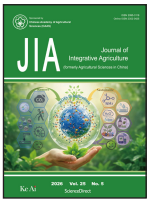
Journal of Integrative Agriculture 2026, 25(5): 1971–1980

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

Simultaneously enhancing plant growth and immunity through the application of engineered *Bacillus subtilis* expressing a microbial pattern

Shuangxi Zhang[1*], Xinlin Wei[1*], Hejing Shen[1], Qinhu Wang[2], Yi Qiang[1], Langjun Cui[1], Hongxing Xu[1], Yuyan An[1#], Meixiang Zhang[1#]

[1] *National Engineering Laboratory for Endangered Medicinal Resource Development in Northwest China/Key Laboratory of Medicinal Resources and Natural Pharmaceutical Chemistry of Ministry of Education, College of Life Sciences, Shaanxi Normal University, Xi'an 710119, China*

[2] *State Key Laboratory for Crop Stress Resistance and High-Efficiency Production, College of Plant Protection, Northwest A&F University, Yangling 712100, China*

Highlights

- The engineered strain, OKB105 (flg22), could secrete the plant immunity inducer flg22.
- Compared to OKB105 (EV), OKB105 (flg22) significantly enhanced plant resistance, while maintaining similar growth-promotion activity.
- Employing an engineered beneficial microbe expressing a microbial pattern is a promising strategy for simultaneously enhancing plant growth and immunity.

Abstract

Simultaneously enhancing plant growth and disease resistance is an ideal goal in agriculture. Significant efforts have been made to promote plant growth or immunity through the use of biological reagents, such as the application of beneficial microbes and plant immunity inducers. However, balancing plant immunity and growth remains a challenging task. In this study, we engineered the plant growth-promoting bacterium *Bacillus subtilis* OKB105 to express a secreted microbial pattern, flg22, and assessed its activity in enhancing both plant growth and disease resistance. The OKB105 (flg22) strain exhibited plant growth-promoting activity similar to the OKB105 strain containing an empty vector, OKB105 (EV). Furthermore, the OKB105 (flg22) strain significantly enhanced plant resistance against two distinct pathogens, *Pseudomonas syringae* DC3000 Δ*hopQ1-1* and *Phytophthora parasitica*, compared to OKB105 (EV), confirming that the engineered OKB105 (flg22) effectively enhances plant disease resistance. Interestingly, root irrigation with OKB105 (flg22) also markedly boosted the plant's aboveground resistance to pathogens compared to OKB105 (EV). We further demonstrated that OKB105 (flg22) can be applied to confer resistance to pathogens in other plants that recognize flg22. Finally, RNA-Seq and qRT-PCR analyses illustrated that OKB105 (flg22) effectively induced the expression of defense-related genes in pattern-triggered immunity. Our results prove that employing an engineered beneficial microbe expressing a microbial pattern is a promising strategy for simultaneously enhancing plant growth and immunity.

Keywords: plant growth promotion, immunity induction, beneficial microbe, microbial pattern, disease resistance

1. Introduction

Crops face a range of plant diseases each year, often leading to crop failure (Savary *et al*. 2019). Currently, chemical pesticides are the primary methods used to ensure crop yields, but this raises concerns about environmental and food safety (Yang *et al.* 2022; Zhu *et al.* 2024). In response to these challenges, there is a growing need to prioritize biological methods, which offer a more environmentally friendly approach (Yang *et al.* 2022; Zhu *et al.* 2024).

The use of plant immunity inducers is increasingly

Received 20 February 2024; Received in revised form 7 April 2024; Accepted 23 April 2024; Available online 1 May 2024
Shuangxi Zhang, E-mail: sxzhang2022@snnu.edu.cn; Xinlin Wei, E-mail: xinlinwei@snnu.edu.cn; #Correspondence Yuyan An, E-mail: anyuyan@snnu.edu.cn; Meixiang Zhang, E-mail: meixiangzhang@snnu.edu.cn
* These authors contributed equally to this study.

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2024.04.034

recognized as a vital aspect of biological disease management (Zhu *et al.* 2024). These inducers activate plant defense, thereby reducing disease severity. Plants possess a sophisticated and two-layered immune system, comprising pattern-triggered immunity (PTI) and effector-triggered immunity (ETI), to combat pathogens (Chisholm *et al.* 2006; Zipfel 2014; Wang *et al.* 2020). Recognized patterns include pathogen-associated molecular patterns (PAMPs) such as bacterial flagellin and fungal chitin (Chisholm *et al.* 2006), as well as cell wall fragments known as damage-associated molecular patterns (DAMPs) (Ferrari *et al.* 2013). Both PAMPs and DAMPs activate plant defense and offer potential as immunity inducers for disease prevention. These inducers possess several advantages, including broad-spectrum efficacy, durability, stability, and safety (Zhu *et al.* 2024). However, a fundamental trade-off exists between plant growth and defense, and immunity inducers often inadvertently suppress growth (Belkhadir *et al.* 2014; Huot *et al.* 2014). Consequently, the simultaneous enhancement of plant growth and immunity using microbial patterns remains a significant challenge in agricultural practice.

The 22-amino acid flagellin peptide (flg22), a conserved PAMP, has been extensively studied for its ability to induce plant immunity (Felix *et al.* 1999; Gómez-Gómez and Boller 2000; Xue *et al.* 2020). Recent research has demonstrated the potential of flg22 in suppressing plant diseases caused by pathogens like *Pseudomonas syringae* DC3000, through mechanisms involving reactive oxygen species (ROS) accumulation (Sun *et al.* 2006). Despite its well-defined mode of action, challenges remain regarding the practical use of flg22 as an immunity inducer. Key concerns include economic feasibility and potential adverse effects on plant growth, as highlighted by recent studies (Yang *et al.* 2022; Zhu *et al.* 2024). Balancing effective pathogen resistance, cost-effectiveness, and minimal growth inhibition remains a major hurdle in harnessing the full potential of flg22 in agricultural applications.

Instead of solely relying on immunity inducers with their limitations, harnessing beneficial offers a promising alternative. Plant growth-promoting rhizobacteria (PGPR), environmentally friendly and non-pathogenic microbes, are well-known for boosting plant growth (Lugtenberg and Kamilova 2009; Miao *et al.* 2015). *Bacillus subtilis*, a common Gram-positive bacterium in plant rhizospheres, exemplifies as a potent PGPR (Hashem *et al.* 2019; Blake *et al.* 2021). This bacterium enhances plant growth through various mechanisms, including atmospheric nitrogen fixation, phosphorus solubilization, and organic acid production for improved nutrient uptake (Elkoca *et al.* 2007; Saeid *et al.* 2018). Additionally, *B. subtilis* strain, OKB105, has been shown to promote plant growth by producing spermidine, which stimulates expansin synthesis and reduces ethylene levels (Xie *et al.* 2014). Recent studies delve into utilizing *B. subtilis* as a delivery system for immune inducers (Zhang and Gleason 2020). Its advantages include its high product yields, lack of toxic byproducts, and favorable fermentation properties for cost-effectiveness (van Dijl and Hecker 2013; Kovács 2019). For instance, *B. subtilis* has been successfully employed to produce StPep1, enhancing potato resistance against root-knot nematodes (Zhang and Gleason 2020). Notably, the flg22 from *B. subtilis* strain OKB105 (derived from strain 168) exhibits weaker immune-inducing properties compared to *Pseudomonas* flg22, potentially making it suitable for expressing a secreted microbial pattern flg22 with reduced impact on plant growth (Nakano *et al.* 1988; Felix *et al.* 1999; Deng *et al.* 2019). The combined characteristics of *B. subtilis* strain OKB105 and plant immunity inducers suggest their potential synergy in simultaneously enhancing plant growth and disease resistance.

In this study, we conducted a proof-of-concept investigation by modifying the plant growth-promoting bacterium *B. subtilis* OKB105 (Nakano *et al.* 1988) to produce a secreted microbial pattern flg22 (Felix *et al.* 1999). The engineered strain, OKB105 (flg22), displayed similar growth rates and colony morphology to the wild-type OKB105, indicating its robustness. Similar to OKB105 (EV), pretreatment of *Nicotiana benthamiana* with OKB105 (flg22) significantly increased the root lengths and the plant heights compared to the water control. Additionally, tobacco pre-treated with OKB105 (flg22) displayed improved resistance against *P. syringae* DC3000 Δ*hopQ1-1* and *Phytophthora parasitica* compared to OKB105 (EV). Root irrigation with OKB105 (flg22) also enhanced aboveground resistance to pathogens compared to OKB105 (EV), indicating its potential to bolster systemic defense activation. Our findings suggest that OKB105 (flg22) can confer resistance to pathogens in various plants that recognize flg22. RNA-Seq and qRT-PCR analyses demonstrated that OKB105 (flg22) effectively induced the expression of immune-related genes. Our findings suggest that employing an engineered beneficial microbe expressing a secreted microbial pattern could be a promising strategy to simultaneously enhance plant growth and disease resistance.

2. Materials and methods

2.1. Plant materials and bacterial strains

Nicotiana benthamiana, *Nicotiana tabacum*, *Solanum lycopersicum*, and *Capsicum annuum* plants were grown at 24°C under a long-day (16 h light *vs.* 8 h dark) photoperiod.

Bacillus subtilis OKB105 strains and *Escherichia coli* with different vectors were grown at 37°C in Luria-Bertani (LB) medium supplemented with 50 μmol L^{-1} kanamycin. *Pseudomonas syringae* DC3000 Δ*hopQ1-1* was grown in LM medium at 28°C. *Phytophthora parasitica and Phytophthora capsici* were cultured at 28°C in V8 Juice Broth medium.

2.2. Characterization of *B. subtilis*

The codon-optimized nucleotide sequence of flg22 from *Pseudomonas aeruginosa* was synthesized in Sangon Biotech, Shanghai, China. Subsequently, the sequence of flg22 was recombined into the *B. subtilis*/*E. coli* shuttle vector pBE-S (TaKaRa, Shiga, Japan) which encodes a secretory signal peptide. The accuracy of the sequence was confirmed by Sanger sequencing (Sangon Biotech). Plasmids pBE-S-flg22 and pBE-S were then separately transformed into *B. subtilis* OKB105 according to the method described previously (Spizizen 1958). The growth curves of

OKB105 (WT), OKB105 (EV), and OKB105 (flg22) strains were assessed using the method described by Cao *et al.* (2011) and Zhang *et al.* (2011). Plasmid stability assay was performed according to the previous method with minor modifications (Cao *et al*. 2011). A 0.1% (v/v) overnight culture was successively transferred into LB medium without kanamycin over five cycles. Diluted cultures were then plated on LB agar medium without kanamycin overnight, and then one hundred colonies from the diluted cultures were transferred to LB agar medium with kanamycin. Surviving colonies were counted. After five rounds of continuous transference, three survival colonies were detected using PCR. Additionally, their colonial morphology was compared with that of the wild type.

2.3. Detection of secreted flg22

The presence of secreted flg22 by OKB105 (flg22) was confirmed according to a previously established method (Zhang and Gleason 2020). Samples were boiled for 10 min, and 20 μL was loaded onto a 15% SDS-PAGE gel, then transferred onto PVDF membranes (Thermo Fisher Scientific, Shanghai, China). The PVDF membrane was incubated with 5% defatted milk in TBST buffer at room temperature for 1 h. Subsequently, it was treated with anti-His antibody (dilution 1:10,000, ABclonal Technology, Wuhan, China) for 1 h at room temperature, followed by incubation with horseradish peroxidase-conjugated secondary antibody (dilution 1:10,000, Sigma, USA) for an additional 40 min. Chemiluminescent signals were detected and imaged using the SuperSignal West Pico Chemiluminescent substrate (Thermo Fisher Scientific, Waltham, USA) and the Bio-Rad ChemiDoc Touch Imaging System (Bio-Rad, USA).

2.4. Treatment of plants with engineered OKB105 strains

The evaluation of OKB105's impact on tobacco growth was carried out as previously described with slight modifications (Xie *et al.* 2014). Seeds of *N. benthamiana* and *N. tabacum* were used for assessing the effect of OKB105 on tobacco growth. The seeds were sterilized in 10% NaClO for 10 min, followed by washing with sterilized distilled water. After sterilization, the seeds were subjected to an overnight soak with *B. subtilis* OKB105 suspension or water. Then treated seeds were washed with sterilized distilled water and sown on 1/2 MS medium containing 0.8% (w/v) agar under a long-day (16 h light *vs*. 8 h dark) photoperiod at 24°C. The root lengths of 7-day-old and 17-day-old *N. benthamiana* were measured. For root-irrigation treatment, seeds of *N. benthamiana* and *N. tabacum* were sown in the soil, and then each seedling was irrigated with 80 mL of OKB105 (EV) suspension (OD_{600}=0.1) or OKB105 (flg22) suspension (OD_{600}=0.1). The plant heights of 27-day-old *N. benthamiana* were subsequently measured.

To assess the effect of engineered OKB105 on plant resistance, a suspension of OKB105 (flg22) (OD_{600}=0.5) was infiltrated into one side of a 4-week-old *N. benthamiana* leaf, while a suspension of OKB105 (EV) (OD_{600}=0.5) was infiltrated into the other side using a needleless syringe. After 24 h, these plant leaves were used for the subsequent experiment. For the spray treatment, each *N. benthamiana* plant was sprayed with 5 mL OKB105 (flg22) suspension (OD_{600}=1.0) or OKB105 (EV) suspension (OD_{600}=1.0). After 24 h, these plants were used for the following experiment. In the soil-irrigation treatment, each 21-day-old *N. benthamiana*, tomato, or pepper plant grown in soil was irrigated with 80 mL of OKB105 (flg22) suspension (OD_{600}=0.1) or OKB105 (EV) suspension (OD_{600}=0.1) by drop irrigation. After 7 d, these plants were used for the subsequent experiment.

2.5. Bacterial inoculation assay

The bacterial inoculation assay of *P. syringae* strain DC3000 Δ*hopQ1-1* was performed according to the previously described protocol (Ouyang *et al.* 2023).

2.6. *Phytophthora* inoculations

Mycelial plugs were transferred from the original *Phytophthora* plate to the new V8 agar medium and incubated in a constant temperature incubator at 28°C for 3–4 days. An inoculation tray with three layers of filter paper was prepared by adding 80 mL of sterile water to fully moisten the paper. For inoculation, at least six leaves from 4-week-old plants were used. Each leaf, retaining a 2 cm-length petiole, was wrapped with moistened cotton and placed on the inoculation plate. Mycelial plugs of *Phytophthora* were placed on the back of the leaf. To ensure full contact, 18 μL of sterile water was added to the contact surface between the mycelial plug and the leaf. The inoculation tray was sealed, wrapped with plastic, and incubated in a constant temperature incubator at 22°C for 36–60 h in the dark. Leaf lesions at 2 days post-inoculation (dpi) were observed under a handheld ultraviolet lamp, and the size of each lesion was measured by using ImageJ (http://rsb.info.nih.gov/ij).

For *Phytophthora* inoculation in tomato or pepper stems, the procedure was carried out as previously described (Ahmed *et al.* 2000). Stem lesions were assessed at 2 dpi for tomato and 5 dpi for pepper.

2.7. RNA-Seq analysis

Suspensions of OKB105 (flg22) (OD_{600}=0.5) and OKB105 (EV) (OD_{600}=0.5) were infiltrated into 4-week-old *N. benthamiana* leaves, respectively. Three hours later, these leaves were collected for RNA extraction. Each leaf was treated as an individual biological replicate, and three biological replicates were collected for each treatment. High-quality total RNA (3 μg) from each sample was sent to Sangon Biotech for library construction and Illumina platform 2500 sequencing (Sangon Biotech). The quality of the raw sequencing data was evaluated using FastQC-v0.11.2. Trimmomatic-v0.36 was then used to trim raw data by removing adapters and low-quality sequences. The trimmed data was mapped to the reference genome of *N. benthamiana* (https://nbenthamiana.jp/) using HISAT2-v2.1.0v, and read counts of tobacco genes were retrieved using RSeQC-v2.6.1. Deferentially expressed genes (DEGs, FDR<0.05, Log_2FC>1) between different treatments were analyzed using

DEGseq-v1.26.0. Gene ontology (GO) and transcription factor (TF) enrichment analysis on the DEGs was performed using Ontologizer-v2.1 (FDR<0.05). Gene function was annotated based on the National Center for Biotechnology Information (NCBI) non-redundant protein sequences NR database (ftp://ftp.ncbi.nlm.nih.gov/blast/db/). The heatmap was plotted using TBtools-II (Chen *et al.* 2023).

2.8. Quantitative RT-PCR

Total RNA was extracted using the Total RNA Extraction Kit following the protocol provided by Sangon Biotech. Subsequently, RNA samples were reverse transcribed in a 20 μL reaction using the ABScript II cDNA First-Strand Synthesis Kit (ABclonal Technology, Wuhan, China). PCR was performed with SYBR Green to determine the relative expression levels of defense-related and growth-related genes, and *NbEF1α* was selected as an internal control. Relative changes in gene transcript levels were quantified using the $2^{-\Delta\Delta Ct}$ method. The primers for RT-qPCR are listed in Appendix A.

2.9. Statistical analysis

Each experiment was performed with at least three biological replicates. Duncan's multiple range tests were used (P<0.05). The statistical analysis for the comparison between the two groups was carried out using Student's *t*-test, with $^{*}P$<0.05, $^{**}P$<0.01, and $^{***}P$<0.001 indicating significance.

3. Results

3.1. Generation of the flg22-secreting *B. subtilis* strain

The microbial pattern flg22 from *P. aeruginosa* can elicit defense responses in numerous plants (Felix *et al.* 1999). Compared with the flg22 derived from *P. aeruginosa* used in this study, the flg22 from OKB105, identical to that from *B. subtilis* 168, contains two residue variations that diminish its ability to elicit a defensive response (Appendix B), thereby making OKB105 suitable for expressing the flg22 from *P. aeruginosa*. To produce flg22, the flg22 sequence was integrated into the *B. subtilis*/*E. coli* shuttle vector and subsequently introduced into the *B. subtilis* strain OKB105. The growth rate and colony appearance of OKB105 (flg22) and OKB105 (EV) were comparable to those of OKB105 (WT) (Fig. 1-A and B). To evaluate the stability of the plasmids in OKB105, *B. subtilis* strains were cultured in LB medium without selective pressure and then transferred to fresh LB medium every 12 h. After five rounds of continuous subculturing, over 90% of *B. subtilis* still displayed resistance to kanamycin (Fig. 1-C). Additionally, PCR analysis confirmed the presence of the DNA fragment of flg22 in the detected OKB105 (flg22) (Fig. 1-D). Finally, the Western blot analysis confirmed the presence of flg22 in the supernatant of OKB105 (flg22), whereas it was not detected in the supernatant of OKB105, demonstrating that OKB105 (flg22) is indeed capable of secreting flg22 (Fig. 1-E). Overall, the secretion of flg22 by the *B. subtilis* strain OKB105 did not alter the biological characteristics of *B. subtilis*, and there was no loss of plasmids after five generations of subculturing.

3.2. OKB105 (flg22) boosts tobacco growth similarly to OKB105 (EV)

OKB105 can synthesize spermine, which promotes plant root growth (Xie *et al.* 2014). However, flg22, acting as a PAMP, typically induces plant immunity and inhibits plant growth (Gomez-Gomez *et al.* 1999; Huang *et al.* 2023). To investigate whether the synthesis of flg22 by OKB105 hinders

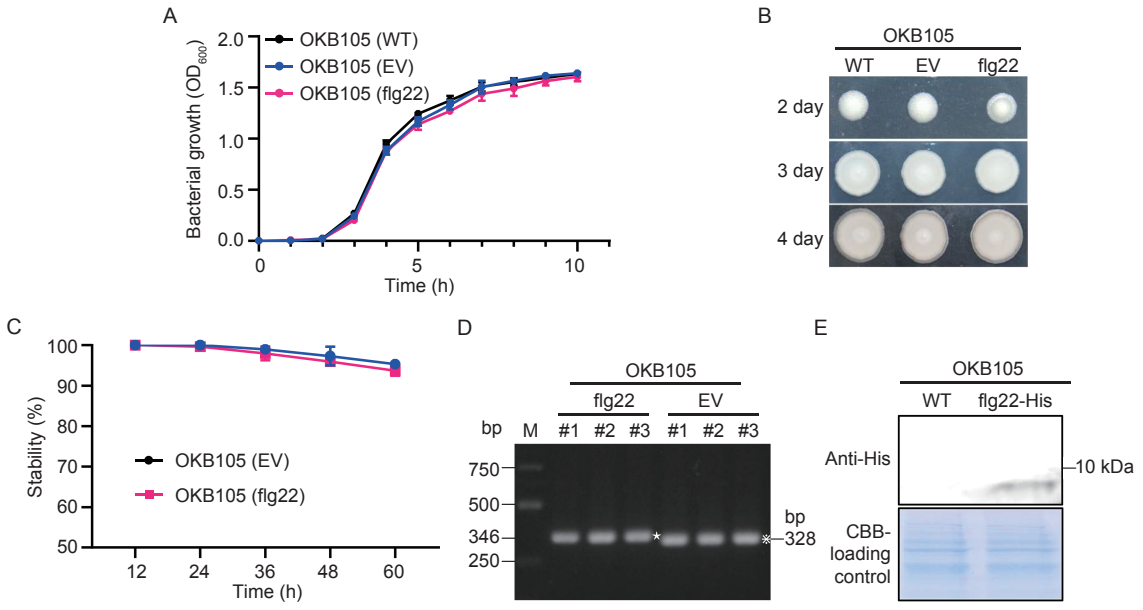


Fig. 1 Characterization of *Bacillus subtilis* OKB105 strains. A, growth curves of OKB105 (flg22), OKB105 (EV), and OKB105 (WT). B, bacterial morphology of OKB105 (flg22), OKB105 (EV), and OKB105 (WT). C, plasmid stability assessment for strains OKB105 (flg22) and OKB105 (EV). Data are presented as the mean±SD (n=3). D, identification of recombinant bacteria in C by PCR. E, detection of secreted flg22 by OKB105 (flg22) using anti-His tag antibody.

its plant growth-promoting effect, we soaked *N. benthamiana* seeds in the suspension of OKB105 (flg22) or OKB105 (EV) for 12 h and then spotted them on 1/2 MS plates. The root length of plants was observed at 7 and 17 days, respectively. Similar to OKB105 (EV), pretreatment with OKB105 (flg22) significantly increased the root lengths of 7- or 17-day-old *N. benthamiana* plants, compared to the water control (Fig. 2-A and B; Appendix C).

OKB105 strain not only promotes root growth but also increases shoot growth (Xie *et al.* 2015). The height of 27-day-old *N. benthamiana* plants treated with OKB105 (flg22) or OKB105 (EV) was significantly higher than that of the control (Fig. 2-C and D). It has been reported that OKB105 promotes the growth of *N. tabacum* seedlings (Xie *et al.* 2014). We also detected the impact of OKB105 (flg22) on *N. tabacum*. Compared with water treatment, pretreatment with OKB105 (flg22) and OKB105 (EV) significantly increased the root and aerial growth of *N. tabacum* (Appendix D).

In addition, two expansin genes, *EXPA1* and *EXPA2*, which are associated with plant growth, are induced by OKB105 in tobacco (Cosgrove 2005; Xie *et al.* 2014). The transcript levels of *EXPA1* and *EXPA2* were significantly higher in 7-day-old *N. benthamiana* seedlings treated with OKB105 (flg22) and OKB105 (EV), compared to the control (Fig. 2-E and F). These results confirmed that the engineered OKB105 expressing flg22 maintains the activity of plant growth promotion.

3.3. OKB105 (flg22) enhances tobacco disease resistance

To assess the impact of OKB105 (flg22) on disease resistance, 4-week-old tobacco plants were sprayed with OKB105 (flg22) or OKB105 (EV), followed by inoculation with *P. syringae* DC3000 Δ*hopQ1-1* or *P. parasitica* after a 24-hour treatment. Leaves pretreated with OKB105 (flg22) exhibited significantly smaller bacterial populations compared to those pretreated with OKB105 (EV) (Fig. 3-A). Additionally, the relative expression level of a PTI marker gene, *CYP71D20*, was higher upon pretreatment with OKB105 (flg22) than with OKB105 (EV) after inoculation with DC3000 Δ*hopQ1-1* (Fig. 3-B). Similar results were observed when inoculated with *P. paracitica* (Fig. 3-C–E). Tobacco leaves infiltrated with OKB105 (flg22) also exhibited enhanced resistance against DC3000 Δ*hopQ1-1* and *P. paracitica* compared to infiltration with OKB105 (EV) (Appendix E), further elucidating the effect of OKB105 (flg22) on plant resistance. Moreover, leaves of tobacco plants root-irrigated with OKB105 (flg22) displayed stronger resistance against DC3000 Δ*hopQ1-1* and *P. parasitica* than those root-irrigated with OKB105 (EV) (Fig. 3-F–H), suggesting that root irrigation with OKB105 (flg22) also markedly boosts plant's aboveground resistance to pathogens compared to OKB105 (EV). These results confirmed that OKB105 (flg22) induces disease resistance against multiple pathogens in tobacco.

3.4. OKB105 (flg22) promotes disease resistance in tomato and pepper

Phytophthora parasitica and *P. capsici* are prevalent plant pathogens that cause severe diseases in tobacco, tomato, pepper, and other crops, leading to significant losses (Blaker and Hewitt 1987; Granke *et al.* 2012; Meng *et al.* 2014). Therefore, to assess the impact of OKB105 (flg22) on crops, four-week-old tomato and pepper plants were root-irrigated with OKB105 (flg22) or OKB105 (EV).

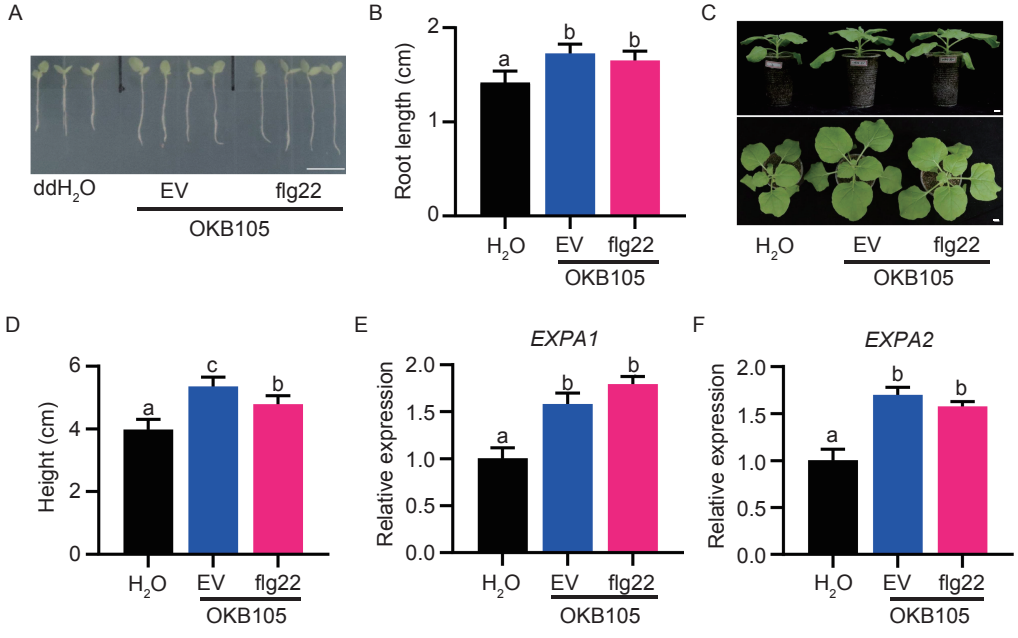


Fig. 2 Growth promotion of *Nicotiana benthamiana* by treatment with OKB105 (flg22). A, phenotype of 7-day-old tobacco seedlings treated with water, OKB105 (EV), and OKB105 (flg22). Bar, 1 cm. B, quantification of root lengths of tobacco seedlings in A (n=16). C, phenotype of 27-day-old tobacco plants treated with water, OKB105 (flg22), and OKB105 (EV). Bar, 1 cm. D, quantification of plant heights of tobacco plants in C (n=16). E, relative expression levels of *EXPA1* of seedlings in A (n=3). F, relative expression levels of *EXPA2* of seedlings in A (n=3). Data are shown as the mean±SD. The same letters indicate no significant difference at P<0.05.

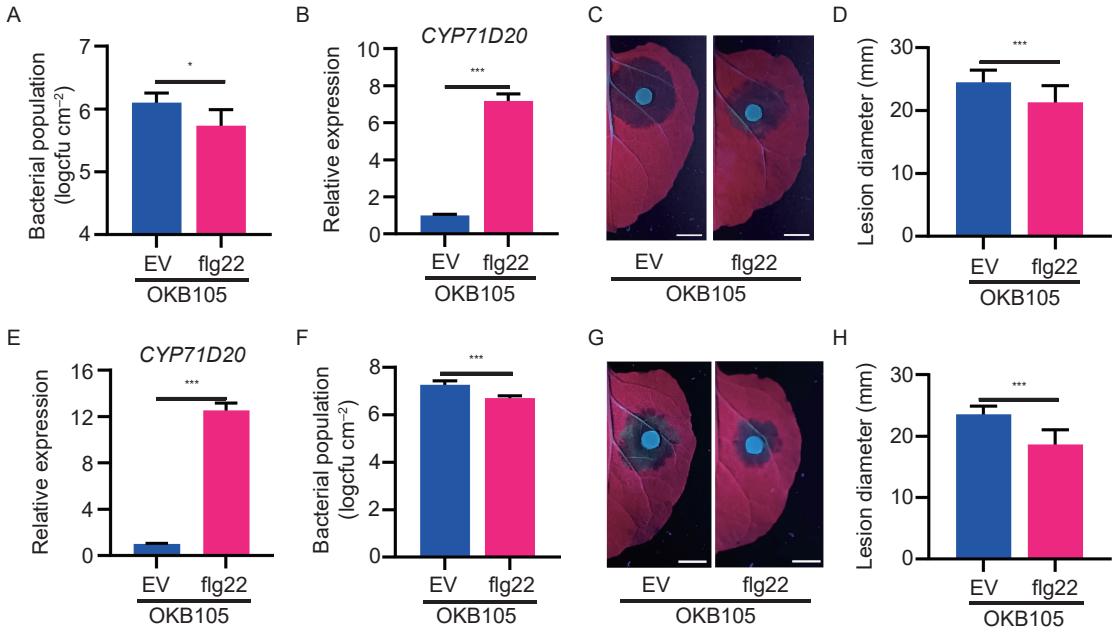


Fig. 3 OKB105 (flg22) induced plant resistance against DC3000 Δ*hopQ1-1* and *Phytophthora parasitica* in *Nicotiana benthamiana*. A, spray with OKB105 (flg22) enhanced tobacco resistance against DC3000 Δ*hopQ1-1*. The tobacco leaves sprayed with OKB105 (flg22) or OKB105 (EV) were inoculated with DC3000 Δ*hopQ1-1* at 24 hours post-OKB105 treatment. Bacterial colonization was measured at 2 days post-inoculation (dpi). B, the relative expression levels of *CYP71D20* were examined upon inoculation with DC3000 Δ*hopQ1-1*. C, OKB105 (flg22) enhanced tobacco resistance against *P. parasitica* by spraying with OKB105 (flg22). The treated leaves were inoculated with *P. parasitica* at 24 hours post-OKB105 treatment. The phenotype of *P. parasitica*-inoculated leaves was observed at 2 dpi under the handheld ultraviolet lamp. D, quantification of lesion size after infestation mold in C. E, the relative expression levels of *CYP71D20* inoculated with *P. parasitica*. F, tobacco plants root-irrigated with OKB105 (flg22) demonstrated increased resistance against DC3000 Δ*hopQ1-1*. The leaves of tobacco plants root-irrigated with OKB105 (flg22) or OKB105 (EV) were inoculated with DC3000 Δ*hopQ1-1* at 7 days post-OKB105 treatment. Bacterial colonization was measured at 2 dpi. G, tobacco plants root-irrigated with OKB105 (flg22) demonstrated increased resistance against *P. parasitica*. The leaves of tobacco plants root-irrigated with OKB105 (flg22) or OKB105 (EV) were inoculated with *P. parasitica* at 7 days post-OKB105 treatment. The phenotype of *P. parasitica*-inoculated leaves was observed at 2 dpi under the handheld ultraviolet lamp. Bar, 1 cm. H, quantification of lesion size in G. Data are shown as the mean±SD (A and F, n=6; D and H, n=12; B and E, n=3). * and *** indicate significant differences at P<0.05 and P<0.001, respectively.

Seven days post-treatment, *P. capsici* or *P. parasitica* were inoculated. Results showed that pretreatment with OKB105 (flg22) alleviated lesion size of leaves and stems in pepper plants (Fig. 4-A–D), compared to those of OKB105 (EV). Furthermore, pretreatment with OKB105 (flg22) alleviated disease symptoms in the leaves and stems of tomato plants (Fig. 4-E–H) after *Phytophthora* inoculation. These results suggested that OKB105 (flg22) has the potential to confer resistance to pathogens in crops that recognize flg22.

3.5. OKB105 (flg22) induces expression of many defense-related genes

It is well known that certain plants can recognize flg22 and activate defense (Gómez-Gómez and Boller 2000). To identify the genes induced by OKB105 (flg22) at the transcriptome level, we separately infiltrated OKB105 (flg22) and OKB105 (EV) into tobacco leaves to detect changes in the transcriptome. Compared to OKB105 (EV), OKB105 (flg22) upregulated the expression of 452 genes and downregulated the expression of 78 genes (Appendix F). Gene ontology enrichment analysis revealed that OKB105 (flg22) treatment promoted the expression of genes involved in interspecies interactions between organisms and plant response to biotic stimulus (Fig. 5-A). Further analysis revealed that OKB105 (flg22) induced the transcription of PTI-related genes, such as PTI marker genes *PTI5*, *CYPD71D* (Fig. 5-B). In addition, the transcription levels of *WIPK* and *MEK5* in the MAPK pathway were significantly upregulated (Fig. 5-B). The transcription levels of SA-related genes, such as homologous genes of *SARD1* and *WRKY30*, were also significantly upregulated (Fig. 5-B). Gene ontology enrichment analysis also revealed that OKB105 (flg22) treatment promoted the expression of genes involved in transcription regulatory region nucleic acid binding (Fig. 5-A). Transcription factors are well-known to play a crucial role in plant defense (Alves *et al.* 2014). The transcription factor enrichment analysis revealed a significant enrichment of ERF, C2H2, WRKY, and NAC transcription factor families among upregulated genes (Appendix G). Many of these transcriptional factors have been linked to plant defense (Kiełbowicz-Matuk 2012; Alves *et al.* 2014), indicating that the application of OKB105 (flg22) could enhance the expression of defense-related genes, through these transcription factors. Overall, these results indicate that OKB105 (flg22) enhances plant disease resistance by upregulating the expression of defense-related genes.

To validate the transcriptome results, we assessed the transcriptional levels of defense-related genes, including

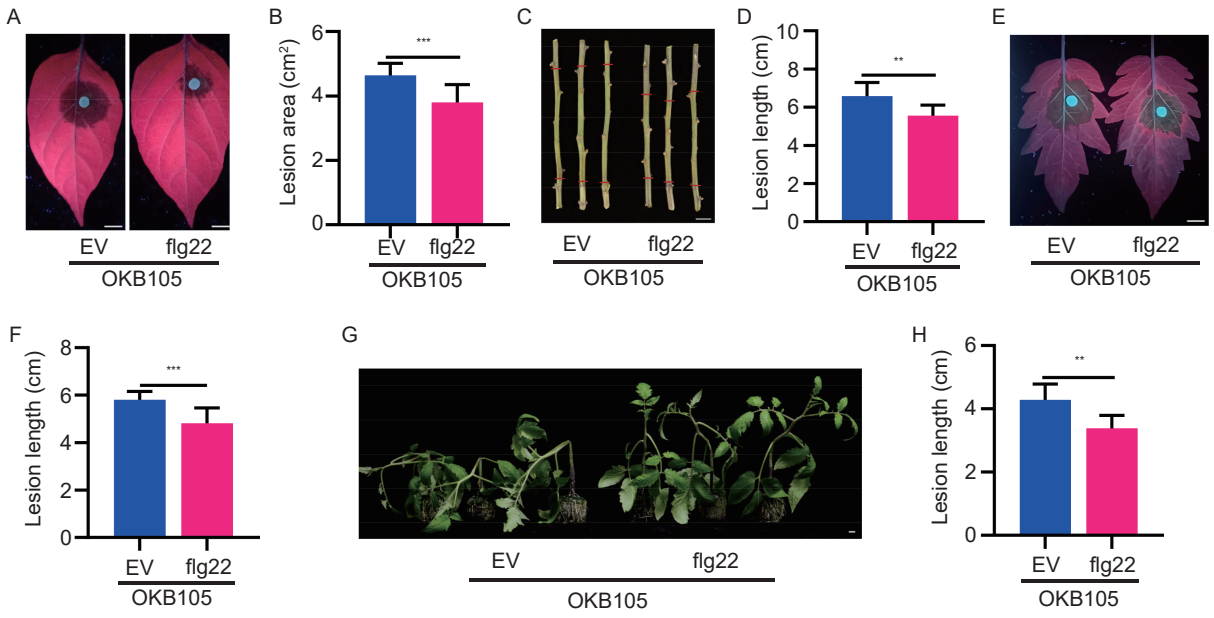


Fig. 4 OKB105 (flg22) induced plant resistance in tomato and pepper. A, the pepper plants were root-irrigated with OKB105 (flg22) or OKB105 (EV). After 7 days, the leaves of treated plants were inoculated with *Phytophthora capsici*. The phenotype of *P. capsici*-inoculated leaves was observed at 2 days post-inoculation (dpi) under the handheld ultraviolet lamp. Bar, 1 cm. B, quantification of lesion size in A. C, the stems of treated pepper plants were inoculated with *P. capsici* at 7 days post-OKB105 treatment. The phenotype of *P. capsici*-inoculated stems was observed at 5 dpi. The red line indicates the edge of the lesion. Bar, 1 cm. D, quantification of lesion size in C. E, the tomato plants were root-irrigated with OKB105 (flg22) or OKB105 (EV). After 7 days, the leaves of treated plants were inoculated with *P. parasitica*. The phenotype of *P. parasitica*-inoculated leaves was observed at 2 dpi under the handheld ultraviolet lamp. Bar, 1 cm. F, quantification of lesion size in E. G, the stems of treated tomato plants were inoculated with *P. parasitica* at 7 days post-OKB105 treatment. The phenotype of *P. parasitica*-inoculated stems was observed at 2 dpi. Bar, 1 cm. H, quantification of lesion size in G. Data are shown as the mean±SD (B and F, n=12; D and H, n=8). ** and *** indicate significant differences at P<0.01 and P<0.001, respectively.

PTI5, *CYPD71D20*, *MEK5*, and *SARD1*. The qRT-PCR results were consistent with the transcriptome data. The relative expression levels of *PTI5*, *CYPD71D20*, *MEK5*, and *SARD1* in plants treated with OKB105 (flg22) were significantly upregulated (Fig. 6). These results were consistent with the transcriptome data. These findings further support the idea that OKB105 (flg22) promotes stronger plant resistance by activating the plant's immune response compared to OKB105 (EV).

4. Discussion

Chemical pesticides pose environmental and health concerns, prompting the exploration of sustainable alternatives like plant immunity inducers (Yang *et al.* 2022; Zhu *et al.* 2024). However, these inducers can negatively impact plant growth and incur significant costs (Huot *et al.* 2014). This study proposes a novel approach: combining plant immunity inducers with plant growth-promoting rhizobacteria to overcome these drawbacks. We engineered a *B. subtilis* strain, OKB105, to secrete the immune-inducing flagellin peptide flg22. This reduces the cost and potential drawbacks of using external flg22. Our results not only confirm the stronger effect of OKB105 (flg22) in activating plant immunity against pathogens compared to OKB105 (EV) but also demonstrate that employing an engineered beneficial microbe expressing a microbial pattern is a promising strategy for simultaneously enhancing plant growth and resistance.

OKB105 is a derivative of *B. subtilis* 168, and it possesses a flagellin protein-encoding gene designated as *hag*, which is identical to the corresponding gene found in *B. subtilis* 168. In contrast to the flg22 derived from *P. aeruginosa*, which was utilized in this study, the flg22 from *B. subtilis* 168 or OKB105 exhibits two amino acid residue variations (Appendix B). Research indicates that these differences result in a reduced capacity for immune induction in *Arabidopsis thaliana* (Deng *et al.* 2019). Moreover, to colonize at the roots of plants, endophyte *B. subtilis* also evades plant defense by producing subtilomycin, which binds to self-produced flagellin, not flg22 from *P. aeruginosa* to evade plant defense (Deng *et al.* 2019). Therefore, the *B. subtilis* strain OKB105 (flg22) that secretes flg22 may induce plant resistance. Our results showed that pretreatment with OKB105 (flg22) enhances the disease resistance of tobacco, pepper, and tomato plants to multiple pathogens (Figs. 3 and 4; Appendix E), confirming the hypothesis that OKB105 can be used to secrete flg22 to confer plant resistance.

Although the loss of plasmids during bacterial subculturing poses a potential problem for the bacteria that carry the plasmid (Cao *et al.* 2011), we evaluated the stability of the plasmid and found that *B. subtilis* maintained the vector after five generations of consecutive subculturing without selective pressure (Fig. 1-C and D). Considering the potential loss of the plasmid without selective pressure, inserting the flg22 sequence into *B. subtilis* genome may be an alternative method. In addition, plant root irrigation with OKB105 (flg22)

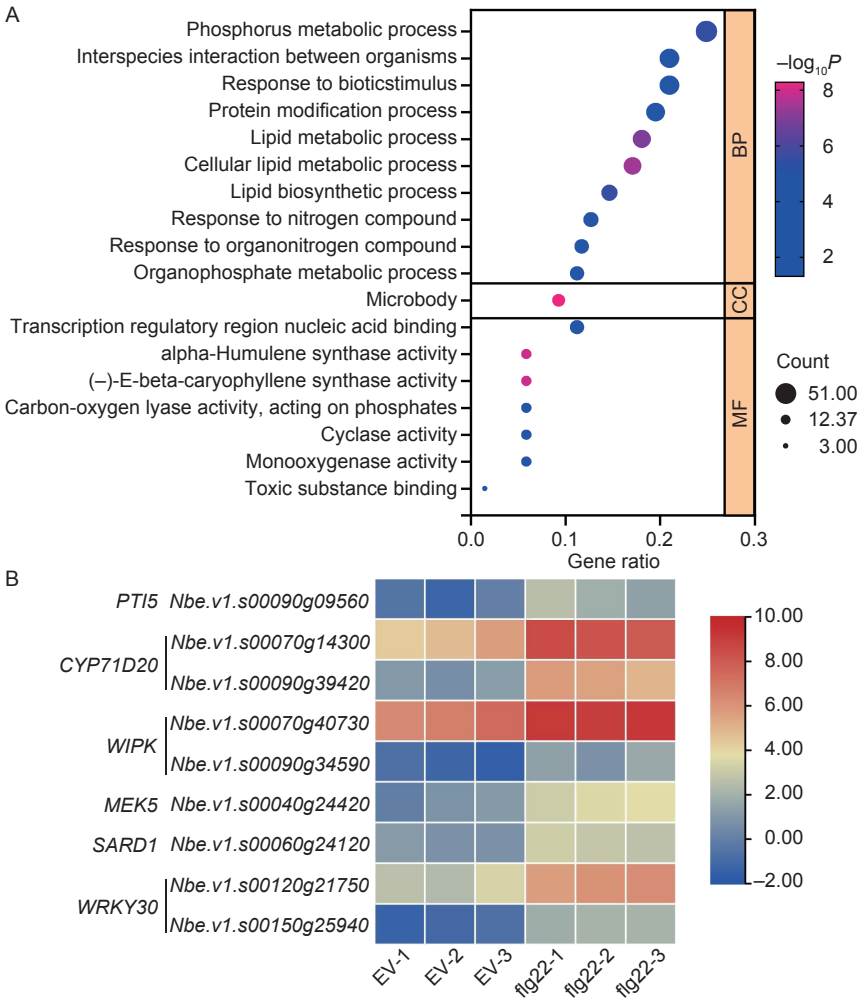


Fig. 5 OKB105 (flg22) induced transcriptome changes. A, Gene Ontology (GO) enrichment analysis of genes upregulated by OKB105 (flg22) compared to OKB105 (EV) revealed involvement in plant immunity, including the biological process involved in interspecies interaction between organisms and response to biotic stimulus. BP, biological processes; CC, cellular components; MF, molecular functions. B, Heatmap of defense-related genes induced by OKB105 (flg22).

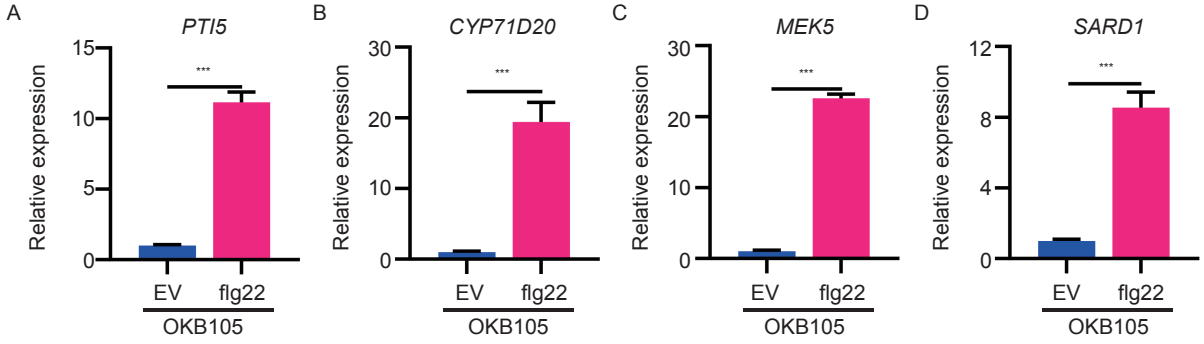


Fig. 6 OKB105 (flg22) induced the expression of defense-related genes using qPCR. A, *PTI5*. B, *CYP71D20*. C, *MEK5*. D, *SARD1*. Data are shown as the mean±SD (n=3). *** indicates significant difference at $P<0.001$, respectively.

displayed stronger resistance than those with OKB105 (EV) (Figs. 3-F–H, and 4), suggesting that OKB105 (flg22) is effective to boost plant immunity against pathogens.

The activation of plant defense always hinders growth (Huot *et al.* 2014). Previous studies have shown that flg22 leads to the suppression of plant primary root growth (Gomez-Gomez *et al.* 1999; Gómez-Gómez and Boller 2000; Huang *et al.* 2023). *Bacillus subtilis* strain OKB105, which fixes atmospheric nitrogen, solubilizes phosphorus and secretes spermidine, improves nutrient availability, stimulates the expression of expansins, and results in plant growth (Cosgrove 2005; Xie *et al.* 2014). Our research

reveals that this root inhibition effect can be alleviated by engineering *B. subtilis* OKB105 to express flg22. Notably, OKB105 alone promotes primary root growth (Fig. 2-A and B; Appendix C). Based on these findings, we propose that the root inhibition effect of flg22 can be neutralized by the growth promotion activity of OKB105. Therefore, we did not observe a significant difference in root length between *B. subtilis* OKB105 (flg22) and *B. subtilis* OKB105 (EV) treatment (Fig. 2-A and B; Appendix C). Furthermore, OKB105 not only stimulates tomato growth, but also enhances tomato resistance against cucumber mosaic virus (CMV), tobacco mosaic virus (TMV), and *Ralstonia solanacearum* (Wang *et al.* 2011; Gao *et al.* 2013; Ren *et al.* 2014). These results suggest that OKB105 can balance plant defense and growth. *B. subtilis* strain RIK1285 expressing StPep1 elicitor enhances potato resistance against root-knot nematodes and doesn't influence potato growth (Zhang and Gleason 2020). Our findings indicated that OKB105 (flg22) can enhance plant resistance against multiple pathogens, compared with OKB105 (EV) (Figs. 3 and 4; Appendix E). Furthermore, the OKB105 (flg22) strain also enhanced plant growth (Fig. 2; Appendices C and D). These results suggest that combining OKB105 with plant immunity inducers is an effective strategy for simultaneously enhancing plant resistance and promoting plant growth. The growth promotion factors in OKB105 might mitigate the plant growth inhibition caused by flg22.

Plant immunity inducers may not only trigger local resistance but also induce systemic resistance (Yang *et al.* 2022; Zhu *et al.* 2024). Previous research has demonstrated that flg22-induced PTI can initiate systemic acquired resistance (SAR). Specifically, localized application of flagellin has been found to enhance SAR levels (Mishina and Zeier 2007). Additionally, studies conducted by Jean T. Greenberg's group have shown that flg22 elicits both local and systemic immune responses (Jelenska *et al.* 2017). Their research further illustrated that flg22 has the capability to migrate to distal organs, thereby inducing SAR (Jelenska *et al.* 2017). Certainly, it is widely acknowledged that *B. subtilis* typically triggers induced systemic resistance (ISR). However, flg22 has the capacity to initiate SAR. Moreover, research has demonstrated that simultaneous activation of ISR and SAR yields an additive effect on the level of induced protection against *P. syringae* pv. *tomato* (van Wees *et al.* 2000). In our research, the leaves treated with OKB105 (flg22) exhibited stronger resistance against DC3000 Δ*hopQ1-1*, *P. parasitica*, and *P. capsici* (Figs. 3 and 4). Transcriptome data also revealed that OKB105 (flg22) induced the transcription of genes associated with PTI (Figs. 5 and 6). These results suggested that flg22 induced local resistance. Tobacco, pepper, and tomato root-irrigated with OKB105 (flg22) enhanced aboveground resistance compared to OKB105 (EV), suggesting systemic defense activation beyond potential local effects from *B. subtilis* (Figs. 3-F–H and 4). Therefore, we propose that the observed stronger aboveground resistance in tobacco plants root-irrigated with OKB105 (flg22) compared to the OKB105 (EV) is likely due to the additive effect of ISR and SAR.

Engineering beneficial microbes to express secreted flg22 may not be a universally feasible approach for promoting plant disease resistance due to the reported lack of flg22 detection by pattern recognition receptors (PRRs) in certain plant species (Wei *et al.* 2018; Wang *et al.* 2022). Therefore, exploring PAMPs with broad recognition profiles across diverse plant species presents a potentially more universal and robust approach, especially when combined with PGPR.

5. Conclusion

In this study, we engineered a *B. subtilis* strain, OKB105, to secrete the plant immunity inducer flg22. Compared to the control strain OKB105 (EV), OKB105 (flg22) significantly enhanced resistance against DC3000 Δ*hopQ1-1* and *P. parasitica* in tobacco, while maintaining similar growth-promotion activity to OKB105 (EV) in tobacco. Furthermore, the OKB105 (flg22) was also effective in enhancing resistance to oomycete pathogens in tomato and pepper plants. Transcriptome data revealed that the OKB105 (flg22) enhances plant resistance by activating plant immune signaling pathways. This study highlights the potential of combining plant immunity inducers and PGPR to enhance plant defense against pathogens.

Acknowledgements

This work was supported by the Technology Innovation Leading Program of Shaanxi, China (2023QYPY2-01), the National Natural Science Foundation of China (32072399 and 32302296), and the Fundamental Research Funds for the Central Universities, China (GK202201017 and GK202207024).

Declaration of competing interest

The authors declare that they have no conflict interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) utilized Grammarly and Wordvice AI to enhance the readability, grammar, and clarity of the manuscript. After using these tools, the author(s) carefully reviewed and edited the content as necessary and take full responsibility for the published article.

Appendices associated with this paper are available at 10.1016/j.jia.2024.04.034

References

Ahmed A S, Sánchez C P, Candela M E. 2000. Evaluation of induction of systemic resistance in pepper plants (*Capsicum annuum*) to *Phytophthora capsici* using *Trichoderma harzianum* and its relation with capsidiol accumulation. *European Journal of Plant Pathology*, **106**, 817–824.

Alves M S, Dadalto S P, Gonçalves A B, De Souza G B, Barros V A, Fietto L G. 2014. Transcription factor functional protein-protein interactions in plant defense responses. *Proteomes*, **2**, 85–106.

Belkhadir Y, Yang L, Hetzel J, Dangl J L, Chory J. 2014. The growth-defense pivot: Crisis management in plants mediated by LRR-RK surface receptors. *Trends in Biochemical Sciences*, **39**, 447–456.

Blake C, Christensen M N, Kovács Á T. 2021. Molecular aspects of plant growth promotion and protection by *Bacillus subtilis*. *Molecular Plant-Microbe Interactions*, **34**, 15–25.

Blaker N, Hewitt J. 1987. A comparison of resistance to *Phytophthora*

parasitica in tomato. *Phytopathology*, **77**, 1113–1116.
Cao Y, Zhang Z, Ling N, Yuan Y, Zheng X, Shen B, Shen Q. 2011. *Bacillus subtilis* SQR9 can control *Fusarium wilt* in cucumber by colonizing plant roots. *Biology and Fertility of Soils*, **47**, 495–506.
Chen C, Wu Y, Li J, Wang X, Zeng Z, Xu J, Liu Y, Feng J, Chen H, He Y. 2023. TBtools-II: A "one for all, all for one" bioinformatics platform for biological big-data mining. *Molecular Plant*, **16**, 1733–1742.
Chisholm S T, Coaker G, Day B, Staskawicz B J. 2006. Host-microbe interactions: Shaping the evolution of the plant immune response. *Cell*, **124**, 803–814.
Cosgrove D J. 2005. Growth of the plant cell wall. *Nature Reviews Molecular Cell Biology*, **6**, 850–861.
Deng Y, Chen H, Li C, Xu J, Qi Q, Xu Y, Zhu Y, Zheng J, Peng D, Ruan L. 2019. Endophyte *Bacillus subtilis* evades plant defense by producing lantibiotic subtilomycin to mask self-produced flagellin. *Communications Biology*, **2**, 368.
van Dijl J, Hecker M. 2013. *Bacillus subtilis*: From soil bacterium to super-secreting cell factory. *Microbial Cell Factories*, **12**, 1–6.
Elkoca E, Kantar F, Sahin F. 2007. Influence of nitrogen fixing and phosphorus solubilizing bacteria on the nodulation, plant growth, and yield of chickpea. *Journal of Plant Nutrition*, **31**, 157–171.
Felix G, Duran J D, Volko S, Boller T. 1999. Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *The Plant Journal*, **18**, 265–276.
Ferrari S, Savatin D V, Sicilia F, Gramegna G, Cervone F, Lorenzo G D. 2013. Oligogalacturonides: Plant damage-associated molecular patterns and regulators of growth and development. *Frontiers in Plant Science*, **4**, 49.
Gao S, Wu H, Wang W, Yang Y, Xie S, Xie Y, Gao X. 2013. Efficient colonization and harpins mediated enhancement in growth and biocontrol of wilt disease in tomato by *Bacillus subtilis*. *Letters in Applied Microbiology*, **57**, 526–533.
Gómez-Gómez L, Boller T. 2000. FLS2: An LRR receptor–like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. *Molecular Cell*, **5**, 1003–1011.
Gomez-Gomez L, Felix G, Boller T. 1999. A single locus determines sensitivity to bacterial flagellin in *Arabidopsis thaliana*. *The Plant Journal*, **18**, 277–284.
Granke L L, Quesada-Ocampo L, Lamour K, Hausbeck M K. 2012. Advances in research on *Phytophthora capsici* on vegetable crops in the United States. *Plant Disease*, **96**, 1588–1600.
Hashem A, Tabassum B, Abd-Allah E F. 2019. *Bacillus subtilis*: A plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi Journal of Biological Sciences*, **26**, 1291–1297.
Huang Y, Cui J, Li M, Yang R, Hu Y, Yu X, Chen Y, Wu Q, Yao H, Yu G. 2023. Conservation and divergence of flg22, pep1 and nlp20 in activation of immune response and inhibition of root development. *Plant Science*, **331**, 111686.
Huot B, Yao J, Montgomery B L, He S Y. 2014. Growth-defense tradeoffs in plants: A balancing act to optimize fitness. *Molecular Plant*, **7**, 1267–1287.
Jelenska J, Davern S M, Standaert R F, Mirzadeh S, Greenberg J T. 2017. Flagellin peptide flg22 gains access to long-distance trafficking in Arabidopsis *via* its receptor, FLS2. *Journal of Experimental Botany*, **68**, 1769–1783.
Kiełbowicz-Matuk A. 2012. Involvement of plant C2H2-type zinc finger transcription factors in stress responses. *Plant Science*, **185**, 78–85.
Kovács Á T. 2019. *Bacillus subtilis*. *Trends in Microbiology*, **27**, 724–725.
Lugtenberg B, Kamilova F. 2009. Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology*, **63**, 541–556.
Meng Y, Zhang Q, Ding W, Shan W. 2014. *Phytophthora parasitica*: A model oomycete plant pathogen. *Mycology*, **5**, 43–51.
Gao M, Zhou J, Wang E, Chen Q, Xu J, Sun J. 2015. Multiphasic characterization of a plant growth promoting bacterial strain, *Burkholderia* sp. 7016 and its effect on tomato growth in the field. *Journal of Integrative Agriculture*, **14**, 1855–1863.
Mishina T E, Zeier J. 2007. Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in Arabidopsis. *Plant Journal*, **50**, 500–513.
Nakano M, Marahiel M, Zuber P. 1988 Identification of a genetic locus required for biosynthesis of the lipopeptide antibiotic surfactin in *Bacillus subtilis*. *Journal of Bacteriology*, **170**, 5662–5668.
Ouyang X, Chen J, Sun Z, Wang R, Wu X, Li B, Song C, Liu P, Zhang M. 2023. Ubiquitin E3 ligase activity of *Ralstonia solanacearum* effector RipAW is not essential for induction of plant defense in *Nicotiana benthamiana*. *Frontiers in Microbiology*, **14**, 1201444.
Ren P, Xie Y, Zhang Y, Wu H, Gao X. 2014. Effect and mechanism of controlling TMV disease on tobacco by surfactin produced by *Bacillus subtilis* OKB105. *Chinese Journal of Biological Control*, **30**, 216.
Saeid A, Prochownik E, Dobrowolska-Iwanek J. 2018. Phosphorus solubilization by *Bacillus species*. *Molecules*, **23**, 2897.
Savary S, Willocquet L, Pethybridge S J, Esker P, McRoberts N, Nelson A. 2019. The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, **3**, 430–439.
Spizizen J. 1958. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. *Proceedings of the National Academy of Sciences of the United States of America*, **44**, 1072–1078.
Sun W, Dunning F, Pfund C, Weingarten R, Bent A F. 2006. Within-species flagellin polymorphism in *Xanthomonas campestris pv campestris* and its impact on elicitation of Arabidopsis FLAGELLIN SENSING2–dependent defenses. *The Plant Cell*, **18**, 764–779.
Wang S, Wu H, Zhan J, Xia Y, Gao S, Wang W, Xue P, Gao X. 2011. The role of synergistic action and molecular mechanism in the effect of genetically engineered strain *Bacillus subtilis* OKBHF in enhancing tomato growth and Cucumber mosaic virus resistance. *BioControl*, **56**, 113–121.
Wang W, Feng B, Zhou J M, Tang D. 2020. Plant immune signaling: Advancing on two frontiers. *Journal of Integrative Plant Biology*, **62**, 2–24.
Wang Y, Pruitt R, Nürnberger T, Wang Y. 2022. Evasion of plant immunity by microbial pathogens. *Nature Reviews Microbiology*, **20**, 449–464.
van Wees S C, de Swart E A, van Pelt J A, van Loon L C, Pieterse C M. 2000. Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*. **97**, 8711–8716.
Wei Y, Caceres-Moreno C, Jimenez-Gongora T, Wang K, Sang Y, Lozano-Duran R, Macho A P. 2018. The *Ralstonia solanacearum* csp22 peptide, but not flagellin-derived peptides, is perceived by plants from the *Solanaceae* family. *Plant Biotechnology Journal*, **16**, 1349–1362.
Xie S, Wu H, Chen L, Zang H, Xie Y, Gao X. 2015. Transcriptome profiling of *Bacillus subtilis* OKB105 in response to rice seedlings. *BMC Microbiology*, **15**, 1–14.
Xie S, Wu H, Zang H, Wu L, Zhu Q, Gao X. 2014. Plant growth promotion by spermidine-producing *Bacillus subtilis* OKB105. *Molecular Plant-Microbe Interactions*, **27**, 655–663.
Xue J, Gong B, Yao X, Huang X, Li J. 2020. BAK1-mediated phosphorylation of canonical G protein alpha during flagellin signaling in Arabidopsis. *Journal of Integrative Plant Biology*, **62**, 690–701.
Yang B, Yang S, Zheng W, Wang Y. 2022. Plant immunity inducers: From discovery to agricultural application. *Stress Biology*, **2**, 5.
Zhang L, Gleason C. 2020. Enhancing potato resistance against root-knot nematodes using a plant-defence elicitor delivered by bacteria. *Nature Plants*, **6**, 625–629.
Zhang N, Wu K, He X, Li S, Zhang Z, Shen B, Yang X, Zhang R, Huang Q, Shen Q. 2011. A new bioorganic fertilizer can effectively control banana wilt by strong colonization with *Bacillus subtilis* N11. *Plant and Soil*, **344**, 87–97.
Zhu F, Cao M, Zhang Q, Mohan R, Schar J, Mitchell M, Chen H, Liu F, Wang D, Fu Z Q. 2024. Join the green team: Inducers of plant immunity in the plant disease sustainable control toolbox. *Journal of Advanced Research*, **57**, 15–42.
Zipfel C. 2014. Plant pattern-recognition receptors. *Trends in Immunology*, **35**, 345–351.

Executive Editor-in-Chief Fanghao Wan
Managing Editor Juan Zhang