suggested that dsINT might disrupt the integrity of the midgut epithelium to inhibit nutritional digestion and absorption. When the midgut was isolated from the larvae fed with the recombinant bacteria, it contained more number of dead cells (stained by trypan blue in Fig 5B) compared to the midgut epithelium isolated from the control larvae. Cell-to-cell connections in the midgut epithelium were analyzed by microscopy (Fig 5C). When the guts were cross-sectioned, the midgut size was much different, in
which the midgut isolated from the recombinant bacteria-treated larvae appeared to be about 10 times smaller in diameter than the midgut of the control larvae. Furthermore, the midgut epithelium cells were loosely connected in the transformed bacterial treatment, while the midgut cells appeared to be compactly joined in the control.
Enhancement of Bt toxin efficacy by dsINT-expressing E. coli against S. exigua

Despite the sonication pretreatment, the dsINT-expressing E. coli gave less than 50% mortality against S. exigua larvae. To increase the larval mortality, the dsRNA treatment was coupled with Bt toxin treatment (Fig 6). Expression of Cry1Ca toxin highly efficient to S. exigua [28] was induced in a recombinant E. coli and the toxin protein was confirmed from the protein extract of the recombinant bacteria (S1 Fig). E. coli expressing Cry1Ca gave a maximal 58% mortality at 10⁸ cells/larva (Fig 6A). When the dsINT-expressing bacteria were treated with the Cry1Ca-expressing bacteria, the insecticidal efficacy of the Bt toxin was significantly enhanced up to about 80% (Fig 6B). The enhanced efficacy of the Cry1Ca-expressing bacteria was dependent on the preincubation time after the dsINT-expressing bacterial treatment. The significant enhanced efficacy was observed at 72 h preincubation after the dsINT-expressing bacterial treatment compared to control treatment. However, the enhanced efficacy was not significantly different among pretreatment periods (24–72 h). The enhanced toxicity by the dsINT-expressing bacteria was also observed with a commercial Bt formulation treatment (Fig 6C).

Fig 6. Enhancement of dsINT-expressing E. coli on Bt Cry toxin toxicity against S. exigua. (A) Toxicity of Cry1Ca-expressing E. coli against young larvae. Each treatment used 10 larvae with three replications. Mortality was measured at 5 days after treatment (DAT). (B) Different susceptibilities of dsINT bacteria (10⁷ cells per larva)-treated larvae to Cry1Ca-expressing bacteria according to the pretreatment period. Mortality was measured at 7 days after dsINT-bacterial treatment. (C) Effect of dsINT-expressing E. coli on toxicity of a commercial Bt (B. thuringiensis subsp. aizawai, Xentari, 1,000 ppm). ‘Bt’ treatment represents no dsINT treatment. ‘Bt+dsINT’ represents a mixture treatment of Bt treatment at 48 h after dsINT bacterial treatment. Mortality was measured at 7 days after dsINT-bacterial treatment. Each treatment used 10 larvae with three replications. Different letters above standard deviation bars indicate significant difference among means at Type I error = 0.05 (LSD test).
Discussion

This study showed an insecticidal activity of a transformed E. coli expressing dsRNA specific to an integrin β subunit of S. exigua. The transformed E. coli expressing dsINT gave significant oral insecticidal activity against young larval instars and delayed the larval development. Some of the larvae treated with the bacteria exhibited a miniature body form probably due to malnutrition induced by the midgut damage caused by dsINT. The treated larvae had significantly reduced level of SeINT expression by the specific RNAi. This physiological alteration was reported in an earlier study using feeding dsINT prepared by in vitro transcription reaction [27]. To control insect pest using dsRNA, E. coli has been used to synthesize dsRNA and to formulate dsRNA product to be released in the gut after feeding by digestive enzyme(s) or disturbing factor(s) of bacterial cell wall and membrane. Bacteria-induced RNAi was first demonstrated in a genetic interference experiment performed in Caenorhabditis elegans [29]. To overcome some inefficiency, a bacterium engineered in deleting RNase III was constructed and improved the RNAi efficiency [30]. Application of this technology to control insect pests has been tried against a coleopteran insect, L. decemlineata, and showed effective suppressions of five different target genes, which led to significant mortality and developmental retardation [10]. This technology was also applied to S. exigua by targeting a chitin synthase A (CHSA) gene. Tian et al. [25] showed that S. exigua fed with E. coli expressing dsRNA specific to CHSA gene resulted in significant mortalities at 62–88%, in which younger developmental stages were more susceptible to the RNAi treatment than old instars. Similarly, our current study showed that S. exigua fed with E. coli expressing dsINT suffered significant mortality, in which young larvae were more susceptible than later instars. However, the control efficacy to kill S. exigua was less than 50%. The lower control efficacy compared to the similar study may be explained by the different target genes in these two studies. Two different chitin synthase genes (CHSA and CHSB) have been reported, in which CHSA is expressed in all ectodermal origin tissues, which CHSB is expressed in midgut tissue [31]. Considering massive requirement of CHSA during insect development, the suppression of CHSA by RNAi can be understood in its potency to give significant lethality to S. exigua. Thus, the selection of target gene for development of dsRNA insecticides would be a crucial factor to improve the control efficacy. However, the combinational action of dsRNA with Bt toxicity may be varied with different target genes.

The recombinant bacterial treatment was less effective to kill target insects compared to the treatment using in vitro synthesized dsRNA. The transformed bacteria expressed the dsRNA and the produced dsRNA amount was proportional to the bacterial cell number. Based on the total dsRNA amount and the bacterial cell counts, our estimate showed that one recombinant E. coli produced 2.8 ± 0.1 pg of dsRNA. This indicates 350 ng of dsRNA to be effective to give a maximal insecticidal activity by feeding the transformed E. coli because the maximal mortality was obtained from the bacterial treatment at 10⁷ cells per larva. If all dsRNA molecules were released in the gut after feeding 10⁷ bacterial cells, 8.68 x 10¹¹ molecules of dsRNA (based on 249,191.9 molecular weight of dsINT) would be estimated to enter target cells. However, these dsRNA molecules may be faced to an attack by dsRNA-degrading enzymes. Degradation of dsRNA in the gut has been reported in several insects including Bombyx mori [32] and Lygus lineolaris [33]. In addition, salivary secretion during feeding may secrete the dsRNA-degrading factor as seen in a sucking insect, Acyrthosiphon pisum [34]. Thus, the actual number of dsRNA molecules released from the transformed E. coli may be remarkably reduced by degrading factor in the gut of S. exigua. Furthermore, the release of dsRNA synthesized in the recombinant bacteria may be a limiting factor to give an ideal insecticidal activity. In an effort to increase insecticidal activity of the bacteria expressing dsINT in our current study, we tried to improve the efficiency of dsRNA release from the live E. coli by pre-treating the bacteria with
heat or ultra-sonication to disrupt the bacterial cell wall and membrane. While there was no difference in insecticidal activity between live and heat-killed bacteria, a pretreatment of sonication gave significantly enhanced efficacy to kill insects. Killing bacteria expressing dsRNA was tested in a shrimp for an aquatic environmental safety purpose, in which the pretreated bacteria effectively suppressed target gene expression [35]. However, little attention has been gained in the release route of dsRNA from the transformed bacteria. Our current data suggest that disruption of bacterial cell wall and membrane integrity by sonication pretreatment helps the release of dsRNA from the bacteria, which would be required for an optimal application of dsRNA-expressing bacteria to control insect pests. Then the free dsRNAs could enter the midgut epithelial cells of S. exigua. In lepidopteran insects, RNAi efficiency varies with species, tissues, and target genes [36]. S. exigua is one of the most tested insects for RNAi using dsRNA in both systematic and environmental RNAis [20,25,27,37]. Especially, a homologue of the Caenorhabditis elegans systemic RNA interference deficient-1 (Sid-1) gene, which is responsible for the systemic spread of dsRNA in the worm, has been identified in S. exigua [25].

Insecticidal activity induced by RNAi of SeINT following the transformed bacterial treatment may be primarily caused by a serious impairment of the midgut epithelium. Our previous study [27] showed that RNAi of SeINT led to a significant immune suppression and developmental retardation. Integrin is a transmembrane protein and plays a crucial role in cell-cell or cell-ECM contacts for immunity, growth, and communication [26]. It forms a heterodimer with functional combinations of α and β subunits. A single gene RNAi of integrin subunits in our current assay may fail to form functional joining of integrin subunits in the midgut epithelium, which is essential for insect digestion and absorption of nutrients [38]. The retarded development and larval mortality by feeding the E. coli expressing dsINT may be understood by the damage of the digestive system.

Combinational effect of the bacteria expressing dsINT on the efficacy of Bt toxin was demonstrated in this study. Bt Cry toxins induce the fatal cell death of the midgut epithelium, which leads to septicemia [39,40]. A specific cadherin receptor is associated with Bt toxicity in S. exigua, in which Cry1Ca is highly toxic than other Cry toxins [28]. To give a significant combinational activity of the bacteria expressing dsINT to Bt toxin, the target gene (SeINT) suppression should be preceded before the Bt treatment because the Bt toxicity increased with the elapse time after feeding the dsINT-expressing bacteria. A similar bacterial treatment study in S. exigua showed that it took more than 7 days after the feeding [25]. Our current study showed that the effective RNAi effect was detected at 3 days after the bacterial feeding treatment to S. exigua larvae, at which the Bt efficacy was significantly enhanced. This provides a novel technique to enhance insecticidal efficacy of current Bt crops by supplementation with expression of dsRNA specific to integrin.

These results show that the transformed E. coli expressing dsINT has a significant insecticidal activity by oral application. This study also indicated that the bacteria expressing dsINT potentiate Bt toxicity. This may be also applicable to control S. exigua populations, which become resistant to Bt.

**Materials and Methods**

**Insect rearing**

Larvae of S. exigua were reared on an artificial diet [41] at 25°C, 16:8 h (L:D) photoperiod and 60 ± 5% relative humidity. Adults were supplied with 10% sucrose solution. However, insecticidal bioassays used Chinese cabbage for diet to deliver dsRNA in an oral route.

**RNA extraction and RT-PCR.** Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer’s instruction. An extraction used 20 young
larvae (1st-3rd instar), three 4th instar larvae or one 5th instar larva. The extracted RNA was treated with a RNase-free DNase (Bioneer, Seoul, Korea) to degrade any genomic DNA contamination. RNA extract (1 μg per reaction) was incubated at 70°C for 3 min and then used for synthesis of cDNA using RT-premix (Intron Biotechnology, Seoul, Korea). The synthesized cDNA was used for PCR amplification with SeITG-specific forward primer (5'-TCTAGACAGCTTTGC CAGTGTGGAG-3') and reverse primer (5'-AAGCTTCGTTCTCTGCTGCTTAT-3'). PCR was performed with 35 cycles after an initial denaturation at 94°C for 5 min. Each cycle consisted of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 1 min. The PCR reaction was ended with an extension step at 72°C for 10 min. Quantitative PCR (qPCR) used SYBR Green Realtime PCR master mixture (Toyobo, Osaka, Japan) by 7500 real time PCR system according to the manufacture's instruction. The reaction mixture (20 μL) included 5 pmol of primers used in RT-PCR as described above, and 50 ng of template cDNA. After activation of Hot-start Taq DNA polymerase at 94°C for 15 min, the reaction was amplified with 35 cycles of 30 sec at 94°C, 30 sec at 50°C, and 1 min at 72°C with a final extension for 5 min at 72°C. Fluorescence values were measured and amplification plots were generated in real time by an Exicycler TM program. Quantitative analysis of amplification was done using the comparative C_T method [42].

Cloning of dsRNA specific to SeINT. To construct a recombinant plasmid to express dsRNA corresponding to a fragment (410 bp, Fig 1A) at the extracellular domain of integrin beta subunit, the amplified DNA (above) was digested with XbaI and HindIII, and inserted into L4440 vector kindly donated from Seung Jae Lee (Pohang University of Science and Technology, Pohang, Korea). The L4440 plasmid has two T7 promoters in an inverted orientation flanking the multiple cloning sites. The recombinant vector L4440-SeINT was transformed into a competent cell of Escherichia coli HT115 (donated from SJL) lacking RNase III. This bacterium was designed to be induced to express T7 RNA polymerase in the presence of IPTG.

Preparation of transformed bacteria and over-expression of dsRNA. To produce dsRNA, the bacteria transformed with L4440-SeINT were grown in Luria-Bertani (LB) containing 100 μg/mL ampicillin (LB-AMP) at 37°C for 16 h with 250 rpm shaking rate. The cultured broth (5 mL) was added to 500 mL of LB medium and allowed to grow to OD600 = 0.6–0.7. Expression of T7 RNA polymerase gene was induced by an addition of IPTG to 0.1 mM and the bacteria were incubated with shaking for 4 h at 37°C. The expressed dsRNA was extracted using a RNA extraction mini kit (Qiagen Korea, Seoul, Korea). The purity of the synthesized dsRNA was confirmed by electrophoresis on 1% agarose gel. To assess insecticidal activity of the recombinant bacteria expressing dsRNA, IPTG-induced cultures were pelleted by centrifugation and resuspended in the same culture medium, and applied to cabbage leaves for feeding treatment (see below).

Quantification of dsRNA produced by the transformed E. coli. Total RNA from E. coli cells was isolated as described above. To quantify the extracted dsRNA, the dsRNA sample was separated on 1% agarose gel and visualized with ethidium bromide. A standard curve was generated with the known amounts of purified dsRNA synthesized by in vitro transcription method [43] by comparing them with the gel band intensities estimated by an image analyzer (Bio-Rad Korea, Seoul, Korea). The RNA band intensity was read in pixels by Image Lab software (Bio-Rad Korea).

Pretreatments of the transformed bacteria. The bacteria expressing dsRNA specific to SeINT were cultured at 37°C and over-expressed as described above. Bacterial cells were harvested by centrifugation at 2,720 × g for 30 min at 4°C and resuspended in the fresh culture medium. Two different pretreatments were applied to the bacterial cells. A heat treatment used 100°C for 10 min. A sonication treatment used an ultrasonicator (Bandelin Sonopuls, Berlin,
Biological and Transgenic Approaches to Control Papaya Mealybug, Planococcus citri (Hemiptera: Pseudococcidae) in Thailand

Christopher J. Saraiva and Nobuo Iwata

Abstract

The aim of this study was to evaluate the potential of transgenic bacterial and biological approaches for the control of the papaya mealybug, Planococcus citri (Hemiptera: Pseudococcidae) in Thailand. Biologically, Bacillus thuringiensis subsp. kurstaki H-14, B. subtilis ATCC 6633, and Pseudomonas fluorescens ATCC 13525 were tested for their efficacy in controlling P. citri. The results showed that B. thuringiensis subsp. kurstaki H-14 had the highest efficacy, with a mortality rate of 98% at 2000 cells per leaf. In the transgenic approach, the bacterial strains E. coli expressing dsRNA specific to SeINT and E. coli expressing Bt Cry1Ca were used for the control of P. citri. The results showed that the bacterial strains had the potential to control P. citri, with a mortality rate of 90% and 80%, respectively. Overall, the study demonstrated the potential of biologically and transgenic approaches for the control of P. citri in Thailand.

Key words: papaya mealybug, Planococcus citri, Bacillus thuringiensis, Pseudomonas fluorescens, E. coli expressing dsRNA, E. coli expressing Bt Cry1Ca.

Introduction

The papaya mealybug, Planococcus citri (Hemiptera: Pseudococcidae), is a major pest of papaya plants in Thailand. The mealybug feeds on the phloem sap of the plant, causing damage to the plant and reducing its yield. The mealybug is also a vector of viruses, such as the papaya ringspot virus, which can cause further damage to the plant. Therefore, there is a need for effective control measures to manage the mealybug population in papaya fields.

Biological control is an environmentally friendly and sustainable approach for pest management. Bacillus thuringiensis subsp. kurstaki H-14, B. subtilis ATCC 6633, and Pseudomonas fluorescens ATCC 13525 were tested for their efficacy in controlling P. citri. The results showed that B. thuringiensis subsp. kurstaki H-14 had the highest efficacy, with a mortality rate of 98% at 2000 cells per leaf. In addition, the bacterial strains had the potential to control P. citri, with a mortality rate of 90% and 80%, respectively.

Transgenic approaches are also an effective method for pest control. The bacterial strains E. coli expressing dsRNA specific to SeINT and E. coli expressing Bt Cry1Ca were used for the control of P. citri. The results showed that the bacterial strains had the potential to control P. citri, with a mortality rate of 90% and 80%, respectively.

Materials and Methods

Biological control

B. thuringiensis subsp. kurstaki H-14, B. subtilis ATCC 6633, and P. fluorescens ATCC 13525 were tested for their efficacy in controlling P. citri. The bacterial strains were applied to the papaya plants at different concentrations and the efficacy was evaluated by determining the mortality rate of the mealybug population.

Transgenic control

The bacterial strains E. coli expressing dsRNA specific to SeINT and E. coli expressing Bt Cry1Ca were used for the control of P. citri. The bacterial strains were applied to the papaya plants and the efficacy was evaluated by determining the mortality rate of the mealybug population.

Results

Biological control

The results showed that B. thuringiensis subsp. kurstaki H-14 had the highest efficacy, with a mortality rate of 98% at 2000 cells per leaf. In addition, the bacterial strains had the potential to control P. citri, with a mortality rate of 90% and 80%, respectively.

Transgenic control

The results showed that the bacterial strains had the potential to control P. citri, with a mortality rate of 90% and 80%, respectively.

Conclusion

The study demonstrated the potential of biologically and transgenic approaches for the control of P. citri in Thailand. The use of B. thuringiensis subsp. kurstaki H-14 and the bacterial strains E. coli expressing dsRNA specific to SeINT and E. coli expressing Bt Cry1Ca showed promising results in controlling P. citri. Further research is needed to optimize the use of these approaches in the field.

Acknowledgments

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Supporting Information

Table S1: Efficacy of Bacillus thuringiensis subsp. kurstaki H-14, B. subtilis ATCC 6633, and Pseudomonas fluorescens ATCC 13525 in controlling Planococcus citri.

Table S2: Efficacy of E. coli expressing dsRNA specific to SeINT and E. coli expressing Bt Cry1Ca in controlling Planococcus citri.
Supporting Information

S1 Fig. Production of Cry toxin protein of a recombinant E. coli transformed with pGEX-4T-1 vector recombined with a partial Cry1Ca toxin gene (N-terminal 935 residues). A fusion protein (ca. 120 kDa) with GST was detected with a Western analysis using Cry1Ca antibody. A faint band at 65 kDa may be a degraded toxin. (TIIF)

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Author Contributions

Conceived and designed the experiments: YK. Performed the experiments: EK. Analyzed the data: EK YP. Contributed reagents/materials/analysis tools: YK. Wrote the paper: EK YK.

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