CROP PROTECTION ACTIVITY AND COLONIZATION BEHAVIOR OF *Allorhizobium vitis* VAR03-1, A BIOCONTROL BACTERIUM FOR GRAPEVINE CROWN GALL DISEASE

September 2024

NIARSI MERRY HEMELDA

Graduate School of Environmental and Life Science

(Doctor Course)

OKAYAMA UNIVERSITY

Crop Protection Activity and Colonization Behavior of *Allorhizobium vitis* VAR03-1, A Biocontrol Bacterium for Grapevine Crown Gall Disease

ブドウ根頭がんしゅ病の拮抗細菌 *Allorhizobium vitis* VAR03-1 の植物保護作用と宿主定着性の解析

Niarsi Merry Hemelda

Graduate School of Environmental and Life Science
Division of Agricultural and Life Science
Okayama University, Japan
September 2024

The dissertation was submitted to the Graduate School of Environmental, Life, and Natural Science of Okayama University in partial fulfillment of the requirements for the Doctor of Philosophy degree.

Written under the supervision of Professor Yoshiteru Noutoshi
and co-supervised by Professor Kazuhiro Toyoda and Professor Yuki Ichinose.

Acknowledgments

First and foremost, I would like to express my deepest gratitude to the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) and The Japan International Cooperation Agency (JICA) for providing me with the scholarship that made my PhD studies and life in Japan possible. Their generous support has been fundamental to the successful completion of my research. I am also grateful to be part of the JICA family. Through this, I have learned and experienced many things in Japan and met fellows from all over the world.

I am profoundly indebted to Professor Yoshiteru Noutoshi, my dedicated supervisor, for his invaluable guidance, encouragement, unwavering support, and endless patience throughout my doctoral journey. His mentorship has been instrumental in shaping my research and academic growth.

I am also grateful to Professor Kazuhiro Toyoda and Professor Yuki Ichinose, my co-supervisors, for their insightful feedback and continuous support. Their expertise and advice have significantly enriched my research. I would also like to extend my thanks to Associate Professor Hidenori Matsui for his valuable discussions and assistance.

I would like to give special thanks to Dr. Akira Kawaguchi from the Western Region Agricultural Research Center, National Agriculture and Food Research Organization (NARO), for providing grapevine seedlings and to Professor Kent D. Chapman from the University of North Texas for the generous gifts of Arabidopsis *Atfaah* knockout mutants. Their contributions have been crucial to the progress of my research.

I am grateful to the members of the Laboratory of Plant Pathology and Genetic Engineering, particularly the Noutoshi Lab members, for their unwavering support and friendship. I am especially thankful to my fellow international students for their companionship and stimulating discussions. The collaborative environment and friendships within the Noutoshi Lab have been a source of motivation and joy.

Lastly, I owe my deepest appreciation to my husband for his relentless support, both emotionally and practically, as I pursued my dream of studying in Japan. His constant presence and encouragement, especially during challenging times, have been my greatest source of strength. I am also deeply grateful to my parents and families in Indonesia for their unconditional love and prayers, which have always been a pillar of support throughout my academic journey.

Summary

Crown gall disease in grapevines is predominantly caused by the tumorigenic (Ti) strain of *Allorhizobium vitis*. A nonpathogenic strain of *A. vitis*, VAR03-1, acts as a biocontrol agent and protects grapevines from this disease. A previous study in our laboratory identified rhizoviticin, a phage tail-like bacteriocin known as a tailocin, as the primary substance responsible for the antagonistic activity of VAR03-1 against Ti in vitro. To further confirm this, the first study (in Chapter 2) aimed to evaluate the antitumorigenic activity of rhizoviticin-deficient VAR03-1 mutants in tomato and its original host, grapevine. The tested mutants largely lost their tumor-suppressing activity, indicating that rhizoviticin plays a critical role in the biocontrol activity of VAR03-1 in planta. Interestingly, the mutants still retained some antagonistic activity in both plant species despite losing almost all of their growth suppression activity against Ti in vitro. Since VAR03-1 and Ti belong to the same bacterial genus and species, their growth habitats on the host are thought to be similar. Therefore, the competition for nutrients and host colonization space could contribute to the residual biocontrol activity of VAR03-1 in planta.

The second study (in Chapter 3) aimed to characterize the colonization behavior of VAR03-1 in the plant rhizosphere. For this purpose, a model plant organism, *Arabidopsis thaliana*, was used due to its small size and the availability of various mutants that facilitate the molecular analysis of the underlying mechanisms. When VAR03-1 was added to MS (Murashige and Skoog) agar, colony formation surrounding primary roots (PR) and enhanced Arabidopsis growth were observed, indicating successful colonization. In contrast, *Pseudomonas protegens* Cab57, another biocontrol agent, appeared to colonize only the root surface with no visible colony formation. To understand this difference in colonization patterns, the growth of both bacterial strains in a liquid MS medium was examined. VAR03-1 was able to grow in MS with sucrose or other sugars, but Cab57 exhibited a low growth rate under these conditions. Further supplementation with four organic acids, components of Arabidopsis root exudates required for virulence induction in the pathogenic bacterium *Pseudomonas syringae*, enhanced the growth of VAR03-1 and significantly restored the lower growth of Cab57. Consistently, in MS agar with sucrose and organic acids, VAR03-1 colonization was enhanced, and Cab57 produced visible colonies even far from the roots. These results suggest that nutrient requirements play a crucial role in shaping colonization patterns in the host rhizosphere.

In this experiment, Arabidopsis growth was severely inhibited by both bacterial species under supplementation of sucrose and organic acids. When increased numbers of bacterial cells

were applied to MS agar without sucrose, plant growth was also hampered, suggesting that bacterial overgrowth with nutrient supplementation causes this negative effect on plant growth. To understand the molecular basis behind this growth suppression, several Arabidopsis mutants that lack the ability to sense microbe-derived molecules or signal through the defense-related phytohormones salicylic acid or ethylene were tested for their response to VAR03-1. These mutants showed growth inhibition similar to that of wild-type plants. Also, the expression of *FRK1* and *PDF1.2*, marker genes for immune responses, which were induced by VAR03-1 in no sucrose condition, became suppressed along with the increased sucrose concentration. Yet, plant growth was still inhibited under these conditions. Taken together, these results suggest that the observed plant growth inhibition is not a consequence of an immune response.

It has been reported that Arabidopsis growth is inhibited by exogenously applied *N*-acyl-L-homoserine lactones (AHLs), bacterial density recognition signal molecules produced during host colonization. This growth inhibition was abolished in the Arabidopsis fatty acid amide hydrolase (*AtFAAH*) mutant (*Atfaah*), which was impaired in converting AHLs to homoserine, a potential cause of the growth inhibition. However, in the presence of VAR03-1 with sucrose, the *Atfaah* mutant exhibited growth inhibition similar to that of wild-type, suggesting that this growth inhibition is independent of VAR03-1-derived AHLs.

Finally, to investigate the bacterial factor involved in this growth inhibition, the *ΔrecA* mutant of VAR03-1 was used. RecA is a regulator of the SOS response to environmental stress, such as UV-induced DNA damage, and is known to be involved in biofilm formation. The inhibition of Arabidopsis growth by VAR03-1 overgrowth was attenuated in the *ΔrecA* mutant, although the growth rates of the *ΔrecA* mutant were comparable to those of the wild-type in vitro. Because the biofilm formation rate of the *ΔrecA* mutant is higher than that of the wild-type, we speculate that biological activities associated with the free-living, rather than the sessile, lifestyle of VAR03-1 are linked to its deleterious effects on plant growth.

In summary, this study has identified multiple modes of action for VAR03-1 as a biocontrol agent. One mechanism involves rhizoviticin as the primary biocontrol agent, and the other involves its host colonization properties. The bacterial colonization habitat in the host rhizosphere is defined, at least in part, by the nutrient requirements of the microbes. This finding provides evidence that non-pathogenic microorganisms of the same genus and species as the pathogens often exhibit antagonistic activity in the field.

Table of Contents

List of figures

List of supplementary figures

List of tables

List of supplementary tables

Abbreviations

AHLs	*N*-acyl homoserine lactones
dpi	days post inoculation
eDNA	environmental DNA
EPS	exopolysaccharide
ETI	effector-triggered immunity
KB medium	King's B medium
kDa	kilo Dalton
MAMP	microbial-associated molecular patterns
MS medium	Murashige and Skoog medium
MTI	MAMP-triggered immunity
OD_{600}	optical density at 600 nm
PR	primary root
PTI	pattern-triggered immunity
SDW	sterile distilled water
SOS	save our soul
Ti	tumor-inducing, tumorigenic
T-DNA	transfer DNA
WT	wild-type

CHAPTER 1:
General Introduction

1.1 *Allorhizobium vitis* VAR03-1

1.1.1 *A. vitis* and crown gall disease in grapevine

Crown gall disease affects a wide range of herbaceous and woody plants, encompassing over 140 genera across more than 60 families. This disease is caused by *Agrobacterium tumefaciens* and certain members of Rhizobiaceae, including *Rhizobium rhizogenes, R. radiobacter* and *Allorhizobium vitis* (Agrios, 2005). While *A. tumefaciens* can induce crown gall disease in angiosperms, it is rarely infected grapevine. In grapevines, crown gall disease is predominantly caused by *Allorhizobium vitis* (=*Rhizobium vitis*), posing a significant threat to grapevine cultivation globally (Burr et al., 1998).

Similar to other crown gall-inducing bacteria, *A. vitis* harbors a tumor-inducing (Ti) plasmid, which is crucial for its pathogenicity. This plasmid contains transfer DNA (T-DNA) that can integrate into plant cells, disrupting their normal physiological processes. The T-DNA includes genes responsible for the synthesis of plant hormones as well as genes encoding the biosynthesis of opines, which are specialized compounds that serve as energy sources for *A. vitis* with Ti-plasmid. Once T-DNA is integrated into the plant genome, infected plant cells begin to produce opines and undergo uncontrolled cell division, resulting in the formation of galls (Kuzmanović et al., 2018).

A. vitis is a soil inhabitant associated with plant residues in the soil, beyond its exclusive presence in galls. Notably, research by Burr et al. (1995) demonstrated that *A. vitis* can survive in decaying roots and canes for up to two years. This saprophytic ability in decaying grapevine tissue is crucial for the bacterium's dissemination. In grapevine nurseries, *A. vitis* spreads between plants primarily through dormant cuttings. Grapevines are propagated via cuttings, which are taken during the winter dormancy period. These dormant cuttings may harbor *A. vitis* asymptomatically (Burr and Katz, 1984). Furthermore, *A. vitis* can be systemically distributed throughout the plant, as evidenced by Lehoczky, (1971), who found *A. vitis* in the sap of grapevine cuttings. This systemic distribution is likely facilitated by sap flow from the infected roots to the plant shoots, carrying *A. vitis* and potentially contaminating the cutting shoots before they are harvested for propagation.

Managing crown gall disease in grapevines encounter significant challenges due to the soil-borne nature of *A. vitis*. One conventional method is grafting, where resistant grapevine cultivars are used as rootstocks and preferred cultivars as scions (Burr et al., 1998; Süle and Burr, 1998). However, infection can still occur at wounding sites, such as the graft union, freeze-injury sites in winter, or wounds caused by nematode attacks (Kuzmanović et al., 2018). Another strategy involves producing *A. vitis*-free cuttings by treating them with hot water (50 °C), a method employed in some commercial nurseries worldwide. Nonetheless, this approach does not completely eradicate *A. vitis* ((Burr et al., 1998).

The use of biological agents to control plant disease is considered promising. One effective mechanism of microbial biocontrol agents is competition for nutrients and space (Köhl et al., 2019). The utilization of nonpathogenic bacterial strains to control related pathogenic species has gained attention due to their shared nutrient preferences and ecological niches. In 1972, a nonpathogenic strain of *Rhizobium rhizogenes*, designated strain K84, was reported to protect peach seedlings effectively from tumorigenic *R. rhizogenes* strain (Kerr, 1972). Subsequent research revealed that K84 produces a bacteriocin called agrocin 84, which can kill other strains of the same and closely related species (Kerr and Htay, 1974). In 1975, K84 was registered as a commercial biopesticide, and it remained in use until 1988, when it was replaced by a genetically modified version, strain K1026, which also produces agrocin K84. These strains have effectively controlled crown gall disease in various crops, including nut trees, roses, and stone fruits. However, they are ineffective in grapevines due to the resistance of *A. vitis*, the causal agent of crown gall disease in grapevines, to agrocin 84 (Kerr and Bullard, 2020). Therefore, searching for effective biocontrol agents against crown gall disease in grapevines remains ongoing.

Some bacterial strains have been reported to have potential ability to antagonize Ti strain of *A. vitis* and protect grapevines from crown gall diseases. Some bacteria strains have been identified as belonging to different genera than *A. vitis*, such as the genus *Curtobacterium* (Ferrigo et al., 2017), *Pseudomonas*, and *Bacillus* (Eastwell et al., 2006). Meanwhile, some of them are bacterial strains belonging to the same species *A. vitis*, such as *A. vitis* F2/5 (Burr et al., 1997) and E26 (Li et al., 2009), as well as VAR03-1 (Kawaguchi et al., 2005) and ARK1 (Kawaguchi, 2013) which were isolated from Japan.

1.1.2 *A. vitis* VAR03-1 as a biocontrol agent for crown gall disease

A. vitis strain VAR03-1, a nonpathogenic strain isolated from graft unions of nursery stock grapevines in Okayama, Japan (Kawaguchi et al., 2005), has shown significant potential as a biocontrol agent. This strain has been reported to suppress gall formation caused by the tumorigenic (Ti) strain of *A. vitis* in grapevine (Kawaguchi et al., 2008, 2007, 2005). In both greenhouse and field experiments, VAR03-1 demonstrated superior protection against the Ti strain (91.8% and 100% protection, respectively), compared to K84 (12% and 15% protection, respectively) (Kawaguchi et al., 2008). Furthermore, VAR03-1 persists in the grapevine root system for approximately two years. Notably, VAR03-1 has shown significantly higher survival colonization than K84, as evidenced by the substantial decline in the K84 population within three months post-application, whereas the population of VAR03-1 remains stable (Kawaguchi et al., 2008). Beyond grapevine, VAR03-1 also exhibits protective effects against a broader range of Ti strains from other species, including *Rhizobium rhizogenes*, *R. radiobacter*, and *Agrobacterium tumefaciens*, in various host plants such as sunflowers, tomatoes, roses, and apples, performing comparably to K84 (Kawaguchi et al., 2012, 2008). Consequently, VAR03-1 is considered a promising biocontrol agent against crown gall disease, not only in grapevine but also in various other crops.

1.1.3 Rhizoviticin

Following the evaluation of the biocontrol activity of VAR03-1, further investigations have been conducted to elucidate its mode of action against Ti strains. In an in vitro dual antagonistic assay, the co-inoculation of VAR03-1 with Ti strains of *A. vitis*, *R. rhizogenes*, *R. radiobacter*, or *Agrobacterium tumefaciens* resulted in the formation of a clear inhibition zone, demonstrating direct antagonistic activity of VAR03-1 against these Ti strains (Kawaguchi et al., 2008, 2005). A more detailed investigation was carried out by Saito et al. (2018), which revealed that the culture filtrate of VAR03-1 significantly reduced both the growth and virulence gene expression of the Ti strain. Interestingly, this inhibitory effect was diminished when the Ti strain was inoculated in VAR03-1 culture filtrate that had been heated at 100 °C for 20 minutes, suggesting that the active factor(s) responsible for the antagonistic effect is heat-sensitive. To narrow down the potential factors, Saito et al. (2018) fractionated the VAR03-1 culture filtrate using a 100 kDa membrane. The antagonistic effect was retained in the >100 kDa fraction, indicating that the responsible molecule(s) is likely proteinaceous and exceeds 100 kDa in size.

An investigation involving phenotypic screening of over 6,000 transposon-insertional mutants revealed that the bioactive molecule produced by VAR03-1 is a prophage-like particle named rhizoviticin (Fig. 1) (Ishii et al., 2024). Genome organization analysis from the whole genome sequencing (WGS) of VAR03-1 (Noutoshi et al., 2020a), VAT03-9, a Ti strain (Noutoshi et al., 2020b), and VAR06-30, a non-pathogenic and non-antagonistic strain (Noutoshi et al., 2020c), and comparative genome analysis indicated that rhizoviticin has a shorter sequence (22,883 bp) than potential homologous prophages in other *A. vitis* strains. This truncation results from the loss of sequences encoding head formation (Fig. 2), categorizing rhizoviticin as a headless phage-like particle or tailocin (Ishii et al., 2024).

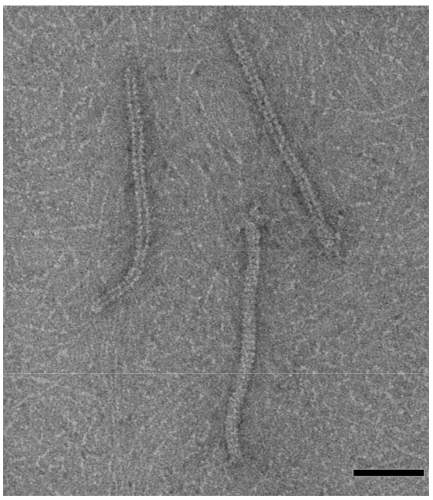

Figure 1. Electron microscopic image of rhizoviticin structure of *A. vitis* VAR03-1 (Ishii et al. 2024).

Bacteria employ various strategies to compete with other bacteria in their environment. One strategy is producing peptides toxic to bacteria, known as bacteriocins. Unlike antibiotic secondary metabolites, which typically affect a broad range of unrelated bacteria, bacteriocins specifically target closely related strains or species. One example of bacteriocin is tailocin, which is a particle that resembles phage tails in morphology (Ghequire and De Mot, 2015).

Certain bacteria have been reported to produce tailocins, particularly those within the genus *Pseudomonas*, including *P. aeruginosa*, *P. putida*, and *P. syringae* (Ghequire et al., 2015; Hockett et al., 2015). Additionally, tailocin production has been documented in *Clostridium difficile* (Gebhart et al., 2012) and *Stenotrophomonas maltophilia* (Liu et al., 2013). Tailocins are classified into two types: R-type, which are rigid and contractile, and F-type, which are flexible and non-contractile (Scholl, 2017). Rhizoviticin is an example of an F-type

tailocin (Ishii et al., 2024). While mechanisms of action of R-type tailocins have been extensively characterized, those of F-type tailocins remain less understood. However, it is generally known that the killing activity of tailocins involves the disruption of target cell membranes, leading to cell death (Woudstra et al., 2024).

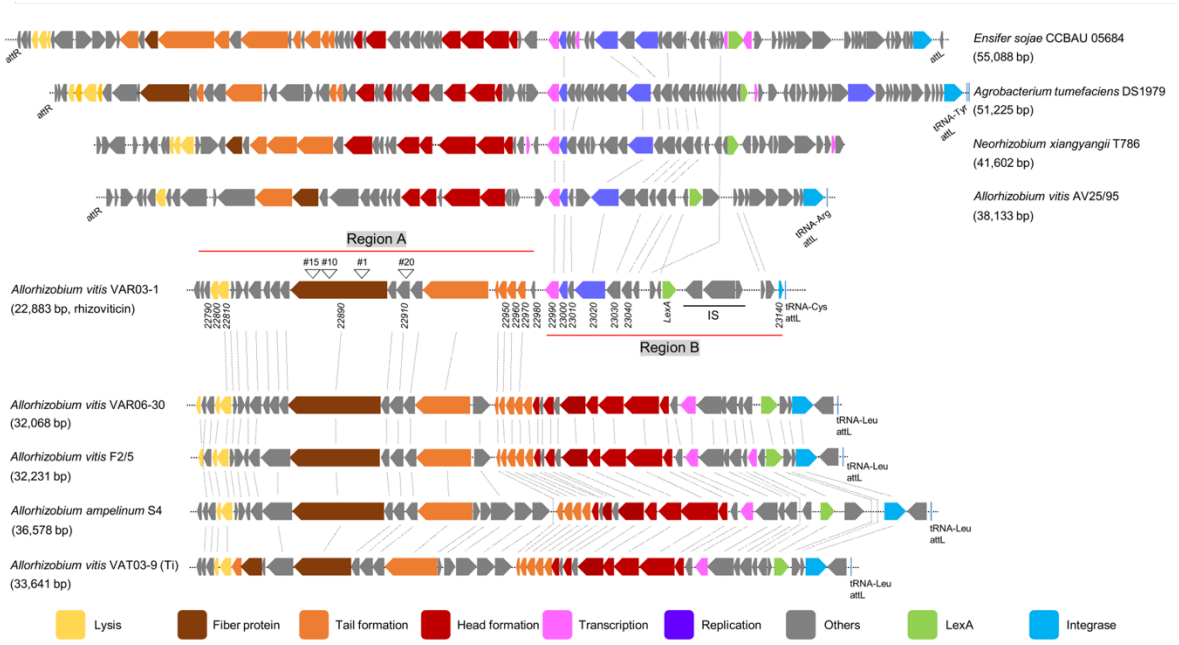

Figure 2. Genomic organization of rhizoviticin (Ishii et al., 2024)

1.2 Root exudates as nutrient reservoirs for bacteria

Carbon is a fundamental nutrient for many bacteria. Plant litter and rhizodeposits serve as primary sources of carbohydrates in soil. Plant litter contributes to carbon availability through microbial decomposition, breaking down cellulose and hemicellulose from plant litter into monomer sugars such as glucose, pentoses, and hexoses (Gunina and Kuzyakov, 2015). In contrast, carbon from rhizodeposition originates from plant photosynthates, including both primary and secondary metabolites (Gunina and Kuzyakov, 2015; Vives-Peris et al., 2020). Approximately 14.4–18.3% of photosynthetically fixed carbon is released into the soil as rhizodeposits (Hütsch et al., 2002).

Root exudation is a rhizodeposition process that profoundly impacts microbial dynamics in the rhizosphere. During root exudation, plants release various compounds, predominantly primary metabolites. Carbohydrates, amino acids, and organic acids are the major compounds secreted in large quantities through this process (Table 1) (Vives-Peris et al., 2020). These compounds are crucial carbon sources for bacteria (Gunina and Kuzyakov, 2015).

Root exudates provide a diverse array yet a limited quantity of nutrients for microbes in the rhizosphere. Carbon sources from root exudates can be consumed rapidly by microbes. In the case of glucose, an investigation by Gunina and Kuzyakov (2015), through a literature overview of 16 studies employing isotope-labeled glucose (^{14}C or ^{13}C), estimated that glucose is uptaken by microbes within seconds to minutes and integrated into microbes' cellular biochemical cycle in approximately 30 minutes. This rapid uptake underscores the intense competition among microbes for nutrients from root exudates.

Table 1. Compound secreted from plant roots (Vives-Peris et al., 2020)

Groups	Compounds
Amino acids	α-Alanine, β-alanine, γ-aminobutyric, α-aminoadipic, arginine, asparagine, aspartic, citrulline, cystathionine, cysteine, cystine, deoxymugineic, 3-epihydroxymugineic, glutamine, glutamic, glycine, histidine, homoserine, isoleucine, leucine, lysine, methionine, mugineic, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine
Sugars	Arabinose, fructose, galactose, glucose, maltose, mannose, mucilages of various compositions, oligosaccharides, raffinose, rhamnose, ribose, sucrose, xylose, deoxyribose
Organic acids	Acetic, aconitic, ascorbic, aldonic, benzoic, butyric, caffeic, citric, *p*-coumaric, erythronic, ferulic, formic, fumaric, glutaric, glycolic, lactic, glyoxilic, malic, malonic, oxalacetic, oxalic, *p*-hydroxybenzoic, piscidic, propionic, pyruvic, succinic, syringic, tartaric, tetronic, valeric, vanillic
Fatty acids	Linoleic, linolenic, oleic, palmitic, stearic
Sterols	Campesterol, cholesterol, sitosterol, stigmasterol
Growth factors and Vitamins	*p*-Amino benzoic acid, biotin, choline, inositol, *N*-methyl nicotinic acid, niacin, pathothenic, pantothenate, pyridoxine riboflavin, strigolactones, thiamine
Enzymes	Amylase, invertase, peroxidase, phenolase, acid/alkaline phosphatase, polygalacturonase, protease
Flavonoids	Chalcone, coumarine, flavones, flavonols, flavanones, flavonones, isoflavones
Nucleotides/purines	Adenine, guanine, uridine/cytidine
Others	Al-induced polypeptides, alcohols, alkyl sulphides, auxins, camalexin, dihydroquinone, ethanol, glucosides, glu- cosinolates, glycinebetaine, hydrocyanic acid, inorganic ions and gaseous molecules (e.g. CO_2, H_2, H^+, OH^-, HCO_3), isothiocyanates, unidentified ninhydrin positive compounds, unidentifiable soluble proteins, reducing compounds, scopoletin, sorgoleone, strigolactones

Interestingly, root exudation is a highly dynamic process. The composition and quantity of exudates change in response to the plant's physiological status. Root exudates can vary depending on the developmental stages of the plants (Chaparro et al., 2013; Lopes et al., 2022a; Zhao et al., 2021). Remarkably, even within a single plant, metabolites secreted along the primary root axis can differ (Loo et al., 2024). A meticulous investigation by Loo et al. revealed that this variation was affected by the distribution of sugar transporters along the root axis. Environmental factors such as nutrient availability (Carvalhais et al., 2011; Tawaraya et al., 2013) and the presence of surrounding microbes (Ankati et al., 2019; Gu et al., 2016) also have an impact on root exudates. These dynamic changes in root exudates, in turn, influence the microbial communities in the rhizosphere.

1.3 Roles of sugars in plant-microbe interaction

Sugars are among the most abundant metabolites in root exudates (Vives-Peris et al., 2020). Sugars Will Eventually be Exported Transporters (SWEETs) are a family of sugar transporters involved in many sugar efflux mechanisms, including root exudation (Breia et al., 2021). As sugars are critical nutrients for many pathogens, plants often limit carbon secretion from roots by regulating *SWEET* genes. Chen et al. (2015) reported that *SWEET2* has a role in preventing sugar loss from roots by increasing sugar accumulation in the vacuole. Upon *Phytium* infection, this gene expression was elevated to restrict sugar sequestration. However, some pathogens have developed strategies to control plant *SWEET* genes for their benefit. *Xanthomonas* spp. secreted transcription-activator-like (TAL) effectors to enhance some *SWEET* genes that facilitate sugar leakage (Breia et al., 2021).

From the microbial point of view, sugars do not simply serve as crucial carbon sources for them. Studies have reported that sugars also contribute to various processes associated with the interaction between bacteria and their host plants, such as virulence (Hamilton et al., 2021; Schulte and Bonas, 1992; Zhang et al., 2023), quorum sensing mechanisms (Zhang et al., 2019), motility, and root colonization (Tian et al., 2021).

1.4 Plant immunity and commensal bacteria

Plant immunity comprises a sophisticated, multi-layered defense system against various pathogens. In contrast to animals, plants lack specialized immune cells; hence, they depend on the ability of each cell to recognize and respond to pathogenic threats. The plant immune

system is organized into two primary layers: microbe-associated molecular pattern (MAMP)-triggered immunity (MTI) and effector-triggered immunity (ETI). MTI, also known as pattern-triggered immunity (PTI), serves as the initial defense mechanism, activated by the recognition of MAMPs through pattern recognition receptors (PRRs) located on the plasma membrane of plant cells. However, pathogens have evolved to evade MTI by delivering effector proteins into the plant cells. In response, ETI, the second layer of plant immunity, is activated by the recognition of these effector proteins via intracellular nucleotide-binding leucine-rich repeat (NLR) receptors (Ngou et al., 2022). Further explanation is provided for MTI as we focused on this type of plant immunity in this study.

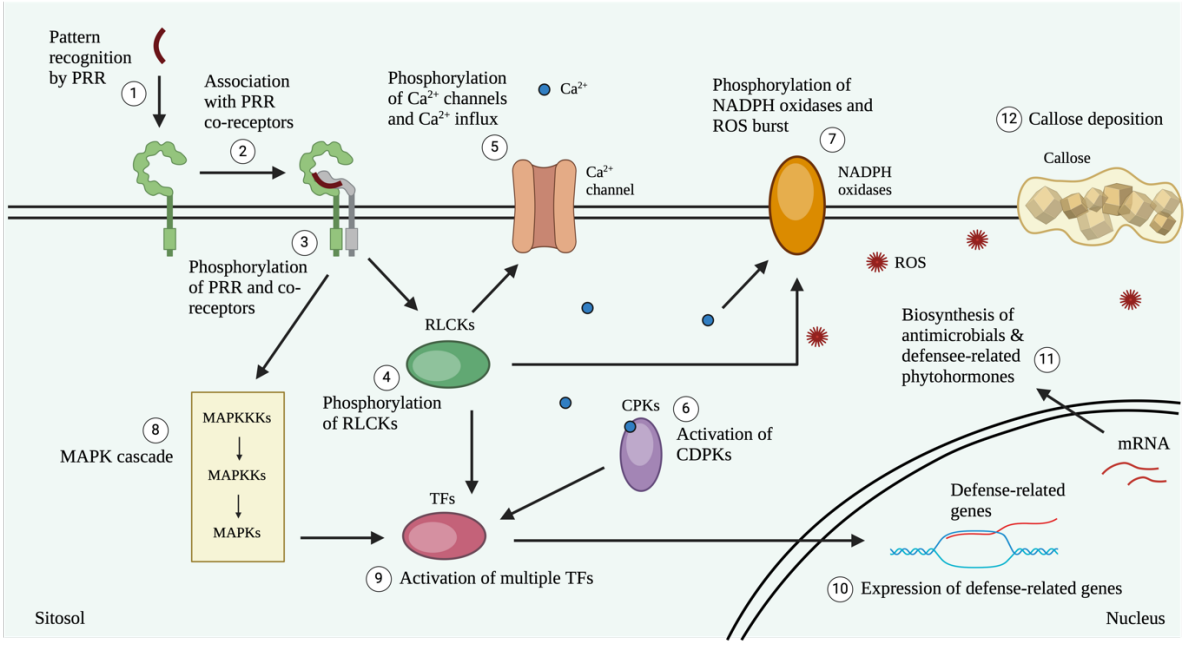

Figure 3. MTI responses upon MAMPs recognition.
This image was adopted from Ngou et al. (2022) and created with BioRender.com.

Plants recognize surrounding microbes through MAMPs, which are conserved across various microbial groups (e.g. bacteria, fungi). Among the best-characterized bacterial MAMPs are peptides derived from flagellin (flg22) and elongation factor-Tu (EF-Tu) (elf18). These MAMPs are recognized by plants through their corresponding PRRs: leucine-rich repeat receptor kinase (LRR-RK) FLS2, and LRR-RK EFR, respectively (Zipfel, 2009). In the absence of MAMP ligands, the PRRs and their co-receptors (e.g. BAK1), are inactive and separately associated with receptor-like cytoplasmic kinases (RLCKs) (e.g. BIK1). Upon MAMP recognition (e.g., flg22), the PRR FLS2 and its co-receptor BAK1 are activated and

form a heterodimeric complex. This activated FLS2-BAK1 complex phosphorylates BIK1, releasing it from the receptor complex to activate downstream signaling pathways (Lu et al., 2010). These pathways include the phosphorylation of calcium channels and NADPH oxidase respiratory burst oxidase protein D (RbohD), triggering Ca^{2+} influx and reactive oxygen species (ROS) burst, respectively. Additionally, activated BIK1 phosphorylates mitogen-activated protein kinase kinase kinases (MAPKKKs), leading to the activation of defense-related transcription factors (TFs) and the expression of defense-related genes. This cascade ultimately results in the biosynthesis of defense-related phytohormones, antimicrobial compounds, and callose deposition (Fig. 3). Notably, this response is rapid: Ca^{2+} influx and ROS burst occur within minutes after MAMP recognition, followed by the synthesis of defense-related phytohormones and callose deposition at a later time within hours (Ngou et al., 2022).

Plants are associated with a diverse array of microbes in both their shoots and roots. These associated microbes are not always harmful; some establish a mutual relationship with plants, while others maintain a commensal lifestyle without inducing detrimental effects on plants (Drew et al., 2021). Regardless of their lifestyle, bacteria are recognized by plants through their MAMPs. Interestingly, the flg22 peptide from commensal bacteria is mainly nonimmunogenic; it does not elicit MTI responses, such as ROS burst, unlike the flg22 from pathogenic bacteria (Colaianni et al., 2021). However, the ROS burst can still be activated through the recognition of other MAMPs, such as elf18 or chitin (Entila et al., 2024).

Even though a commensal bacterium does not induce plant immunity, plant immune response may be activated by MAMPs of other commensal or pathogenic strains as they exist together in the rhizosphere or the phylosphere. Hence, commensal bacteria should develop strategies to evade this activated host immunity. Some commensals have been reported to have the ability to evade MTI by suppressing the MTI responses via type II secretion system (T2SS) (Teixeira et al., 2021) or producing metabolites that can lower root pH (Yu et al., 2019).

1.5 The *recA* gene

Bacteria can suffer from extreme DNA damage due to environmental stress, such as UV irradiation, antimicrobial activity, and other stimuli. RecA is transcriptionally induced by such stresses, resulting in the Save Our Soul (SOS) response induction (Kaushik et al., 2022). RecA can degrade LexA, a repressor of prophage genes, therefore, phage production is activated in the cell. However, an alternative route also occurs, in which the phage repressor becomes inactive. Phage production is also accompanied by the induction of cell lysis by the

lytic enzymes encoded by the prophage, which releases the phage particles (Fig. 4). This phage-induced cell lysis is crucial in bacterial life because it facilitates important processes, such as biofilm formation, pathogen virulence, and horizontal gene transfer (Nanda et al., 2015).

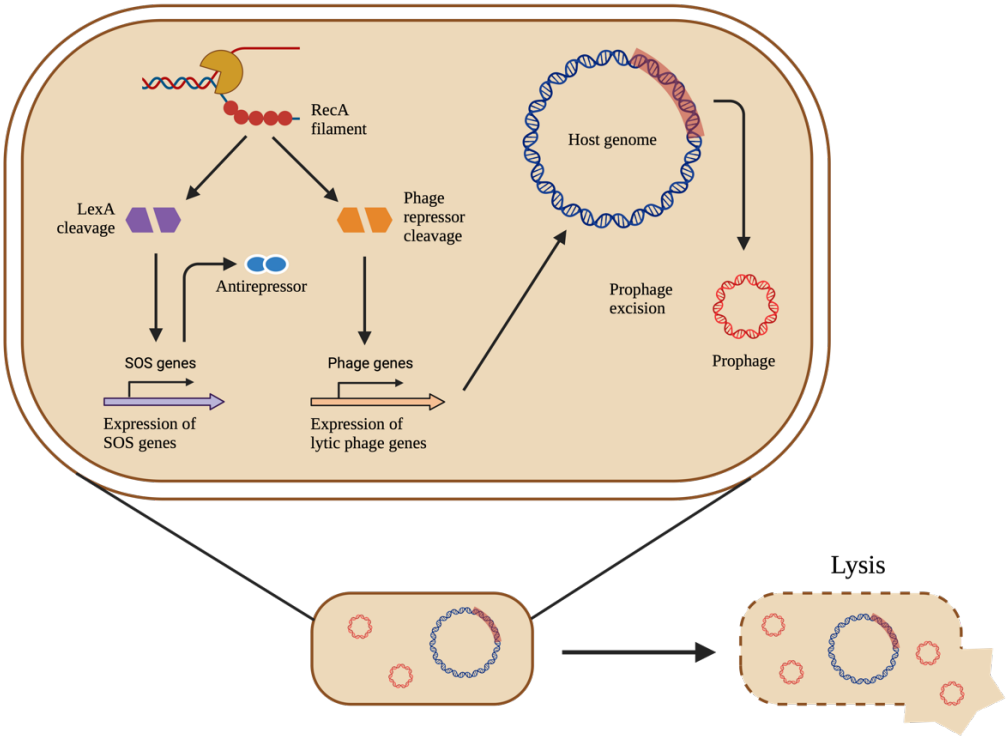

Figure 4. RecA-mediated SOS responses and prophage induction.
This image was adopted from Nanda et al. (2015) and created with BioRender.com.

Biofilm formation is an essential process for bacterial colonization on the surface of host tissues. Many studies provide evidence for the involvement of RecA in biofilm formation (Gao et al., 2020; Turnbull et al., 2016; Wu et al., 2022). As RecA-mediated SOS responses, bacterial cells undergo cell lysis and release all the bacterial-derived molecules into the environments, including nucleic acids. This mechanism provides important resources for environmental DNA (eDNA) (Turnbull et al., 2016; Yasuda et al., 2022), which is one of the important components of biofilm matrix (Verstraeten et al., 2008). Interestingly, loss of *recA* has been reported to not always result in reduced biofilm formation. Apparently, the effect depends on each bacterial species (Table 2).

Table 2. Effects of *recA* deficiency on biofilm formation.

Bacterial strain	Effect of loss of *recA* on biofilm	Mechanisms	Reference
Acinetobacter baumannii	Increased	Increased biofilm in recA mutants induced more exopolysaccharide (EPS) production.	Ching et al. (2023)
Pseudomonas aeruginosa PA14	Increased	Unknown mechanism(s) that did not associated with increased c-di-GMP.	Yahya et al. (2023)
Escherichia coli K-12	Decreased	Unknown	Beloin et al. (2004)
Escherichia coli ATCC 25922	Decreased	Unknown	Recacha et al. (2019)
Streptococcus mutants	Decreased	Unknown	Inagaki et al. (2009)
Bacillus cereus 905	Decreased	Unknown	Gao et al. (2020)

1.6 Objectives

In a prior investigation conducted within our laboratory, rhizoviticin was identified as the primary agent responsible for the antagonistic properties of VAR03-1 against Ti in vitro. Rhizoviticin, a phage tail-like bacteriocin also known as a tailocin, was central to this antagonistic activity. The first study (in Chapter 2) aimed to evaluate the antitumorigenic activity of rhizoviticin-deficient VAR03-1 mutants in both tomato and its native host, grapevine. The results demonstrated that the tested mutants exhibited a significant reduction in tumor-suppressing activity, underscoring the pivotal role of rhizoviticin in the biocontrol function of VAR03-1 in planta. Notably, despite the substantial loss of growth suppression activity against Ti in vitro, the mutants retained some antagonistic activity in both plant species. Given that VAR03-1 and Ti are of the same bacterial genus and species, it is postulated that their similar growth habitats on the host may lead to competition for nutrients and colonization space, thereby contributing to the residual biocontrol activity observed in planta. This finding has led us to the purpose of our second study (in Chapter 3), which aimed to characterize the colonization behavior of VAR03-1 in the plant rhizosphere. The investigation used *Arabidopsis thaliana* as a model plant organism due to its small size and the availability of various mutants that facilitate the molecular analysis of the underlying mechanisms.

CHAPTER 2:
Contribution of Rhizoviticin in the Antitumorigenic Activity of *Allorhizobium vitis* VAR03-1 against Crown Gall Disease in Tomato and Grapevine

2.1 Introduction

A. vitis is a significant pathogen responsible for crown gall disease in grapevines. This bacterium typically infects grapevines through wounds caused by freeze-injury, grafting, and nematode attack (Kuzmanović et al., 2018). Currently, effective control methods for crown gall disease in fields are unavailable. The commercial biocontrol agent for crown gall disease, *Rhizobium rhizogense* K84, is ineffective against *A. vitis* due to the resistance of *A. vitis* to agrocin 84 (Kawaguchi et al., 2007). In Japan, two nonpathogenic *A. vitis* strains, VAR03-1 (Kawaguchi et al., 2007) and ARK1 (Kawaguchi and Inoue, 2012), isolated from grapevine stocks, have demonstrated effective biocontrol activity against the Ti strains in greenhouse and field conditions. Furthermore, strain VAR03-1 has shown significant suppression of crown gall disease not only in grapevine but also in other crops, such as sunflower, tomato, rose, and apple, with performance comparable to the commercial K84 (Kawaguchi et al., 2012, 2008).

Our previous research identified that the bioactive molecule responsible for the antagonistic activity of VAR03-1 is a tailocin named rhizoviticin (Ishii et al., 2024). This rhizoviticin is unique to VAR03-1. Potential prophage homologs from other *A. vitis* strains showed similarity only in the region A of rhizovitin that encodes genes associated with cell lysis, fiber proteins, and tail formation. Region B, on the other hand, displayed similarity to the prophage homologs from *A. vitis* AV25/95 and other bacterial species such as *Ensifer sojae*, *Agrobacterium tumefaciens*, and *Neorhizobium xiangyangii* and provides regulatory elements for the expressions of region A genes (Ishii et al., 2024).

To verify that rhizoviticin is the responsible factor for the antagonistic activity of VAR03-1, deletion mutants of various genes in regions A and B were constructed and tested for their antagonism against Ti strain in vitro. Five mutants corresponding to five genes in region A (*Δ22790*, *Δ22800*, *Δ22810*, *Δ22950*, Δ22960) were evaluated. All mutants, except *Δ22790*, exhibited partial or complete abolishment of antagonistic activity. Additionally, two other mutants from region A (*Δ22970* and *Δ22980*) and six mutants from region B (*Δ22990*,

Δ23000, *Δ23010*, *Δ23020*, *Δ23030*, and *Δ23040*) were also tested. Six of these mutants, excluding *Δ22980* and *Δ23040*, showed abolished antagonistic activity. This in vitro evaluation confirms that rhizoviticin is indeed the factor responsible for the bioactivity of VAR03-1, and underscores the importance of both regions for this activity (Ishii et al., 2024).

To further confirm the role of rhizoviticin in the biocontrol activity of VAR03-1, an in planta evaluation is essential. In this study, we assessed the biocontrol efficacy of several representative deletion mutants, as reported by Ishii et al. (2024), against the Ti strain in tomato and grapevine. Our results confirmed that rhizoviticin is the primary factor responsible for the antagonistic activity of VAR03-1.

2.2 Material and methods

2.2.1 Plant materials and culture conditions

Tomato (*Lycopersicon esculentum* Mill. cv. Ponderosa) seeds were sterilized using a 1% (v/v) sodium hypochlorite solution, followed by shaking for 10 min at room temperature. Seeds were rinsed repeatedly with sterile distilled water (SDW) and sown on wet filter paper in round plates. Plates were kept in a growth chamber to induce germination. Seven-day-old tomato seedlings were transferred to soil in a pot and grown for another 2 weeks before assay. For grapevine (*Vitis vinifera* L. cv. Neo Muscat), six-month-old grapevine seedlings were provided by Dr. Akira Kawaguchi from the Western Region Agricultural Research Center, National Agricultural and Food Research Organization (NARO).

2.2.2 Bacterial strains, media, and culture conditions

The bacterial strains utilized in this study included *A. vitis* VAR03-1 (a biocontrol, rhizoviticin-producing strain), VAT03-9 (a Ti strain), and VAR06-30 (a non-biocontrol and nonpathogenic strain). The *A. vitis* mutants used were constructed in a previous study (Ishii et al. 2024). One transposon mutant (#1) and three deletion mutants (*Δ22980*, *Δ23020*, and *Δ23040*) were selected for this study. Bacterial glycerol cultures were streaked on King's B (KB) agar plates and incubated at 27 °C for 24 hours. Colonies were then transferred into KB liquid medium and incubated overnight at 27 °C with shaking (165 rpm) before harvesting for the crown gall assay.

2.2.3 Crown gall assay in tomato and grapevine

Crown gall assay was conducted on tomato and grapevine seedlings. Overnight bacterial cultures were harvested by centrifugation at 3500 × g for 10 min. Bacterial cell pellet was resuspended in fresh KB medium to a final OD_{600} of 0.2. Bacterial suspensions of the Ti strain (VAT03-9) were mixed in a 1:1 ratio with suspensions of VAR03-1, VAR06-30, or the mutants and then immediately inoculated into the plant stems using a sterilized wooden toothpick for tomatoes or a sterile syringe needle for grapevines. Ti strain suspension mixed with SDW was used as inoculation control. The seedlings were maintained in a growth chamber for 4 weeks. Galls formed at the inoculation sites were measured and categorized on a 0-4 scale (0 = none, 1 = very small, 2 = small, 3 = medium, and 4 = large). Data were presented as a percentage of the gall index.

2.3 Results

In this study, we analyzed the antitumorigenic activity of four representative mutants previously identified by Ishii et al. (2024). Mutant #1, a transposon mutant, has a Tn5 insertion in a gene encoding a fiber protein in region A. The three deletion mutants, *Δ22980*, *Δ23020*, and *Δ23040*, have deletions in genes located in region B, encoding an ssDNA-specific exonuclease, a DnaB-like replicative DNA helicase, and a putative DNA-binding protein, respectively (Fig. 5A). In vitro evaluation confirmed that mutant #1 and *Δ23020* had lost their antagonistic effect against the Ti strain, whereas *Δ22980* and *Δ23040* retained this activity (Ishii et al., 2024).

The crown gall assay conducted on tomato plants revealed a significant difference in gall formation between treatments with rhizoviticin mutants and the wild-type (WT) strain VAR03-1. Mutants #1 and *Δ23020*, both of which exhibited a loss of in vitro antagonistic activity, resulted in gall formation at 80% and 66.7% of the inoculation sites, respectively. The galls induced by mutant #1 ranged from very small to medium in size, whereas those induced by mutant *Δ23020* were predominantly larger. In contrast, treatment with the WT strain VAR03-1 led to gall formation in only 46% of inoculation sites, with galls primarily classified as very small to small in size (Fig. 5B, C). These results suggest a marked reduction in the biocontrol efficacy in both rhizoviticin mutants compared to the WT strain.

A similar pattern was observed in grapevine seedlings, where 70% of inoculation sites treated with mutant *Δ23020* developed very small to medium-sized galls. In comparison, WT treatment resulted in gall formation in only 46% of inoculation sites, with galls being

predominantly very small to small in size (Fig. 5D, E). Collectively, these results strongly suggest that rhizoviticin plays a critical role in the biocontrol activity of the VAR03-1 strain.

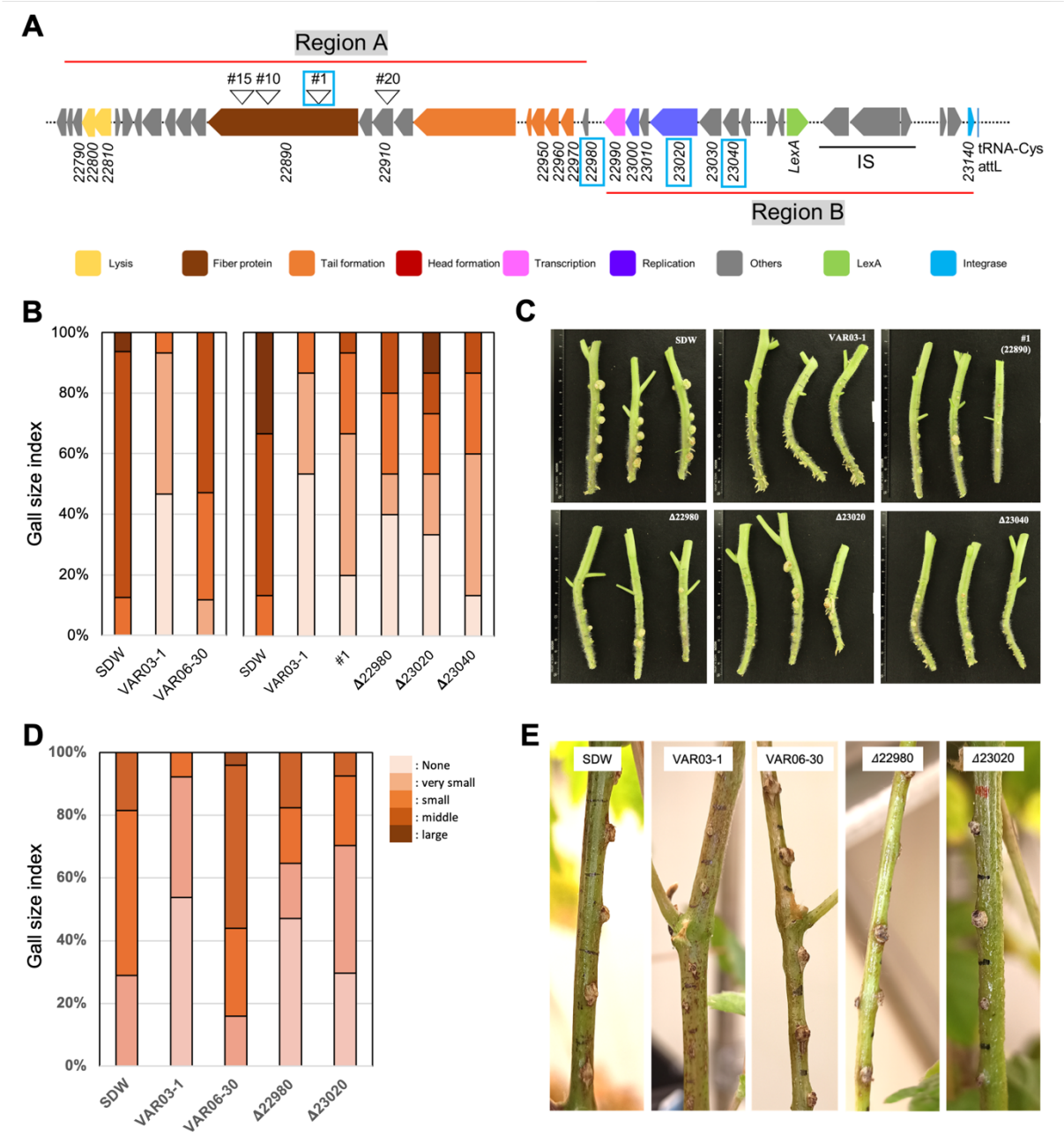

Figure 5. Crown gall assay on tomato and grapevine.

A Genomic organization of rhizovitin. Position of genes affected in rhizoviticin transposon and deletion mutants used in this study were marked in the blue squares. **B, C** crown gall assay on tomato. **D, E** crown gall assay on grapevine.

In contrast, mutants *Δ22980* and *Δ23040*, which retained in vitro antagonistic activity, exhibited less severe gall formation compared to the rhizoviticin mutants with abolished

antagonistic activity (#1 and *⊿23020*). However, the biocontrol efficacy of these mutants was still inferior to that of the WT strain in both tomato and grapevine assays (Fig. 5B-E). These observations suggest that the loss of specific rhizoviticin genes could compromise the anti-tumorigenic efficacy of the strain, despite the retention of antagonistic activity in vitro.

Interestingly, rhizoviticin mutants still displayed a residual antitumorigenic activity in planta, as evidenced by the absence of gall formation in some inoculation spots in both tomato (Fig. 5B) and grapevine (Fig. 5D) stems. This suggests that factors other than rhizoviticin may also contribute to the biocontrol activity of VAR03-1. Since VAR03-1 and Ti strain belong to the same species, we postulated that the colonization ability of VAR03-1 as well as the competition between these strains may be the factors responsible for the residual biocontrol activity.

2.4 Discussion

Bacteria commonly develop strategies to compete with other bacterial strains or species. Plants can benefit from this competition, especially if the bacteria are competing with harmful strains (Köhl et al., 2019). Competition between two bacterial strains or species can be in the form of competition for limited resources (exploitative competition) or competition involving physical and chemical attack (interference competition) (Kinkel and Lindow, 1997). VAR03-1 produces rhizoviticin to attack and compete against its closely related strains, including the Ti strain (Ishii et al., 2024; Saito et al., 2018). At the same time, it benefits the host plant by reducing the Ti strain population, which leads to the inhibition of gall formation. In our study, mutations in genes encoding rhizoviticin components resulted in reduced antagonistic activity of VAR03-1 compared to the WT strain in tomato and grapevine seedlings. This suggests that rhizoviticin is the key factor for the biocontrol activity of VAR03-1.

The use of tailocins has attracted considerable interest in therapeutic applications due to their potent antibacterial activity and narrow spectrum, which minimizes adverse effects on mammalian cells (Woudstra et al., 2024). Although their potential for phytopathogen control is promising, the literature remains sparse. Notably, *Pseudomonas fluorescens* SF4c has been documented to produce tailocins that mitigate symptoms of bacterial-spot disease induced by *Xanthomonas vesicatoria* (Príncipe et al., 2018). However, in that study, the application involved spraying purified tailocin suspensions rather than employing a live bacterial cell suspension. Similarly, *P. chlororaphis* 30-84, a known biocontrol agent against wheat take-all disease caused by *Gaeumannomyces graminis* var. tritici, exerts its biocontrol effects primarily

through the production of phenazines (Maddula et al., 2008). Subsequent research revealed that the 30-84 strain also produces multiple R-tailocin particles, which enhance biocontrol efficacy by contributing to the persistence of the strain in the rhizosphere through competitive interactions with related bacterial species (Dorosky et al., 2017). Our study is, to the best of our knowledge, the first to report on the use of tailocin-producing bacteria as a biocontrol agent where tailocins serve as the primary mechanism of action.

In our study, we confirmed that rhizoviticin is the primary factor responsible for the biocontrol activity of VAR03-1. However, we observed that while rhizoviticin mutants exhibited a reduction in antitumorigenic activity, this activity was not completely abolished, indicating the presence of residual biocontrol effects. WGS analysis revealed that VAR03-1 harbors two additional prophages besides rhizoviticin in its genome (Ishii et al., 2024; Noutoshi et al., 2020a). The residual antitumorigenic activity observed in the rhizoviticin mutants may be attributed to these other prophages. If this hypothesis were correct, in vitro antagonistic analyses of these mutants would be expected to show only a partial reduction in activity. However, the mutants exhibited no antagonistic activity in the in vitro evaluations (Ishii et al., 2024). This suggests that a more plausible explanation for the residual antagonistic activity of the two rhizoviticin mutants may be exploitative competition with the Ti strain within plant tissues, probably due to overlapping ecological niches. Both VAR03-1 and the Ti strain VAT03-9 were isolated from the same grapevine field (Kawaguchi et al., 2005), suggesting that these two strains likely share similar habitats and nutrient sources. Further investigation into the characteristics that enhance VAR03-1’s competitiveness against Ti strains may provide valuable insights.

CHAPTER 3:
Nutrient Requirements Shape the Preferential Habitat of *Allorhizobium vitis* VAR03-1 in the Rhizosphere of *Arabidopsis thaliana*

3.1 Introduction

A. vitis VAR03-1 is a biocontrol agent for crown disease in grapevines caused by the Ti strain of *A. vitis* (Kawaguchi et al., 2007, 2005; Noutoshi et al., 2020a). Ishii et al. (2024) identified rhizoviticin, a phage-like particle known as a tailocin, as the primary factor behind this biocontrol activity. In Chapter 2 of this study, we explored the antitumorigenic activity of rhizoviticin mutants of VAR03-1 in tomato and grapevine. Our results showed that even rhizoviticin mutants lacking antagonistic effects on the Ti strain in vitro exhibited a degree of antitumorigenic activity in tomato and grapevine seedlings. This phenomenon may be due to competition for habitat within plant tissues between VAR03-1 and the pathogens. Consequently, we are interested in investigating the colonization of VAR03-1 in the host plant.

Root colonization is a critical factor for the long-term sustainability of biocontrol agents in plants. Yet, the mechanisms underlining the colonization of VAR03-1 in host plants remain underexplored. The biocontrol activity of VAR03-1 has been evaluated in different host plants against a broad range of Ti strains (Kawaguchi et al., 2012, 2008). These studies highlight the potential colonization of VAR03-1 in host plants beyond grapevine. Therefore, in this study, we used *A. thaliana* as a model plant due to its small size and the availability of various mutants that facilitate the molecular analysis of the underlying mechanisms.

Plant immunity serves as the primary barrier that must be overcome for successful colonization within plant host tissues. Research involving the application of VAR03-1 in grapevine, tomato, sunflower, rose, and apple has demonstrated no detrimental effects on these host plants (Kawaguchi et al., 2008, 2012), suggesting that VAR03-1 adopts a commensal lifestyle in these species. Typically, commensal bacteria possess non-immunogenic flg22 (Colaianni et al., 2021), yet they can still trigger plant immunity through the detection of other MAMPs such as elf18 or chitin (Entila et al., 2024). Furthermore, plant immunity can be activated by MAMPs from other commensal or pathogenic strains present in the surrounding environment. To evade this induced plant immunity, some commensal bacteria have evolved

mechanisms such as secreting specific metabolites to lower root pH (Yu et al., 2019) or utilizing type II secretion systems to suppress plant immune responses (Teixeira et al., 2021).

Following the evasion of plant immunity, commensal bacteria must compete with other microbes for plant-derived nutrients. Certain commensal bacteria exhibit preferences for specific host plants (Wippel et al., 2021), while others show distinct habitat preferences along the root axis (Wei et al., 2021), potentially influenced by the secretion of preferred metabolites (Loo et al., 2024). Consequently, understanding these interactions is critical for optimizing the application of biocontrol agents like VAR03-1 and ensuring their effective deployment in grapevine as well as in different plant species.

During colonization, bacteria swarm to the surface of host tissues to initiate rapid colonization and biofilm formation. Biofilms are sessile communities of bacteria embedded within a matrix attached to the surface of host tissues (Verstraeten et al., 2008). Although the composition of the biofilm matrix can vary among bacterial strains or species, it typically includes EPS, eDNA, and protein (Karygianni et al., 2020). EPS is secreted by bacterial cells through specific pathways (Whitney and Howell, 2013). Meanwhile, eDNA is derived from the lysed bacterial cells via spontaneous activation of the SOS pathway mediated by RecA, which leads to prophage production (Turnbull et al., 2016). RecA is a multifunctional protein containing protease activity that degrades the transcriptional repressor LexA to activate prophage genne expression (Kaushik et al., 2022b; Nanda et al., 2015). Accumulating evidence suggests that loss of the *recA* gene can either increase (Ching et al., 2024; Yahya et al., 2023) or decrease (Beloin et al., 2004; Gao et al., 2020; Inagaki et al., 2009; Recacha et al., 2019) biofilm formation, depending on the bacterial species. Loss of *recA* has also been shown to reduce bacterial virulence (Aranda et al., 2011; Corral et al., 2020; Fuchs et al., 1999; Martínez et al., 1997).

In this study, we investigated the growth pattern of VAR03-1 in the rhizosphere of *A. thaliana* using an MS agar system and compared it with that of another biocontrol agent, *P. protegens* Cab57. Their growth areas were different and possibly dependent on their nutrient requirements. Supplementation of exogenous sucrose in the medium caused bacterial overgrowth and subsequent inhibition of Arabidopsis growth, which was unexpectedly independent of plant immunity and metabolic conversion of bacterium-derived AHL. This underscores the importance of plants in strictly regulating the nutrients released from their roots to maintain a balanced microbial community. Furthermore, we discovered that loss of *recA* in VAR03-1 reduced its inhibitory activity on Arabidopsis seedlings, highlighting the role of RecA in the interaction between VAR03-1 and Arabidopsis.

3.2 Material and methods

3.2.1 Plant material and culture conditions

Arabidopsis seeds were sterilized using a 0.1% (v/v) sodium hypochlorite solution, followed by shaking for 10 min at room temperature. Subsequently, seeds were rinsed 7 times with SDW. A portion of the SDW from the final rinse was retained to submerge the seeds. Seeds were then sown on half-strength MS agar (containing 0.1% (v/v) Gamborg vitamins and 0.8% (w/v) agar) supplemented with 1% sucrose after stratification at 4 °C in the dark condition for 3–4 d. Seeds were incubated in a growth chamber under long day conditions (16 h photoperiod) at 22 °C before experiments. Arabidopsis *Atfaah* mutants were a generous gift from Professor Kent D. Chapman from University of North Texas. The information regarding Arabidopsis mutants used in this study is listed in Table S2.

3.2.2 Bacterial strains, media, and culture conditions

Bacterial strains used in this study were *A. vitis* WT strains, including an antagonistic strain VAR03-1, a pathogenic strain VAT03-9 (Ti), and non-antagonistic and non-pathogenic strain VAR06-30, *recA* homologous mutant of VAR03-1 (*ΔrecA*) (Ishii et al., 2024), VAR03-1 transposon mutants (*Δ*65 and *Δ*86) (Bao, 2022), and *P. protegens* Cab57. Information regarding bacterial strains used in this study is provided in Table S1. Glycerol stock cultures were streak-inoculated onto King's B (KB) agar without antibiotics and incubated at 27 °C for 24 h (*A. vitis* strains) or 12 h (*P. protegens* Cab57). Colonies were then transferred to liquid KB without antibiotic (for wild-type strains) and with 30 µg/mL nalidixic acid (nal) (for *ΔrecA*) or 30 µg/mL nalidixic acid (nal) and 170 µg/mL Kanamycin (km). Then, cultures were incubated at 27 °C with shaking (165 rpm) for 18 h (*A. vitis* strains) and 12 h (*P. protegens* Cab57) for further experiments.

3.2.3 Bacterial inoculation on Arabidopsis seedlings in MS agar

Bacterial culture was harvested by centrifugation (3500 × g, 10 min, room temperature). Bacterial cells were then washed twice using 10 mM $MgCl_2$ to remove any bacterial-derived molecules during cultivation and adjusted to an OD_{600} of 0.5 in 10 mM $MgCl_2$. Fifty microliters of the bacterial suspension was mixed with 50 mL of warm half-strength MS agar and poured

onto a square plate (final OD_{600} of 5×10^{-4}, equivalent to approximately 10^5 cfu/mL). Fifty microliters of 10 mM $MgCl_2$ was used to replace the bacterial suspension in the mock treatment. Eight 5-day-old Arabidopsis seedlings were aseptically transplanted onto the square plate and sealed with micropore tape after solidification. For each treatment, two replicate plates were prepared. The plates were then incubated in a growth chamber with a near-vertical orientation to ensure root penetration into the agar. Eight days after transplantation, photographs of plates were taken using a mirrorless camera, and PR length was analyzed using ImageJ software. Bacterial colonies around the root were observed and photographed using a stereomicroscope (Zeiss stemi 305). The shoots were then detached from the roots and weighed.

For inoculation of VAR03-1 at different bacterial concentrations, the harvested bacterial suspension was adjusted to OD_{600} of 5, 0.5, and 0.05. Five hundred microliters of bacterial suspension was mixed with 50 mL of warm MS agar, resulting in a final bacterial OD_{600} of 5×10^{-2}, 5×10^{-3}, and 5×10^{-3}, respectively. For mock treatment, 500 µL of 10 mM $MgCl_2$ was used.

3.2.4 Bacterial growth assay

Bacterial culture was harvested by centrifugation at 3500 × g for 10 min at room temperature and adjusted to an OD_{600} of 10. One hundred and fifty microliters of bacterial suspension were added to 15 mL of liquid MS (supplemented with 0.1% (v/v) Gamborg vitamins with or without sugars) or KB (final OD_{600} = 0.1). Cultures were incubated at 27 °C with shaking (165 rpm), and OD_{600} was measured every 2 h during a 14 or 16 h incubation period. For organic acid treatments, 150 µL of a 20 mM stock solution (final organic acid concentration = 200 µM) was supplemented into 15 mL of liquid MS before bacterial inoculation.

3.2.5 Root colonization assay

A 12-well plate hydroponic culture system was utilized to measure bacterial colonization. Liquid MS medium was supplemented with a bacterial suspension of VAR03-1 to achieve a final OD_{600} of 5×10^{-4}. Five milliliters of VAR03-1-containing MS liquid were transferred to each well. A silicon rubber piece, with a width half the diameter of the well and four centrally aligned slits, was placed at the top of each row. A 7-day-old seedling was aseptically positioned in the slit of each well, ensuring that the shoot part remained above the

slit while the root part was submerged in the medium. Another piece of silicon rubber without slits was placed to cover the remaining space of the wells in each row. This arrangement could minimize medium evaporation and ensure the growth of the shoot part without touching the medium. The plate was carefully placed in a sterile, clear plastic box, sealed with micropore tape, and incubated in a growth chamber.

After 10 to 12 days of incubation, 3 or 4 representative seedlings from each treatment were selected for root colonization measurements. Roots were aseptically harvested, washed twice with SDW by vigorous pipetting, and placed on a sterile filter paper to remove excess moisture before weighing. Roots were then transferred to a 2 mL microtube containing 400 µL SDW and homogenized using a tissue homogenizer. Subsequently, 600 µL of SDW was added to create a 1000 µL root suspension. Simultaneously. 1 mL of medium was collected from the well corresponding to each harvested seedling. Both root suspension and medium samples were serially diluted. Three 6 µL-drops of each dilution were dispensed on square plates containing KB agar and allowed to dry for approximately 3 min. The plates were then sealed with micropore tape and incubated at 10 °C for 3 to 7 days. Colonies on each drop were observed under a stereomicroscope and counted.

3.2.6 flg22 treatments for gene expression

Arabidopsis seedlings were germinated on MS agar supplemented with different concentrations of sucrose to be tested (0, 0.1, 1, 10, and 25 mM) instead of 1% sucrose. This was done to minimize the effect of high sucrose during the germination period on subsequent responses to flg22. For 1, 10, and 25 mM sucrose treatments, 5-day-old seedlings grown in the corresponding sucrose were used. Meanwhile, for 0 and 0.1 mM treatments, 6-day-old seedlings grown in corresponding sucrose were used. We used different ages of the seedlings to standardize seedling size because seeds germinated at lower sucrose concentrations produced smaller seedlings. Seedlings were then transplanted to square plates containing MS agar supplemented with the same sucrose concentration as their germination medium, with or without the addition of flg22 (final concentration at 0.5 µM). For instance, seedlings germinated on MS agar containing 1 mM sucrose were transplanted onto MS agar containing 1 mM sucrose with or without flg22. The plates were then arranged in a nearly vertical position in the growth chamber and incubated for 3 days before RNA extraction.

3.2.7 Gene expression analysis

Root and shoot of inoculated Arabidopsis seedlings were harvested from square plates at designated time points and collected in separate tubes. Root and shoot from 5 to 15 seedlings were polled in one tube as a composite sample and frozen immediately in liquid nitrogen. Three tubes were prepared for each root and shoot composite sample for each treatment. RNA extraction and cDNA synthesis were performed using ISOSPIN Plant RNA kit (Nippon gene) and PrimeScript™ RT reagent kit with gDNA eraser (Takara Bio), respectively. The relative expression levels of *FRK1* and *PDF1.2* were quantified using Luna Universal qPCR Master Mix (New England Biolab) and the LightCycler 96 (Roche). *UBQ10* was selected as a housekeeping gene for normalization. Primers used for gene expression analysis are listed in Table S3.

3.2.8 Biofilm assay and planktonic cells measurement

Biofilm formation assay was performed on a 96-well plate according to the report by Abarca-Grau et al., (2011) with some modifications. Briefly, bacterial culture grown overnight in liquid ATM medium (containing 2 g/L mannitol) supplemented with biotin (2 mg/L) was adjusted to an OD_{600} of 0.04 in fresh liquid ATM medium. Then, 200 µL of this bacterial suspension was added to each well of a 96-well plate and incubated statically at 27 °C. After 6 d, the liquid bacterial culture was transferred to a new 96-well plate for OD_{595} measurement of planktonic cells. The original plate was then thoroughly rinsed three times with distilled water and air dried for 45 min. Two hundred microliters of 0.1% crystal violet was added to each well and incubated for 45 min to stain sessile bacteria. The plates were then rinsed three times with distilled water and air dried for 45 min. Two hundred microliters of 95% ethanol was added to each well, and 150 µL was transferred to a new 96-well plate. Biofilm formation was measured at OD_{595}.

3.3 Results

3.3.1 Colonization patterns of *A. vitis* strains and *P. protegens* Cab57 in the Arabidopsis rhizosphere

To characterize the root colonization behavior of VAR03-1 in the rhizosphere, we employed Arabidopsis as a model plant in a square plate containing MS medium. VAR03-1 cells were mixed into MS agar without any additional carbon source. Then, Arabidopsis

seedlings were cultivated in the plate in a semi-vertical orientation to observe bacterial growth in the rhizosphere. The presence of VAR03-1 (final $OD_{600}=5\times10^{-4}$) promoted the primary root (PR) growth of Arabidopsis (Fig. 6A). Throughout the study, this trend of enhanced root growth by VAR03-1 was consistently observed across different experimental trials. However, due to the instability of seedling growth on MS agar without sucrose after transplantation, statistical significance was not consistently achieved. The shoot weight remained unaffected (Fig. 6B). Observation revealed colonies of VAR03-1 along the PR, with an uneven density distribution, showing a higher population in the upper part compared to the lower part of the PR (Fig. 6C). Next, we investigated if this colonization pattern was specific to VAR03-1 by using other strains of *A. vitis*, namely VAR06-30 (a non-pathogenic, non-antagonistic strain) and VAT03-9 (Ti) (a pathogenic strain). The colonization patterns of these strains were similar to that of VAR03-1, in that they preferred to colonize the upper part of Arabidopsis more than the lower part (Fig. 6D). Moreover, they promoted the Arabidopsis PR length without significantly affecting the shoots (Fig. 6E–G), suggesting that this is a common trait among *A. vitis* strains when interacting with Arabidopsis. To further investigate the colonization activity of VAR03-1, another biocontrol agent from different species, *P. protegens* Cab57, was used as a control in the experimental setting. Interestingly, colonies of Cab57 were invisible around the PR (Fig. 6D). Nevertheless, Cab57 reduced the length of the Arabidopsis PR without significantly affecting the shoots (Fig. 6E–G). This negative impact on root growth suggests that Cab57 likely colonizes the surface or interior of plant root tissue at levels that are not visible under the microscope.

3.3.2 Difference in nutrient requirements of *A. vitis* VAR03-1 and *P. protegens* Cab57

To investigate the differences in colonization patterns between these bacterial genera, we analyzed their growth in a liquid MS medium. No growth of either strain was detected in the MS medium supplemented with vitamins alone (Fig. 7A–E), indicating that there are no nutrients in the MS medium sufficient to support the growth of these strains. However, when the same medium was used in the presence of Arabidopsis seedlings, as shown in Fig. 6, visible colonies were observed surrounding Arabidopsis PR, at least for VAR03-1. This indicates that bacterial growth in the Arabidopsis rhizosphere of this experimental system relies primarily on nutrients—mainly carbon sources—derived from the host roots.

To determine the specific carbon source requirements and preferences for their growth, we supplemented the MS medium with various sugars commonly found in root exudates at

different concentrations (Badri and Vivanco, 2009; Chaparro et al., 2013). Previous work by Song et al. (2022) showed that 5-day-old Arabidopsis Col-0 seedlings grown hydroponically secreted approximately 40 mg/L (0.12 mM) of sucrose in 24 hours. Given that 1% sucrose (29.2 mM) is a standard concentration used in Arabidopsis research, we tested sugar concentrations ranging from 0.1 to 25 mM. Our results showed that VAR03-1 was capable of growing in MS medium supplemented with all tested sugars (glucose, fructose, sucrose, and mannitol) at concentrations of ≥1 mM (Fig. 7A–D). Sucrose was particularly effective, supporting optimal growth of VAR03-1 at a minimum concentration of 10 mM (Fig. 7C). In addition, we compared the growth of VAR03-1 and Cab57 in liquid MS medium supplemented with 1% (approximately 30 mM) sucrose. Under these conditions, VAR03-1 reached an optical density (OD_{600}) of approximately 0.9 within 20 hours, whereas Cab57 only achieved an OD_{600} of 0.2, with no further increase even after prolonged incubation (Fig. 7E).

In addition to sugars, organic acids are known to be present in root exudates, and some are recognized by certain pathogenic bacteria for inducing full virulence (Anderson et al., 2014). To assess the growth of VAR03-1 on these compounds, we supplemented MS liquid medium with 200 μM of five selected organic acids (shikimic acid, citrate, aspartic acid, *p*-hydroxybenzoate, and 5-oxo-proline). These acids, identified in Arabidopsis root exudates, have been shown to influence virulence gene induction in *Pseudomonas syringae* at effective concentrations of 200 μM (Anderson et al., 2014). However, VAR03-1 did not exhibit growth when each organic acid was individually supplemented at 200 μM (Fig. 7F, dotted line). Interestingly, bacterial growth was only observed when 10 mM sucrose was added alongside the organic acids (Fig. 7F), suggesting that sucrose is a critical limiting factor for VAR03-1 growth. In the sucrose-supplemented MS medium, the optical density (OD_{600}) of VAR03-1 reached approximately 0.6 and further increased to 0.7–0.9 with the addition of citrate, L-aspartic acid, *p*-hydroxybenzoate, or 5-oxo-proline (Fig. 7F). Notably, shikimic acid did not produce any additive growth effect (Fig. 7F). Moreover, supplementing the MS medium with sucrose and all organic acids did not enhance VAR03-1 growth beyond the level achieved with individual organic acids, indicating that any one of these four organic acids is sufficient to produce the additive growth effect when combined with sucrose.

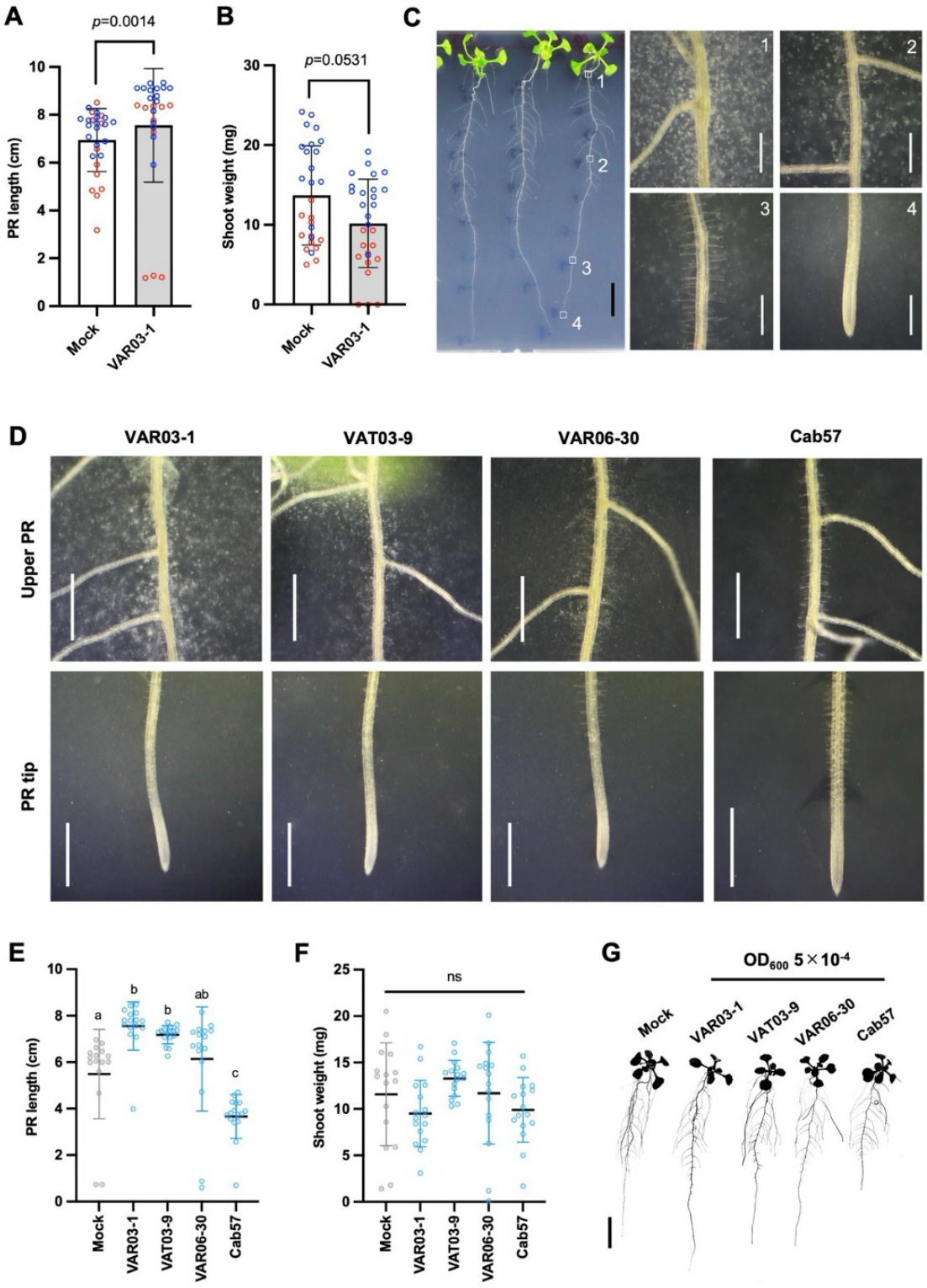

Figure 6. Colonization pattern of *A. vitis* strains and *P. protegens* Cab57 in Arabidopsis rhizosphere.

A, B Primary root (PR) length (**A**) and shoot weight (**B**) of Arabidopsis seedlings grown in sucrose-free MS agar with VAR03-1 ($OD_{600}=5\times10^{-4}$) were measured at 8 days post inoculation (dpi). Mann-Whitney U-test (n=28) was performed to compare the significance between mock and VAR03-1 treatment. Red and blue dots indicate distinct experimental sets. **C** Distribution of VAR03-1 colonies along the root axis of Arabidopsis in MS agar medium.

Four spots indicated by white boxes were further observed with higher magnification, shown in the images of the right side with an indicated number. **D** Bacterial colonies of indicated strains were observed around Arabidopsis PR at upper PR and PR tip regions. **E-G** PR length (**E**), shoot weight (**F**), and morphology (**G**) of Arabidopsis seedlings grown in sucrose-free MS agar containing *A. vitis* strains and *P. protegens* Cab57 (OD_{600}=5×10^{-4} each) at 8 dpi. Different letters indicate statistical significance performed using Brown-Forsythe ANOVA and Dunnett's T3 multiple comparison test (*n*=16) (**E**) and ANOVA followed by Tukey's test (*n*=16) (**F**). Black bars, 1 cm. White bars, 1 mm.

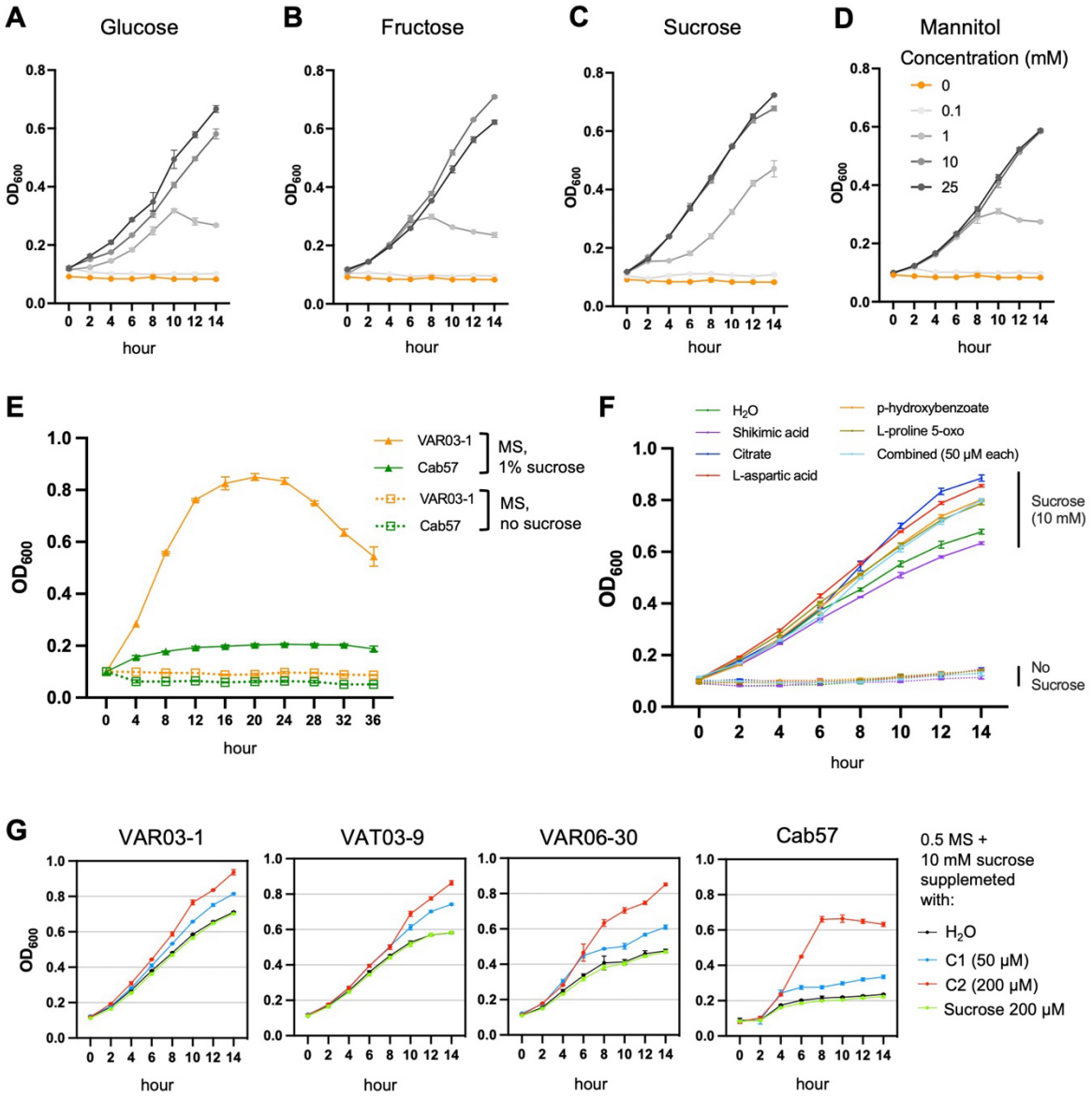

Figure 7. Growth of *A. vitis* strains and *P. protegens* Cab57 in liquid MS supplemented with sugars and/or organic acids.

A-D Growth of *A. vitis* VAR03-1 in liquid MS supplemented with different sugars as indicated. OD_{600} was measured at the indicated time point during cultivation. **E,** Growth of VAR03-1 and Cab57 in liquid MS with or without 1% sucrose. **F** Growth of VAR03-1 in liquid MS supplemented with five individual organic acids (200

μM each) with or without 10 mM sucrose. **G** Growth of *A. vitis* strains and Cab57 in liquid MS supplemented with a mixture of four organic acids (citrate, L-aspartic acid, *p*-hydroxybenzoate, and L-proline 5-oxo) (C1, 50 μM each or C2, 200 μM each) or 200 μM sucrose with 10 mM sucrose.

We then investigated whether this nutrient requirement—specifically the combination of sucrose and organic acids—is common among *A. vitis* strains. To assess this, VAR03-1, VAT03-9, and VAR06-30 were cultured in MS medium containing 10 mM sucrose and/or a mixture of four organic acids (citrate, aspartic acid, *p*-hydroxybenzoate, and 5-oxo-proline) at individual concentrations of 50 or 200 μM. All *A. vitis* strains demonstrated varying levels of growth in the presence of sucrose, with VAR03-1 exhibiting the highest growth compared to the other two strains (Fig. 7G, dark green line). Notably, these growth patterns corresponded to the colonization observed in the upper part of the Arabidopsis PR as depicted in Fig. 6D. VAR03-1, which formed the densest bacterial colonies around the root, also exhibited the greatest growth in sucrose-supplemented medium. In contrast, VAR06-30, which had fewer colonies, demonstrated the least growth. VAT03-9, with a colonization level between VAR03-1 and VAR06-30, showed growth that fell between that of VAR03-1 and VAR06-30. These observations suggest that the upper part of the Arabidopsis PR may be particularly enriched in sucrose compared to other carbon sources.

When organic acids were added in combination with sucrose, the growth of all *A. vitis* strains increased beyond that observed with sucrose alone (Fig. 7G, blue and red lines). Despite this, *A. vitis* strains showed a greater dependence on sucrose than on organic acids. However, the extent to which organic acids enhanced growth varied among the strains, with VAR06-30 showing the greatest increase, followed by VAT03-9 and VAR03-1. Interestingly, the growth-enhancing effects of organic acids compensated for the differences in growth levels induced by sucrose alone, resulting in all *A. vitis* strains achieving similar growth levels in the presence of sucrose and organic acids (Fig. 7G, red line).

Additionally, we examined the nutrient requirements of Cab57 under the same experimental conditions. In MS medium supplemented with both sucrose and organic acids, Cab57 reached an OD_{600} of approximately 0.6, indicating that it also relies on these nutrients for growth, with organic acids playing a particularly important role (Fig. 7G). Overall, these results suggest that *A. vitis* strains and Cab57 have distinct nutrient dependencies: *A. vitis* strains rely primarily on sucrose, while Cab57 shows a greater dependence on organic acids.

3.3.3 Interaction of VAR03-1 and Arabidopsis with exogenously applied sucrose

Our results indicate that sucrose is the primary carbon source crucial for the growth of VAR03-1, suggesting its significant role in the interaction between VAR03-1 and plants. To further explore this interaction, we added a concentration series of sucrose (ranging from 0.1 to 10 mM) to MS agar. In the presence of sucrose at concentrations ≥1 mM, VAR03-1 significantly reduced the primary root (PR) length of Arabidopsis (Fig. 8A). This sucrose-dependent inhibitory effect was also observed in the shoot at 1 mM and became more pronounced with increasing sucrose concentrations up to 10 mM (Fig. 8B). In addition, the number and density of VAR03-1 colonies surrounding the Arabidopsis PR increased in a concentration-dependent manner (Fig. 8C). Although VAR03-1 colonies were distributed throughout the plate, likely due to their ability to utilize sucrose for growth, the highest colony density was observed in the upper-middle regions of the PR in sucrose-supplemented conditions, even at 10 mM (Fig. 8D).

To determine if this sugar-dependent growth inhibition was specific to sucrose, we evaluated the effects of different sugars (glucose, fructose, mannitol, and arabinose) on Arabidopsis growth in the presence of VAR03-1 (Fig. 8E). At a concentration of 10 mM, glucose, mannitol, and arabinose, but not fructose, exhibited statistically significant inhibitory effects on PR length. VAR03-1 colonies also increased in response to these sugars in a manner similar to sucrose (Supplementary Fig. S1).

We hypothesized that the overproliferation of VAR03-1 might contribute to the observed inhibition of Arabidopsis growth. To test this hypothesis, we evaluated Arabidopsis growth on MS agar in the absence of sucrose; instead, we supplemented it with higher concentrations of VAR03-1 (final OD^{600} of 5×10^{-3} and 5×10^{-2}, representing 10- and 100-fold increases, respectively, over the normal condition used in Fig. 6). High concentrations of VAR03-1 significantly reduced both PR length and shoot weight in Arabidopsis, even in the absence of exogenous sucrose (Fig. 8F–H). Although the inhibition observed under these conditions was less pronounced than that observed in the presence of sucrose, it yielded similar outcomes (Fig. 8H). Importantly, inoculation with heat-killed (HK) VAR03-1 did not impede Arabidopsis growth, indicating that the inhibitory effect is specifically associated with the activity of live bacterial cells (Fig. 8F, G). In sucrose-supplemented MS agar inoculated with VAR03-1, PR length inhibition began 4 days after the young Arabidopsis seedlings were planted (Supplementary Fig. S2). This finding suggests that a critical bacterial cell density, reached after 3 days, is required to exert a negative effect on Arabidopsis PR.

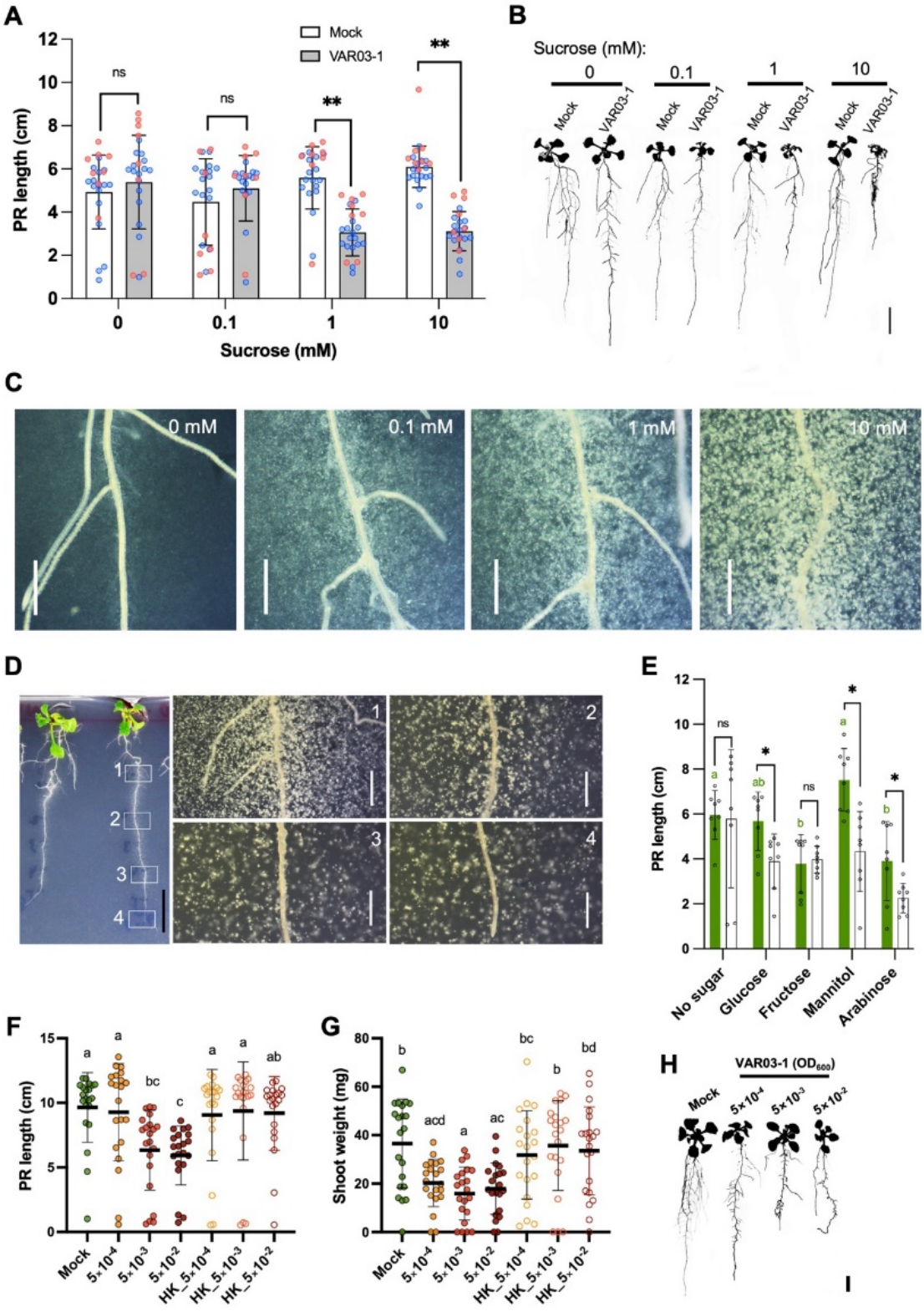

Figure 8. Effect of sucrose on Arabidopsis growth in MS agar mixed with *A. vitis* VAR03-1.

A, B Primary root (PR) length (**A**) and morphology (**B**) of Arabidopsis seedlings grown in MS agar containing VAR03-1 (OD_{600} = 5×10^{-4}) supplemented with sucrose at indicated concentrations (8 dpi). Red and blue dots represent separate experimental sets. Mann-Whitney U test (n = 24) was performed to compare differences between mock and VAR03-1 treatments in **A**. *ns*, not significant; **$p < 0.001$. **C** Colonies of VAR03-1 around the

upper region of Arabidopsis PR in MS medium supplemented with sucrose at indicated concentration. **D** Colonies of VAR03-1 in different parts of Arabidopsis PR in MS medium supplemented with 10 mM sucrose. Four spots along the PR axis were observed for higher magnification, as shown on the right sides with the indicated number. **E** Arabidopsis PR (8 dpi) of transplantation to MS agar containing VAR03-1 ($OD_{600} = 5\times10^{-4}$) supplemented with indicated sugars at 10 mM each. Welch's t-test ($n = 8$) was performed to compare the difference between mock and VAR03-1 treatments in each sugar treatment. *ns*, not significant; $*p < 0.05$. Green letters indicate significant differences analyzed using ANOVA test followed by Tukey's test ($n = 8$, $\alpha = 0.05$) to compare mock treatments of all sugar treatments. **F-H** length of PR (**F**), shoot weight (**G**), and morphology (**H**) of Arabidopsis seedlings grown in sucrose-free MS agar containing various concentrations of VAR03-1 (14 dpi). Different letters indicate statistical differences analyzed using Kruskal-Wallis followed by Dunn's test ($n = 21$) (**F**) and Brown-Forsythe ANOVA followed by Dunnett's T3 test ($n = 21$) (**G**). HK, heat-killed. Black and white bars represent 1 cm and 1 mm scale, respectively.

To further elucidate this point, we used two transposon-mediated random mutants, namely Δ65 and Δ86, which exhibited reduced inhibitory effects under supplementation of 1% sucrose (Bao, 2022). Upon supplementation with 10 mM sucrose, Δ86 completely lost its inhibitory effect on both root and shoot growth, whereas Δ65 showed a partial reduction, in which the inhibitory effect on shoot growth was largely reduced, but the effect on root growth remained comparable to the WT. Both mutants showed slower growth than the WT in KB medium (Supplementary Fig. S3C). In MS medium containing sucrose, Δ86 exhibited almost no growth, and Δ65 showed less growth, reaching only 24.2% and 63.5% of the growth of the WT strains, respectively, over a 16 h culture period (Supplementary Fig. S3D).

Regarding their colonization patterns, Δ65 exhibited a similar pattern to the WT, with visible colonies around the primary root (PR), and the colony density increased with sucrose supplementation (Supplementary Fig. S3E). In contrast, Δ86 showed no visible colonies around the PR, but appeared to be attached to the root surface (Supplementary Fig. S3E). The density of Δ86 colonies on the root surface progressively increased with 10 mM sucrose (Supplementary Fig. S3E). Higher magnification revealed that Δ86 produced a small number of colonies a short distance from the PR, with a relatively thicker bacterial layer observed only at 10 mM sucrose.

Using the plasmid rescue method, Bao (2022) identified the transposon insertion sites at Δ65 and Δ86 within the *cobJ* and *mprF* genes, respectively (Supplementary Fig. S3G, H). The *cobJ* gene encodes precorrin-3B methyltransferase, which is involved in the biosynthesis of cobalamin, a cofactor for several enzymatic reactions, including deoxynucleotide synthesis by cobalamin-dependent ribonucleotide reductase (Cowles et al., 1969). The *mprF* gene encodes the multiple peptide resistance factor (MprF), a bifunctional protein that synthesizes

aminoacyl phospholipids or ions and translocates them to the outer leaflets of bacterial membranes (Song et al., 2021). This function is crucial for bacterial survival against cationic antimicrobial peptides (CAMPs) (Ernst and Peschel, 2011) and adaptation to acidic environments (Sohlenkamp et al., 2007).

While it remains unclear whether the function of each causal protein directly influences the sucrose-dependent growth inhibition of Arabidopsis plants, the defective proliferation phenotypes in sucrose-containing medium were consistent with their diminished impact on Arabidopsis growth inhibition. Our results align with previous studies that screened for less colonizing bacterial mutants and identified causal genes related to metabolism (Cole et al., 2017; Sivakumar et al., 2019). Furthermore, the extent of the growth defects in both mutants corresponded to their respective effects. These results support the hypothesis that bacterial overgrowth is a primary cause of the inhibitory effect on Arabidopsis growth.

3.3.4 Bacterial-induced inhibition of Arabidopsis growth in the presence of exogenous sucrose exhibits non-strain-specific behavior

To assess whether the growth inhibition observed in the presence of sucrose was specific to VAR03-1, we extended our investigation to include two additional *A. vitis* strains, VAT03-9 and VAR06-30. Under sucrose-free conditions, all *A. vitis* strains, including the pathogenic VAT03-9, demonstrated a slight enhancement in primary root (PR) growth, indicating a generally consistent effect across strains regardless of their pathogenicity (Fig. 6E, G). In contrast, sucrose supplementation led to significant growth inhibition in both the root and shoot tissues of seedlings treated with any of the *A. vitis* strains, compared to the control conditions (Fig. 9A–C). Notably, the extent of growth inhibition varied among the strains: VAT03-9 exhibited more severe PR and shoot inhibition than VAR03-1, whereas VAR06-30 induced a comparable level of PR inhibition but caused significantly less shoot growth inhibition than VAR03-1.

In addition, *P. protegens* Cab57 was also evaluated. Under sucrose-free conditions, Cab57 caused a significant reduction in PR length (Fig. 6E, G). Upon sucrose supplementation, Cab57 displayed a concentration-dependent inhibitory effect on PR length, with increasing sucrose concentrations amplifying this effect (Supplementary Fig. S4A). While shoot growth was similarly affected by Cab57 across different sucrose concentrations, the growth patterns showed that at 10 mM sucrose, Cab57 induced more severe PR inhibition than any of the *A.*

vitis strains. However, its inhibitory effect on shoot growth was comparable to that of the *A. vitis* strains (Fig. 9A–C).

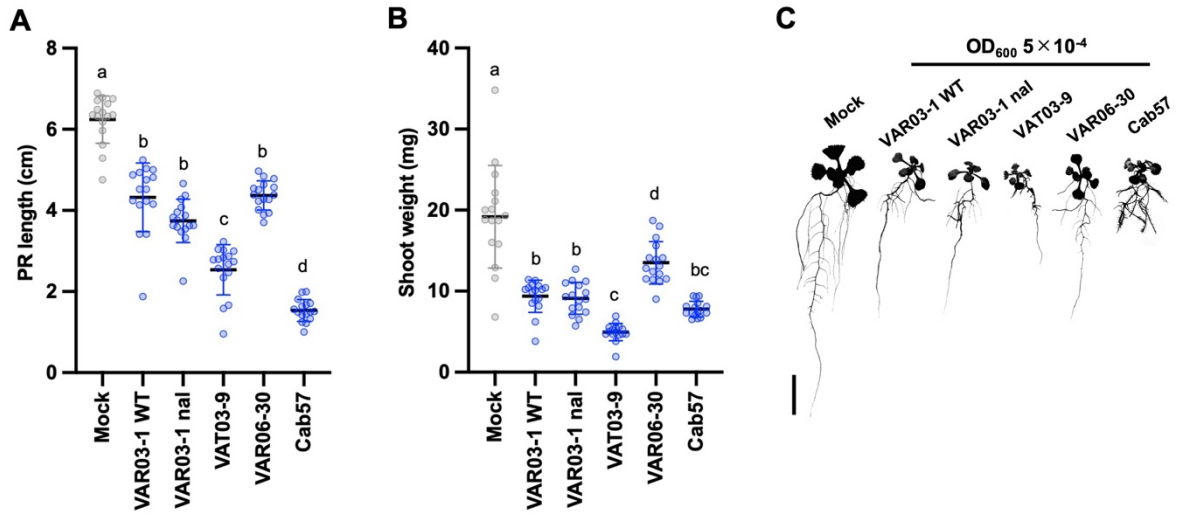

Figure 9. Bacterial-induced inhibition of Arabidopsis growth in presence of sucrose is not specific to VAR03-1.

Length of primary root (PR) (**A**), shoot weight (**B**), and morphology (**C**) of Arabidopsis seedlings grown in MS agar containing indicated bacterial strains ($OD_{600} = 5\times10^{-4}$) supplemented with 10 mM sucrose (8 dpi). Different letters indicate statistical significance analyzed using Brown-Forsythe ANOVA followed by Dunnett's T3 multiple comparison test ($n = 16$) (**A**) and ANOVA followed by Tukey's test ($n = 16$) (**B**). Bars, 1 cm.

Interestingly, while no visible colonies of Cab57 were detected on MS agar medium surrounding the PR under sucrose-free conditions, a small number of colonies were observed in the presence of sucrose, with the number of colonies observed increasing at higher sucrose concentrations (Supplementary Fig. S4C). At both 1 mM and 10 mM sucrose, Cab57 caused severe alterations in root morphology, including a pronounced reduction in PR length and an increase in lateral root formation. Notably, under 1 mM sucrose conditions, swelling was observed at the PR tip, which may be attributed to mucilage accumulation (Supplementary Fig. S4C–E). These observations suggest that the growth inhibitory effects induced by sucrose are not unique to VAR03-1, as similar inhibitory effects were evident across all tested *A. vitis* strains and *P. protegens* Cab57.

3.3.5 Relationship between nutrient requirements and bacterial growth patterns in the Arabidopsis rhizosphere

To further elucidate the relationship between nutrient requirements and bacterial colonization patterns in the Arabidopsis rhizosphere, we examined the effects of organic acid supplementation on the growth and colonization behavior of VAR03-1 and *P. protegens* Cab57.

We transplanted Arabidopsis seedlings onto MS agar medium containing 10 mM sucrose and a mixture of four organic acids—citrate, aspartic acid, p-hydroxybenzoate, and 5-oxo-proline—at varying concentrations (50, 100, or 200 μM of each organic acid). This experimental setup was designed to investigate how the combined presence of sucrose and organic acids influences bacterial growth and seedling development (Fig. 7F).

Our results demonstrated that the presence of organic acids exacerbated the inhibition of seedling growth in a concentration-dependent manner. Specifically, the growth of Arabidopsis seedlings was further suppressed by the addition of organic acids compared to the effect of sucrose alone (Fig. 10; Supplementary Fig. S5). In the case of VAR03-1, the inhibition of seedling growth was significantly amplified by organic acid supplementation, as shown by the increased severity of growth inhibition in both root and shoot tissues (Fig. 10A, top row). Although VAR03-1 colonies were present around the Arabidopsis PR in the presence of both sucrose and organic acids, there was no noticeable increase in colony density at any specific location along the PR due to organic acid supplementation (Fig. 10A; upper part, second row; lower part, third row; tip, fourth row). While direct quantification of bacterial titers in the agar medium could reveal changes in bacterial density, this method remains technically challenging.

For Cab57, we also observed growth inhibition in Arabidopsis seedlings due to organic acid supplementation, though statistical differences in PR length and shoot weight compared to sucrose alone were not detected (Fig. 10B; top row; Supplementary Fig. S5). Notably, at higher magnification, Cab57 colonies surrounding the PR and even in the agar medium away from the roots exhibited increased density and larger colony size with higher concentrations of organic acids (Fig. 10B; middle and bottom rows). These observations are consistent with the nutrient requirements for growth identified in liquid MS culture experiments (Fig. 7G). Our results indicate that both VAR03-1 and Cab57 exhibit distinct patterns of growth and colonization in the presence of sucrose and organic acids. For both bacterial species, the colony density was consistently higher around the plant roots compared to more distant areas of the agar medium, suggesting that additional plant-derived nutrients beyond sucrose and the tested organic acids are essential for their full growth in the rhizosphere.

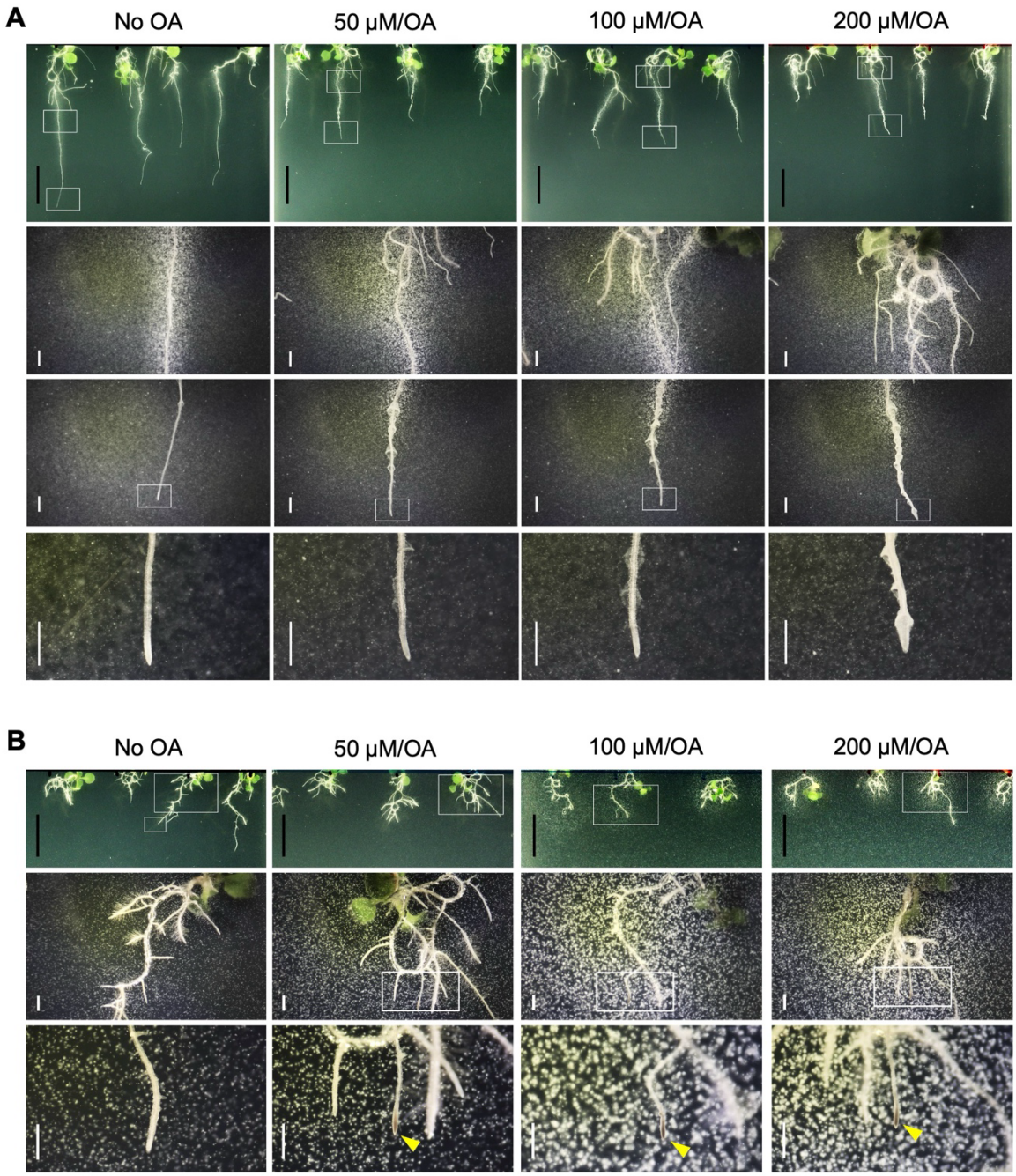

Figure 10. Growth and bacterial colonization of VAR03-1 and Cab57 in presence of sucrose and organic acids

VAR03-1 (**A**) and Cab57 (**B**) colonies around Arabidopsis seedlings in MS agar supplemented with 10 mM sucrose with or without a combination of four organic acids (citrate, aspartic acid, *p*-hydroxybenzoate, and proline 5-oxo) at different concentrations (50, 100, or 200 μM of each) (5 dpi). Seedling morphology and bacterial colonies on MS agar plate (First row), and the enlarged image at upper (second row) and tip part (third row) of primary root (PR). The fourth row in (**A**) provides a further magnified view of the PR tip of the boxes in the third row. Black bars, 1 cm. White bars, 1 mm. All experiments were repeated at least twice with similar results.

3.3.6 VAR03-1 induced Arabidopsis growth inhibition in the mutants for PTI

A trade-off between growth and defense is a typical causal factor for plant growth inhibition in response to microbial infection (Boller and Felix, 2009). Plant mutants that are impaired in the immune response corresponding to the causal factors should not exhibit inhibition as they do not elicit functional defense responses (Okada et al., 2021). We then investigated whether the inhibitory activity of VAR03-1 in presence of sucrose was associated with PTI. Arabidopsis mutants defective in elf18 recognition (*efr1*) and MAMP-triggered signaling (*bak1-5/bkk1-1* and *bik1*) were tested (Kunze et al., 2004; Lu et al., 2010; Roux et al., 2011). The results showed that all mutants displayed growth inhibition in response to VAR03-1 at levels similar to WT in the presence of sucrose (Fig. 11A, B). Supplementation of high concentrations of VAR03-1 in the absence of sucrose also resulted in the inhibition of PR and shoot of Arabidopsis mutants at least at $OD_{600}=5\times10^{-2}$, and in some cases at 5×10^{-3} (Fig. 11C, D). These results indicate that the inhibitory activity of VAR03-1 on Arabidopsis, regardless of the addition of exogenous sucrose, was not dependent on PTI, at least from the gene products tested.

Further investigation of the involvement of PTI in the interaction between VAR03-1 and Arabidopsis was carried out in a hydroponic culture system. This system allowed us to measure the root colonization of VAR03-1 and VAR03-1 populations in the medium (free-living cells). First, Arabidopsis PTI mutants were grown in sucrose-free liquid MS containing VAR03-1 at $OD_{600}=5\times10^{-4}$ (normal concentration) and 5×10^{-3} (high concentration) and tested whether inhibitory activity was also observed similarly to the agar system. Morphological analysis showed that the root and shoot weight of all Arabidopsis mutants treated with VAR03-1 at OD_{600} of 5×10^{-3} were reduced compared to WT, indicating consistent results with the agar system (Fig. 11C, D). Moreover, the *bak1-5/bkk1-1* mutant showed a more pronounced inhibitory effect than *efr1* or WT.

To investigate the reason for this difference, we measured the number of cells both on the root surface and in the liquid medium. Higher numbers of colonies were detected on the roots of *bak1-5/bkk1-1* compared to WT at both VAR03-1 concentrations (Fig. 11G). Additionally, increased colony numbers were observed in all plant genotypes at $OD_{600}=5\times10^{-3}$ compared to those at 5×10^{-4}. It is noteworthy that the increased population of VAR03-1 in the mutant was observed only on the root surface and not in the medium (Fig. 10H). This result implies that plant immunity, particularly that mediated by BAK1-BKK1, plays a specific role in regulating the VAR03-1 population in Arabidopsis roots. Regarding morphological

alterations, the *bak1-5/bkk1-1* mutant allowed VAR03-1 to colonize the roots at a higher level than the WT, and this increased population of VAR03-1 resulted in more severe growth inhibition in the mutant.

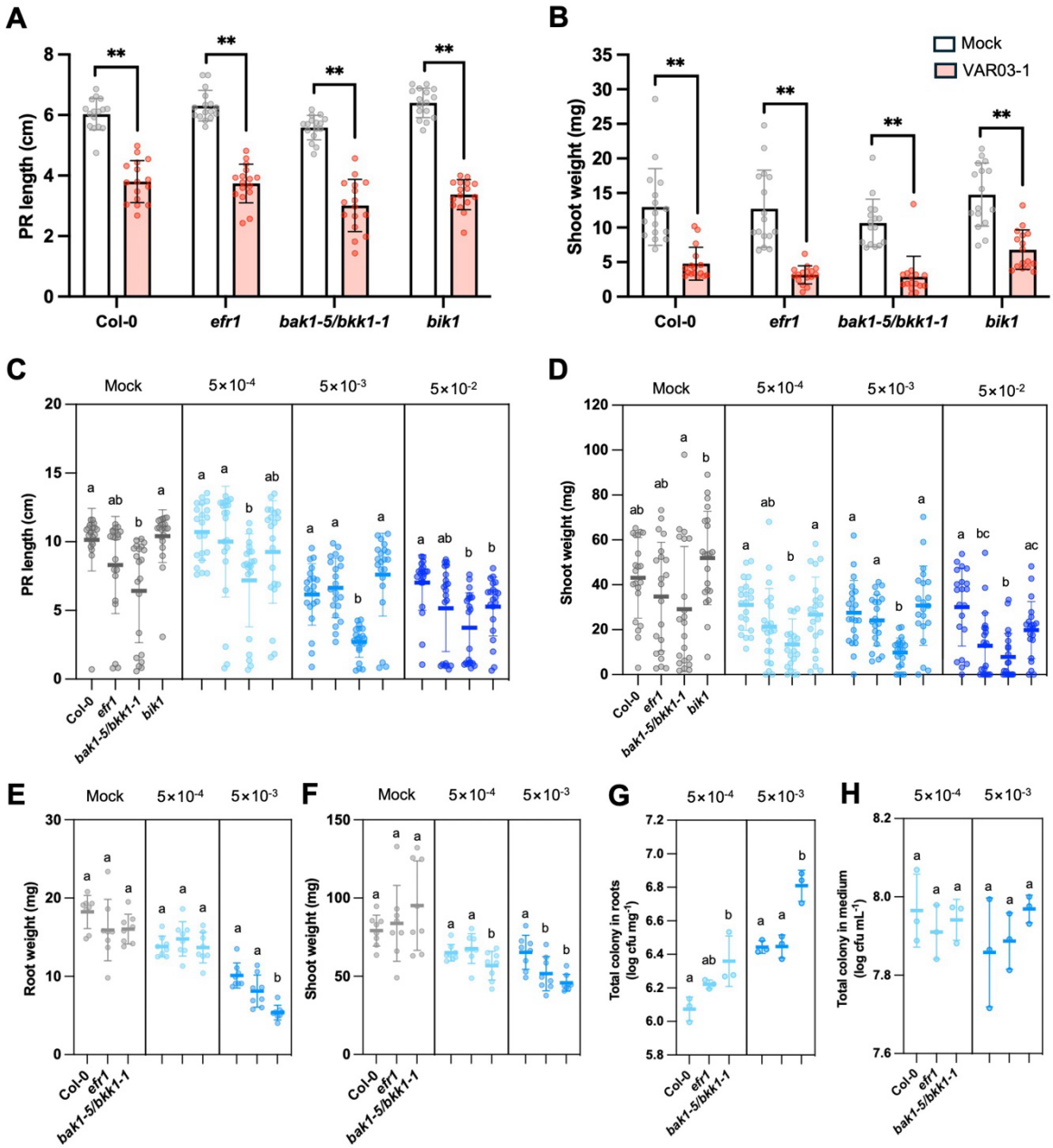

Figure 11. Effect of sucrose in the presence of VAR03-1 or high concentrations of VAR03-1 cells on the growth of Arabidopsis MTI mutants.

A, B Length of primary root (PR) (**A**) and shoot weight (**B**) of indicated Arabidopsis mutants grown in MS agar containing VAR03-1 (OD_{600} = 5×10^{-4}) supplemented with 10 mM sucrose (8 dpi). Statistical significance was analyzed using Student's t-test (n = 32) (**A**) and Mann-Whitney test (n = 16) (**B**). **C, D** PR length (**C**) and shoot weight (**D**) of indicated Arabidopsis mutants in sucrose-free MS agar containing VAR03-1 at different

concentrations (OD_{600}=$5\times10^{-4,}$ 5×10^{-3}, 5×10^{-2}) (14 dpi). Statistical significance was analyzed using Kruskal-Wallis followed by Dunn's comparison test (n = 21) (**C**) and Brown-Forsythe ANOVA followed by Dunnett's T3 (n = 21) (**D**). **E, F** Weight of root (**E**) and shoot (**F**) of indicated Arabidopsis mutants grown in sucrose-free hydroponic culture containing various concentrations of VAR03-1 (OD_{600} = $5\times10^{-4,}$ 5×10^{-3}) for 10 days. One-way ANOVA and Tukey's test (n = 8) were performed to determine statistical significance between treatments. **G, H** Colony performing units (CFU) on root (**G**) and medium (**H**) of indicated Arabidopsis mutants grown hydroponically in sucrose-free MS containing high concentrations of VAR03-1. Statistical significance in different genotypes for the same VAR03-1 concentrations and in different concentration samples for the same genotypes using two-way ANOVA and Tukey's test (n = 3). Letter and asterisks indicate significant differences. *ns*, not significant; * $p < 0.05$; ** $p < 0.001$.

3.3.7 Growth inhibition induced by VAR03-1 in Arabidopsis mutants for defense hormones

To further investigate the role of plant immunity in the interaction between VAR03-1 and Arabidopsis, we employed mutants with defects in salicylic acid biosynthesis and signaling (*npr1-5*, *sid2-2*, and *eds1-2*) as well as a mutant impaired in ethylene signaling (*etr1-1*). These mutants were tested for reduced inhibition of primary root (PR) growth and shoot weight when treated with VAR03-1 under sucrose-supplemented conditions. Our results showed that none of these mutants exhibited a significant decrease in the inhibitory effects compared to the WT, as measured by both PR length and shoot weight (Fig. 12A, B).

To further explore the involvement of plant immune responses, we measured the expression levels of *FRK1* and *PDF1.2*, which are marker genes for PTI, in both root and shoot tissues of Arabidopsis seedlings after 8 days of incubation on MS agar containing VAR03-1 and varying concentrations of sucrose. The expression of these genes was significantly higher in the presence of VAR03-1 compared to the uninoculated controls, with the exception of *FRK1* expression in roots where it was notably downregulated at both 1 mM and 10 mM sucrose (Fig. 12C, D). Specifically, *FRK1* was strongly induced in the shoots in response to VAR03-1 in the absence of sucrose, but this induction was markedly reduced at 1 mM and 10 mM sucrose. Conversely, *PDF1.2* expression was induced by VAR03-1 in both root and shoot tissues, and this induction was diminished in a concentration-dependent manner with increasing sucrose levels, showing a significant reduction even at 0.1 mM sucrose (Fig. 12D).

To assess the impact of sucrose on the plant's ability to mount a PTI response, we examined *FRK1* expression in response to the flg22 peptide in Arabidopsis roots grown on media containing different sucrose concentrations. Our results indicated that there was a non-significant reduction in *FRK1* induction in response to flg22 in the presence of 0.1 mM to 10

mM sucrose, compared to no sucrose treatment (Fig. 12E). This observation suggests that the suppression of plant growth observed in the presence of VAR03-1 under sucrose supplementation is not primarily due to alterations in the PTI response. Instead, it suggests that the inhibitory effects of VAR03-1 on Arabidopsis growth might be more closely related to the overproliferation of the bacteria rather than to activation of plant immune responses.

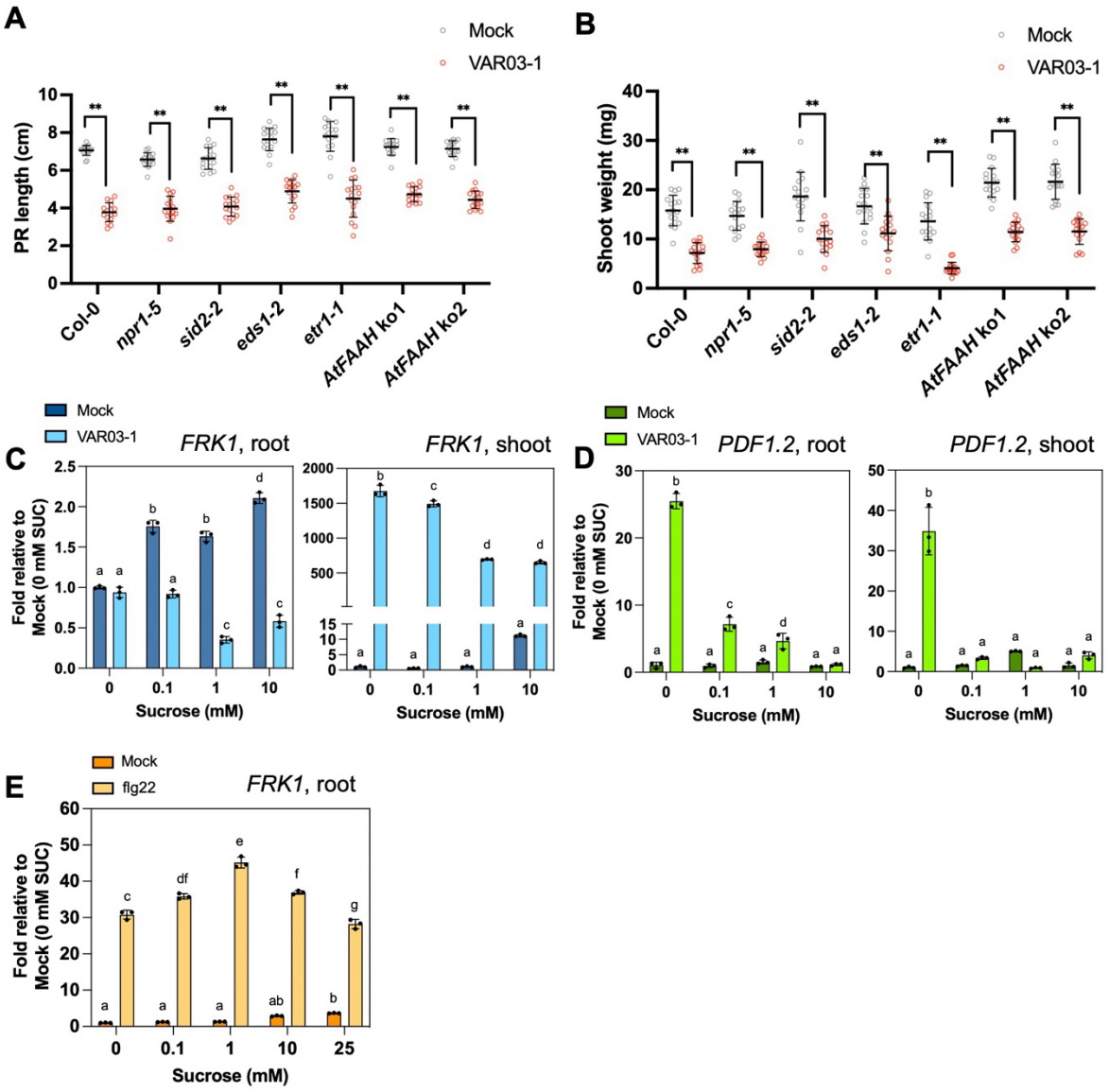

Figure 12. Effect of VAR03-1 on the growth of Arabidopsis mutants related to defense hormones and fatty acid metabolism in presence of sucrose.

A, B Primary root (PR) length (**A**) and shoot weight (**B**) of indicated Arabidopsis plants (8 dpi) in MS agar medium containing VAR03-1 ($OD_{600} = 5\times10^{-4}$), supplemented with 10 mM sucrose. Statistical significance was performed using Student's *t*-test (n = 16), ** $p < 0.001$. **C, D** Expression of *FRK1* (**C**) and *PDF1.2* (**D**) in shoot and root parts of Arabidopsis seedlings in VAR03-1-inoculated MS agar supplemented with indicated sucrose

concentration (8 dpi). **E** Expression of *FRK1* in roots of Arabidopsis seedlings 3 d after application of 0.5 µM flg22 in MS medium supplemented with different concentrations of sucrose. Statitsical analysis in **E-D** was carried out using ANOVA and Tukey's test (n = 3).

3.3.7 AHLs seem not to be involved in VAR03-1-induced Arabidopsis growth inhibition

Bacteria utilize *N*-acyl-L-homoserine lactones (AHLs) as signaling molecules to regulate population density and the transition from free-living states to biofilm formation (Verstraeten et al., 2008). It has been demonstrated that exogenous application of long-chain AHLs can inhibit Arabidopsis growth, a phenomenon attributed to increased ethylene production through the metabolic conversion of AHLs by the enzyme fatty acid amide hydrolase (*AtFAAH*) in Arabidopsis (Ortíz-Castro et al., 2008; Palmer et al., 2014). Specifically, growth inhibition by AHLs is diminished in *Atfaah* mutants and exacerbated in *AtFAAH* overexpressors, indicating that AHL-induced ethylene production is a key factor in this inhibitory effect (Palmer et al., 2014).

To investigate whether AHLs contribute to the growth inhibition induced by VAR03-1 in the presence of sucrose, we compared the growth responses of *Atfaah* mutants to those of WT under our experimental conditions. Both *Atfaah* mutants and WT exhibited similar reductions in root length on MS medium containing both VAR03-1 and sucrose (Fig. 12A, B). This finding suggests that AHLs produced by VAR03-1 do not significantly influence the growth inhibition of Arabidopsis.

The production of long-chain AHLs by *A. vitis* strains, including both virulent and non-virulent strains, has been linked to the *avsI* gene located on their chromosome (Li et al., 2006; Savka et al., 2011; Wang et al., 2008). Our confirmation of the presence of the *avsI* gene in the VAR03-1 genome (Noutoshi et al., 2020b) supports the hypothesis that VAR03-1 may also produce long-chain AHLs. Studies with *Pseudomonas aeruginosa* have shown that long-chain AHLs are generally impermeable through bacterial membranes (Pearson et al., 1999), and this may similarly apply to VAR03-1. Consequently, it is plausible that the external concentration of AHLs produced by VAR03-1 might not significantly affect Arabidopsis growth, despite the known growth-inhibitory effects of exogenously applied long-chain AHLs.

3.3.8 Involvement of *recA* gene in VAR03-1-induced inhibition of Arabidopsis growth in the presence of sucrose

Commensal bacteria establish biofilms on the root surface during colonization, with extracellular DNAs and polysaccharides serving as essential structural components for biofilm formation (Ravaioli et al., 2020; Verstraeten et al., 2008). This biofilm formation process is regulated by RecA, a key positive regulator of the SOS response pathway (Kaushik et al., 2022a; Turnbull et al., 2016; Wu et al., 2022). Previous studies have demonstrated that the loss of RecA function can diminish virulence in pathogenic bacteria (Aranda et al., 2011; Corral et al., 2020; Fuchs et al., 1999; Martínez et al., 1997). To explore whether biofilm formation contributes to VAR03-1-induced growth inhibition of Arabidopsis in the presence of sucrose, we investigated the effects of the *ΔrecA* mutant of VAR03-1 on Arabidopsis seedling growth in MS agar supplemented with sucrose.

Our experiments showed that the *ΔrecA* mutant induced significantly less growth inhibition in both root and shoot parts of Arabidopsis compared to the WT strain (Fig. 13A–C). Additionally, the growth rate of *ΔrecA* in liquid MS medium with sucrose was comparable to that of the WT strain (Supplementary Fig. S3D). Although not measured quantitatively, the colony density of *ΔrecA* around the PR appeared to be reduced compared to the WT strain, with an observed increase in cell layering on the root surface (Fig. 13D). To elucidate the underlying mechanism for this observation, we assessed the biofilm formation capability of the *ΔrecA* mutant. Contrary to our expectations, we found that *ΔrecA* exhibited enhanced biofilm formation compared to the WT strain (Fig. 13E). Furthermore, a significant reduction in the number of free-moving (planktonic) cells was observed in the *ΔrecA* mutant relative to the WT strain (Fig. 13F). These findings suggest that RecA negatively regulates biofilm formation in VAR03-1. The observed attenuation of sucrose-dependent growth inhibition of Arabidopsis seedlings in the *ΔrecA* mutant implies that the detrimental effects of the increased VAR03-1 population are more closely associated with a free-living lifestyle rather than a sessile biofilm state.

3.4 Discussion

In this study, we investigated the interaction dynamics between VAR03-1 and Arabidopsis under gnotobiotic conditions. VAR03-1 is a biocontrol agent for crown gall disease in grapevine. Biocontrol bacteria typically suppress disease through antibiotic activity against

pathogens and induction of disease resistance in host plants. In addition, competition for niche space in plant tissues plays a crucial role in this biocontrol mechanism. Therefore, a deeper understanding of the principles governing the rhizosphere colonization by biocontrol agents is essential for expanding the use of biocontrol strategies in sustainable and environmentally friendly agriculture.

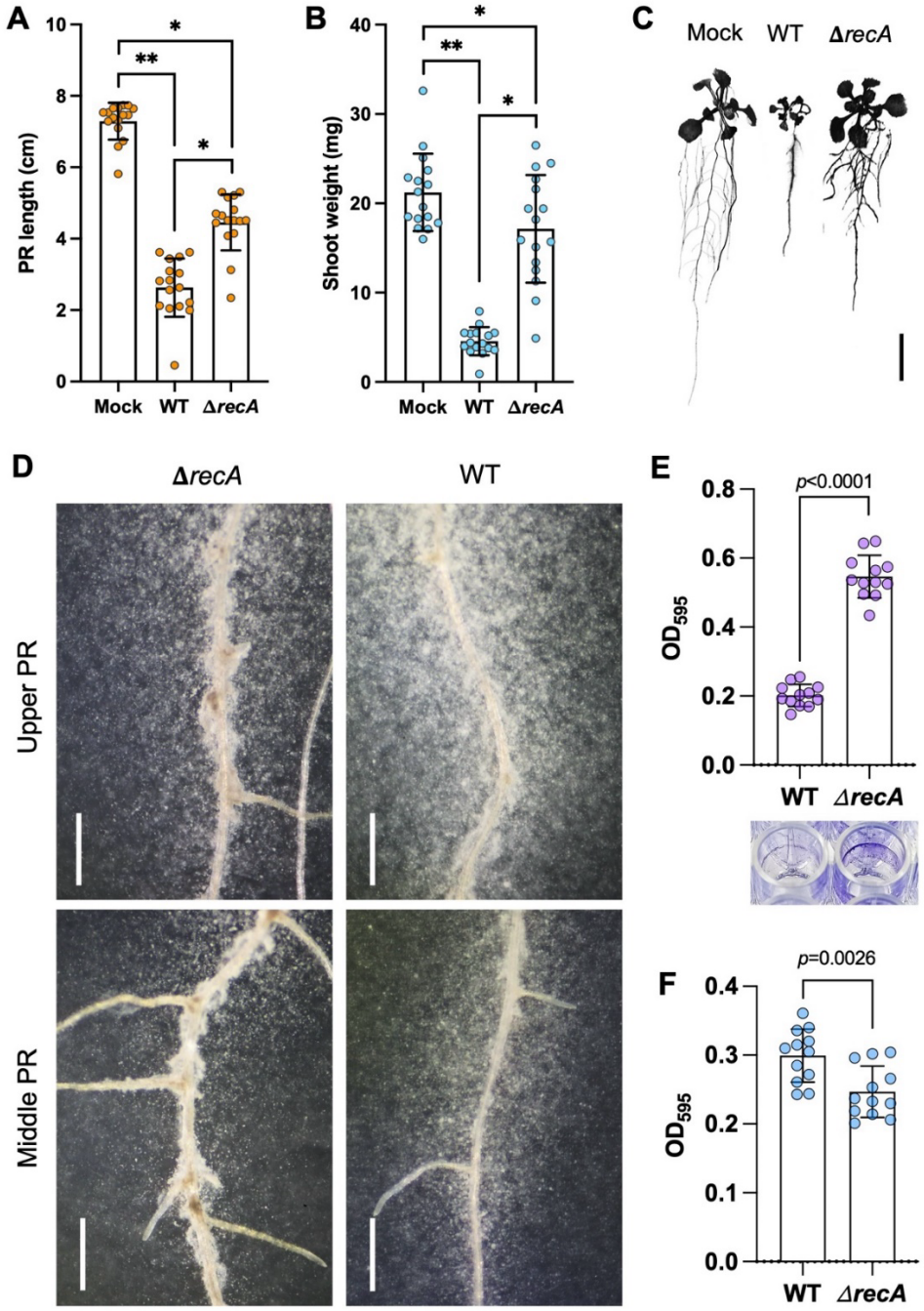

Figure 13. VAR03-1 *ΔrecA* showed a reduced inhibition effect on Arabidopsis growth in the presence of sucrose.

A-C Primary root (PR) length (**A**), shoot weight (**B**), and morphology (**C**) of Arabidopsis seedlings in 10 mM sucrose-containing MS agar medium inoculated with VAR03-1 WT or *ΔrecA* ($OD_{600} = 5\times10^{-4}$) (8 dpi). Statistical significance was analyzed using Kruskal-Wallis test followed by Dunn's test (**A**) and ANOVA followed by Tukey's test (**B**) ($n = 16$). * $p < 0.05$; ** $p < 0.001$; *ns*, not significant. Bar, 1 cm. **D** Colonies of VAR03-1 WT or *ΔrecA* ($OD_{600} = 5\times10^{-4}$) around the upper region (above row) and middle region (below row) of Arabidopsis PR in the 10 mM sucrose-containing MS agar medium (8 dpi). **E, F** Biofilm (**E**) and planktonic cells (**F**) of VAR03-1 *ΔrecA* and WT in biofilm formation assay for 6 days of incubation time.

We utilized Arabidopsis as a model system to investigate the interaction between VAR03-1 and plants. While *A. vitis* strains are commonly isolated from grapevines as the causative agents of crown gall disease, these strains have also been shown to protect other plants, such as apple, pear, rose, and sunflower, from similar diseases (Kawaguchi et al., 2007, 2008, 2012). This broader plant protection suggests that VAR03-1 can colonize plant species outside the Vitaceae family. Under our experimental conditions, VAR03-1 demonstrated the ability to significantly increase its population in the vicinity of the Arabidopsis root system and promoted elongation of the Arabidopsis primary root (PR). These observations indicate that VAR03-1 can establish a functional interaction with Arabidopsis, exhibiting a commensal-like relationship. This model system provides a useful framework for investigating the root colonization patterns of VAR03-1 and the dynamics of plant-microbe interactions, although the applicability of these findings to target crops needs to be validated through further research.

We compared the colonization behavior of two distinct bacterial biocontrol agents, VAR03-1 and Cab57, which belong to different genera, in the Arabidopsis rhizosphere. Surprisingly, the colonization patterns of these bacteria in the root zone differed significantly. This variation appears to be related to their distinct nutrient requirements. Recent research by (Loo et al., 2024) demonstrated that the composition of Arabidopsis root metabolites varies along the root length, with SWEET transporters facilitating sugar sequestration more prominently in the regions closer to the shoot compared to the root tip. This differential distribution of sugars, including sucrose, creates a concentration gradient in our experimental setup. Since sucrose and other sugars are key limiting factors for VAR03-1 growth, the bacterium formed visible colonies where sugar concentrations met its growth demands. These results are consistent with previous studies showing that root exudates, particularly sucrose, can significantly influence the dynamics of microbial communities in the rhizosphere during the early vegetative stages of plants (Lopes et al., 2022).

The supplementation of sugars to the medium facilitated the proliferation of VAR03-1, resulting in an elevated number and density of colonies even at positions distant from

Arabidopsis roots, although a population gradient favoring the upper root regions was still evident. This finding suggests the presence of additional limiting factors, such as other root exudates, that influence VAR03-1 growth. This notion is supported by our observations of differential effects among *A. vitis* strains on PR length and shoot weight under 10 mM sucrose conditions, which cannot be explained solely by growth rates in sucrose-containing liquid MS medium. Variations in the necessity for organic acids and other metabolites may contribute to the observed host specificity and competition within the rhizosphere microbial community (McLaughlin et al., 2023; Shimasaki et al., 2021).

For example, the colonization pattern of Cab57 was notably different from that of VAR03-1. Cab57 predominantly colonized the root surface but showed limited growth in the surrounding PR area. This observation aligns with the fact that organic acids, which are released predominantly from the root tips (Loo et al., 2024), support the Cab57 growth. Indeed, Cab57 began to form visible colonies near the roots and exhibited significant growth at the root tips when 1 mM sucrose was added. However, Cab57 could not form visible colonies in the vicinity of the PR without sucrose, suggesting that Arabidopsis may limit the availability of nutrients necessary for Cab57 growth, potentially due to its negative effects on plant growth. This concept is supported by previous reports indicating that the transcriptional regulation of SWEET transporters in root tissues is closely linked to pathogen infection (Loo et al., 2024; Zhou et al., 2023) and that plants modulate the root exudate composition in response to microbial infection (Gu et al., 2016).

Our study suggests a relationship between the habitat preference of commensal bacteria and their nutrient requirements in the rhizosphere. However, further analysis is needed to confirm whether this relationship occurs in soil environments. A previous study demonstrated a skewed distribution pattern of bacterial communities associated with particular genera in the apical, middle, and rear parts of root sections in the rhizosphere of *Brachypodium distachyon* (Wei et al., 2021). For the biocontrol of plant diseases, potential antagonistic microorganisms belonging to the same genus as the pathogens have been empirically used. For instance, VAR03-1 can effectively suppress crown gall caused by the pathogenic *A. vitis* (Couillerot et al., 2009). Our study provides evidence supporting this experiential knowledge. Given the variability in habitat preference of soil bacteria based on their metabolic characteristics, as demonstrated in this study, candidate biocontrol agents for particular diseases should be selected from microbes with antagonistic activity belonging to the same genus as the target pathogen.

Some strains of *P. fluorescens* have been identified as effective biocontrol agents for several crop diseases (Couillerot et al., 2009). *P. protegens* strains, including CHA0, Pf-5, and Cab57, provide protection to various crops against fungal pathogens and oomycetes (Défago et al., 1990; Pfender et al., 1993; Takeuchi et al., 2014). The colonizing nature of *P. fluorescens* has already been shown to be important for the protection of Arabidopsis from *Pseudomonas* pathogens (Wang et al., 2022). Particularly for soil-borne pathogens, these bacteria may offer crop protection, particularly in an epiphytic or endophytic manner near the root tip.

A study by (Burr et al., 1995) discovered that *A. vitis* survived in decaying grapevine roots and canes buried in the soil for 23 months. Most of the decaying plant material consisted mainly of cellulose and hemicellulose, which decomposed into their monomeric sugars within a few months (Gunina and Kuzyakov, 2015). The metabolic features of *A. vitis* strains may contribute to their long-lasting survival. Additionally, they may play a role in the grapevine-associated microbiome by producing various metabolites after consuming sugars.

Sucrose supplementation resulted in the suppression of host growth by both VAR03-1 and Cab57. A study by Liu et al., (2012)in an aquatic ecosystem demonstrated similar results, indicating that changes in nutrient availability that favor bacterial growth can alter the interaction between aquatic algae and bacteria, transitioning their relationship from commensalism to competition. This shift leads to a decrease in algal biomass, driven by the increased proliferation of bacterial populations and subsequent competition for nutrients. Similar nutrient depletion by the overgrown bacteria may occur in our experimental setting. Alternatively, bacterial-derived molecules other than AHLs, such as bacterial toxins, could contribute to this inhibition (Bolton et al., 1989; Stroo et al., 1988).

In our results, the *ΔrecA* mutant of VAR03-1 reduced its harmful activity to Arabidopsis in the presence of sucrose. This reduced activity appears to correlate with an increased rate of biofilm formation. The effect of RecA on biofilm formation varies between bacterial species. For instance, the loss of *recA* leads to decreased biofilm formation in *Escherichia coli* (Beloin et al., 2004; Recacha et al., 2019), *Streptococcus mutans* (Inagaki et al., 2009), and *Bacillus cereus* (Gao et al., 2020), while it increases biofilm formation in *Acinetobacter baumannii* (Ching et al., 2024) and *P. aeruginosa* (Yahya et al., 2023). As demonstrated in *Staphylococcus lugdunensis* (Ravaioli et al., 2020), the biofilm formation in *A. vitis* may depend more on EPS rather than eDNA from spontaneously bursting cells via RecA activity. The increased biofilm formation observed in the *ΔrecA* mutant of VAR03-1 could be due to an increase in EPS or a behavioral change.

Nevertheless, our study suggests that the growth inhibitory activity caused by the increased population of VAR03-1 in the rhizosphere is more dependent on the biological activity associated with its free-living style. Disturbances affecting persistence and stability of commensalism with host plants, such as sucrose supplementation, should provide clues to understanding the molecular mechanisms and biological significance underlying commensalism. Simplified and controllable experimental settings, in addition to various studies involving synthetic communities or soil systems, should be employed to achieve this goal.

CHAPTER 4: General Discussion and Conclusions

Plants harbor a diverse range of microbes throughout their lifetimes, including pathogenic, commensal, and mutualistic organisms. Notably, lifestyle transitions along the parasitic-mutualist continuum can occur during evolution (Drew et al., 2021). In bacteria, such transitions have been observed during rapid evolution, as evidenced by *P. protegens* CHA0, which underwent significant changes within only six months (Li et al., 2021). Horizontal gene transfer (HGT) and homologous recombination play crucial roles in facilitating these lifestyle transitions (Goyal, 2022; Melnyk et al., 2019). For example, in *Pseudomonas* sp. N2C3, the gain and loss of pathogenicity genomic islands, including lipopeptide/quorum sensing islands, have been shown to result in transitions between commensal and pathogenic states (Melnyk et al., 2019). Similarly, rapid evolution in *P. protegens* CHA0 has shifted its lifestyle from pathogenic to mutualistic, enhancing its catabolic capabilities to utilize diverse carbon sources and thus conferring competitive advantages over the ancestral strain (Li et al., 2021). The transition from a pathogenic to a commensal or mutualistic lifestyle can benefit the host plant, because these microbes can compete with pathogens for limited resources due to their similar ecological niches (Kinkel and Lindow, 1997).

A. vitis VAR03-1 is a non-pathogenic strain. Whole-genome analysis has revealed that this strain lacks the Ti plasmid and does not contain genes associated with virulence (Noutoshi et al., 2020b). In addition, VAR03-1 shows biocontrol activity against crown gall disease in grapevine (Kawaguchi et al. 2007). Previous research in our laboratory identified rhizoviticin, a phage-like particle, as the primary antagonistic factor of VAR03-1 against Ti strains in vitro (Ishii et al., 2024).

Our study shows that while rhizoviticin is the main factor contributing to the biocontrol activity of VAR03-1 in plants, it is not the only one. VAR03-1 tends to colonize the upper part of the Arabidopsis PR, an area likely to be rich in sucrose. This finding is consistent with research by Loo et al. (2024), which suggests that sugars are more concentrated in the upper PR, while organic acids are more abundant at the root tip. All *A. vitis* strains studied, including the Ti strain (VAT03-9), showed a preference for sugars over organic acids, which may explain their tendency to colonize the upper PR (Fig. 14A).

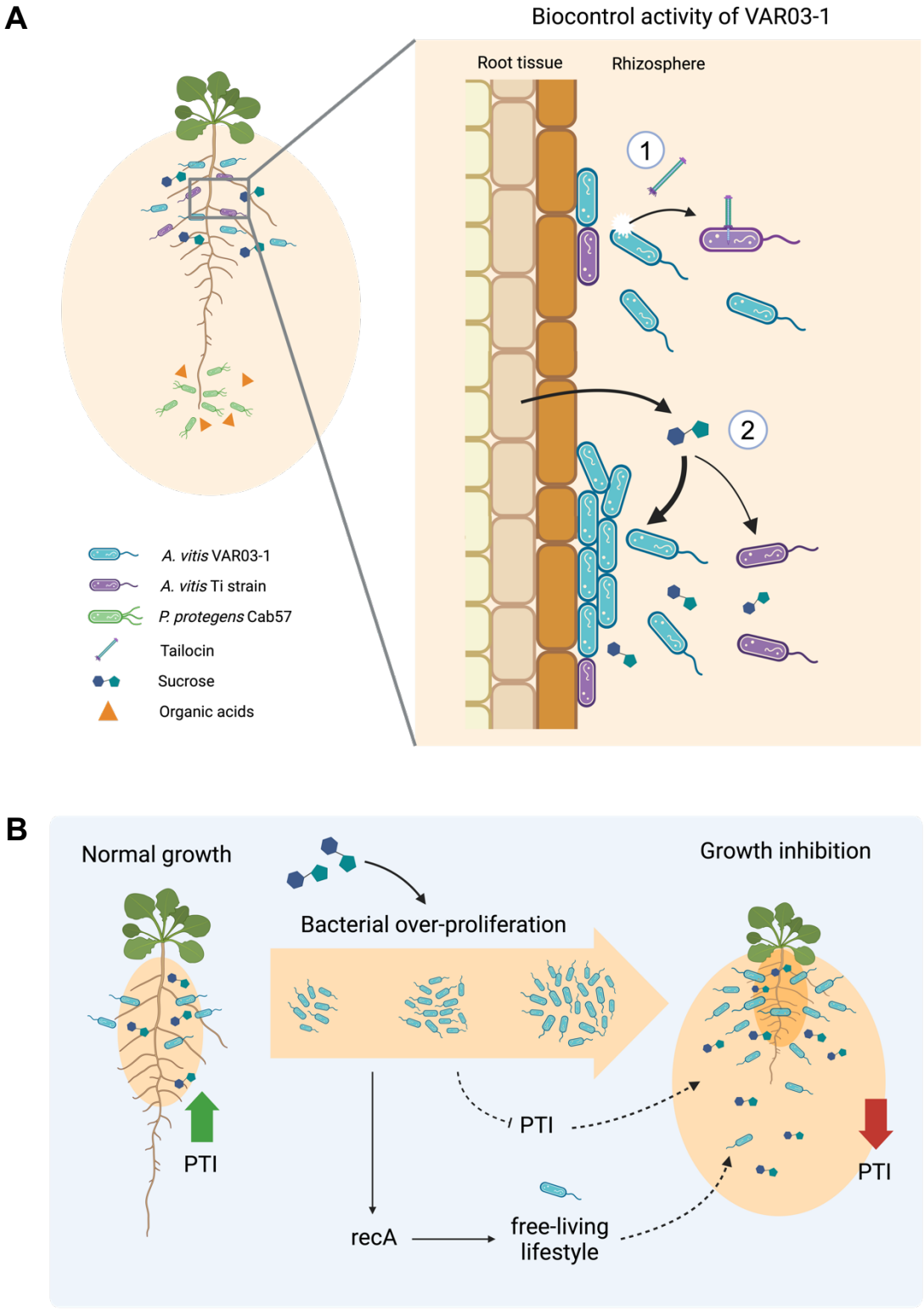

Figure 14. Mechanisms of action of VAR03-1's biocontrol activity.

A Two strategies of biocontrol activity of VAR03-1. VAR03-1 shares similar nutrient requirements to the Ti strainss, relying more heavily on sugars than organic acids for growth. In Arabidopsis roots, sugars are enriched in the upper part of the primary root (PR), the preferred colonization site for *A. vitis* strains, including VAR03-1

and VAT03-9 (Ti). In contrast, bacterial strains that require more organic acids than sugars, such as *P. protegens* Cab57, tend to colonize the lower PR, including the PR tip. As a result, competition for nutrients and space between VAR03-1 and the Ti strain is likely to be inevitable. VAR03-1 outcompetes the pathogen primarily through the production of rhizoviticin (1), which is toxic to the Ti strain. Tailocin serves as the main weapon in this competition. In addition, VAR03-1 outcompetes the Ti strain due to its superior sucrose catabolism (2), which is probably abundant in the upper part of the root. **B** Impact of excess nutrients on VAR03-1 and its interaction with Arabidopsis. In MS medium, VAR03-1 relies on Arabidopsis-derived nutrients for growth. Under these conditions, Arabidopsis recognizes VAR03-1 in the root vicinity by triggering an increase in PTI responses. This may lead the plant to limit nutrient release to control the VAR03-1 population around the roots and maintain a balanced interaction. However, when exogenous nutrients are available, VAR03-1 utilizes them to increase its population, leading to over-proliferation. This excessive population of VAR03-1 induces growth inhibition in Arabidopsis. While over-proliferation suppresses the PTI response, the observed growth inhibition is independent of the plant's immune response. During nutrient-induced over-proliferation, RecA facilitates a more free-living (planktonic) lifestyle. The biological activities associated with the free-living cells may be related to the observed growth inhibition in Arabidopsis. Images were created with BioRender.com

These shared habitat preferences and nutrient requirements imply inevitable competition between VAR03-1 and pathogenic strains. This idea is supported by Russel et al. (2017), who found that antagonism correlated with phylogenetic proximity and increased with greater metabolic similarity. Even rhizoviticin-deficient mutants of VAR03-1 retained partial antitumorigenic activity, highlighting their competitive advantage, likely due to higher metabolic activity compared to the Ti strain. This advantage is evident in Fig. 7G, where VAR03-1 showed superior growth over the Ti strain in sucrose-supplemented media. The ability to thrive in sugar-rich environments is crucial for colonization, particularly in the upper PR where sugars are more abundant. Our observations confirm that VAR03-1 forms more abundant colonies in this region compared to the Ti strain or VAR06-30, which has slower growth on sucrose (Fig. 6D). This finding is consistent with studies by Li et al. (2021) and Russell et al. (2017), emphasizing that enhanced catabolic capacity is essential for competitiveness against closely related strains (Fig. 14A).

In our experimental setup using MS medium without additional sugars, we confirmed the establishment of VAR03-1 colonies in the upper part of the Arabidopsis PR, relying on root-derived nutrients. In this condition, VAR03-1 slightly promoted Arabidopsis PR growth without affecting shoot growth, indicating a balanced interaction between VAR03-1 and Arabidopsis. Since VAR03-1 depends on root-derived nutrients, plants can control the bacterial population around the roots by limiting carbon secretion. Studies have shown that plants modulate root exudation via SWEET transporters upon microbial recognition (Chen et al.,

2015; Loo et al., 2024). Alternatively, VAR03-1 populations can be controlled by activating plant immunity, as VAR03-1 induces PTI responses as evidenced by increased expression of PTI gene markers in both roots and shoots. This activation of plant immunity affects bacterial physiology, including iron metabolism, by suppressing siderophore biosynthesis, leading to bacterial growth restriction (Nobori et al., 2018).

This study highlights the importance of nutrient limitation for VAR03-1. Upon exogenous sucrose addition, VAR03-1 overproliferated and subsequently inhibited the growth of Arabidopsis seedlings. Our data suggest that this inhibition is independent of plant immunity, in contrast to the growth-defense trade-off where growth inhibition results from strong induction of plant immune responses (Boller and Felix, 2009). Given that VAR03-1 lacks genes associated with virulence, we hypothesize that the overproliferated population may indirectly induce this inhibition. One possibility is the production of AHLs, as suggested by Ortíz-Castro et al. (2008) and Palmer et al. (2014); however, our results showed that AHLs did not directly contribute to this inhibition. Instead, our data indicated that RecA played a role in this inhibition, likely by promoting a free-living rather than a sessile lifestyle for VAR03-1 (Fig. 14B). Free-living (planktonic) and sessile cells have distinct gene expression profiles, including virulence genes (Dar et al., 2021; Levipan and Avendaño-Herrera, 2017; Oggioni et al., 2006). For instance, planktonic and sessile cells of *Streptococcus pneumoniae* induce different diseases in humans (Oggioni et al., 2006). A detailed study by by Dar et al. (2021) using single-cell transcriptome imaging revealed that planktonic cells of *P. aeruginosa* express more rhamnolipid (*rhlA*) and *lasA* than biofilm (sessile) cells. RhlA and lasA are part of the *rhl* and *las* quorum sensing systems responsible for the induction of virulence factors in *P. aeruginosa* (Ahmed et al., 2019).

Nevertheless, sucrose itself has been reported to affect the physiology of bacteria. For example, in *Ralstonia solanacearum*, sucrose serves as one of the major catabolic sources for virulence in the xylem sap (Hamilton et al., 2021). In *P. aeruginosa*, host-derived sucrose induces pellicle production through sigma factor SigX, which is important for virulence (Bouffartigues et al., 2014). In *Xanthomonas campestris*, sucrose also induces the biosynthesis of the diffusible signal factor and pathogenicity (Schulte and Bonas, 1992; Zhang et al., 2019). In a nonpathogenic bacterium, *Bacillus subtilis*, sucrose has been reported to enhance bacterial colonization by promoting motility and biofilm formation (Tian et al., 2021). Considering these studies, although our current data only showed that sucrose affected VAR03-1 proliferation, there should be more physiological effects induced by sucrose in VAR03-1. At least our transposon mutants provide evidence for sucrose-induced physiological changes in VAR03-1

that may involve *cobJ* and *mprF* genes. Further research exploring the mechanisms of these two genes in the involvement of sucrose-induced inhibitory activity of VAR03-1 should provide more insight into the VAR03-1 colonization mechanism and its effect on the host plant.

In conclusion, this study has identified several mechanisms through which VAR03-1 functions as a biocontrol agent. One key mechanism involves rhizoviticin as the primary biocontrol factor, while another relates to its colonization properties on the host. Bacterial colonization patterns in the host rhizosphere are influenced, at least in part, by their nutrient requirements. This finding provides evidence that non-pathogenic microorganisms of the same genus and species as pathogens often exhibit antagonistic activity in the field.

Supplementary Information

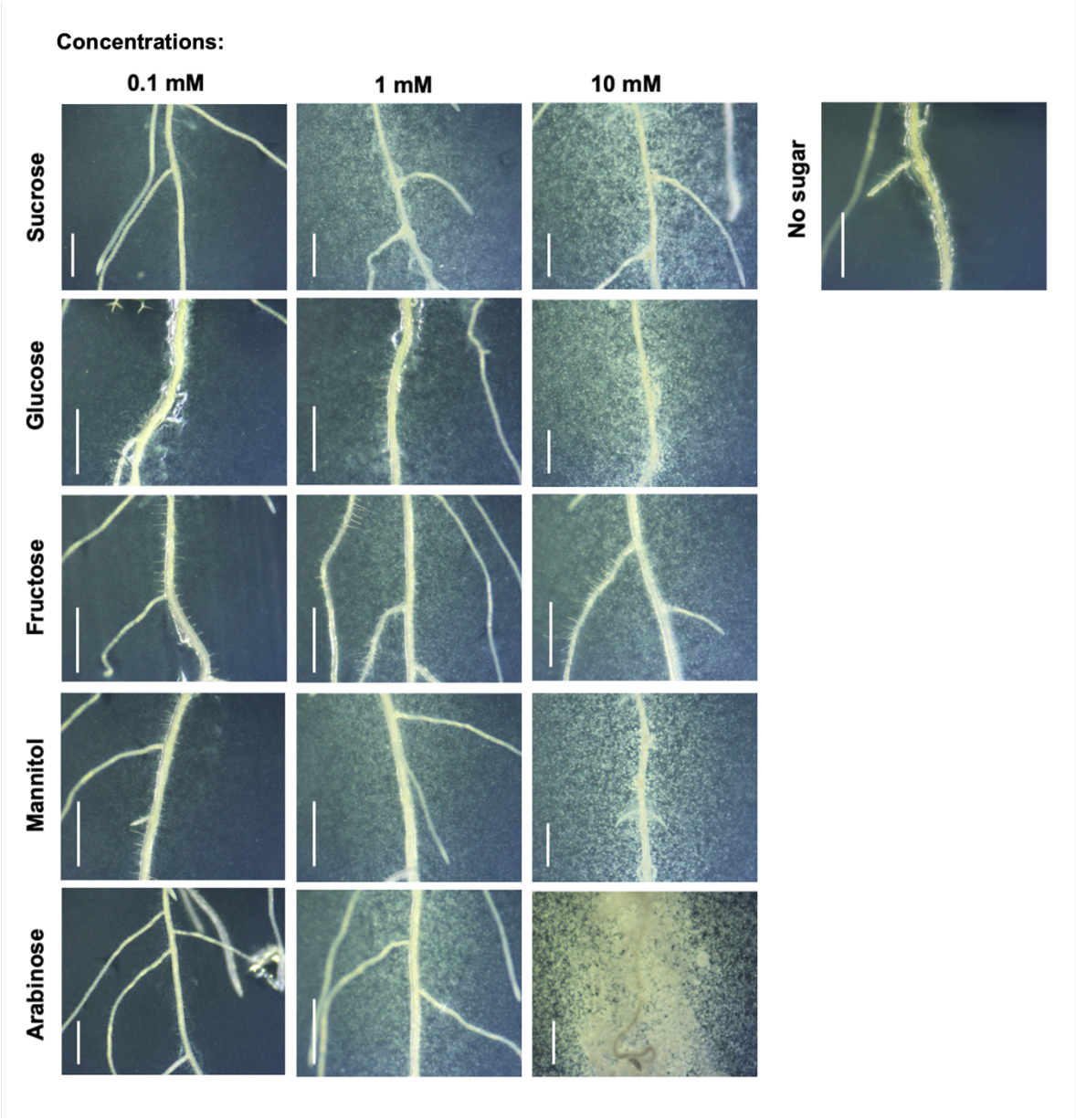

Figure S1. Colonies of VAR03-1 around the upper region of Arabidopsis PR in MS agar containing sugars.

Arabidopsis seedlings were transferred to MS agar containing VAR03-1 ($OD_{600} = 5\times10^{-4}$) supplemented by various sugars at indicated concentrations. Colonies were observed 8 d after transplantation. These images were taken from the same experimental set in Fig. 6E.

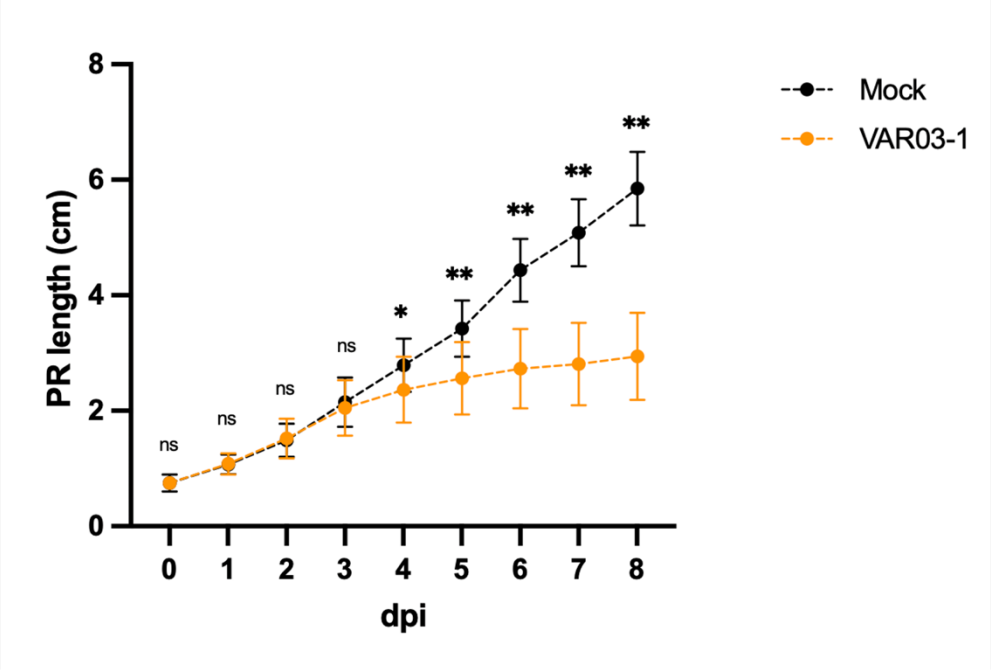

Figure S2. Arabidopsis root inhibition induced by VAR03-1 in the presence of sucrose.

Arabidopsis seedlings were transferred to MS agar containing VAR03-1 ($OD_{600} = 5\times10^{-4}$) supplemented with 10 mM sucrose. Primary root (PR) was measured at indicated time points (days after inoculation, dpi). Student *t*-test ($n = 16$) was performed to compare the significant differences between mock and VAR03-1 treatment at each time point. *ns*, not significant; * $p < 0.05$; ** $p < 0.001$.

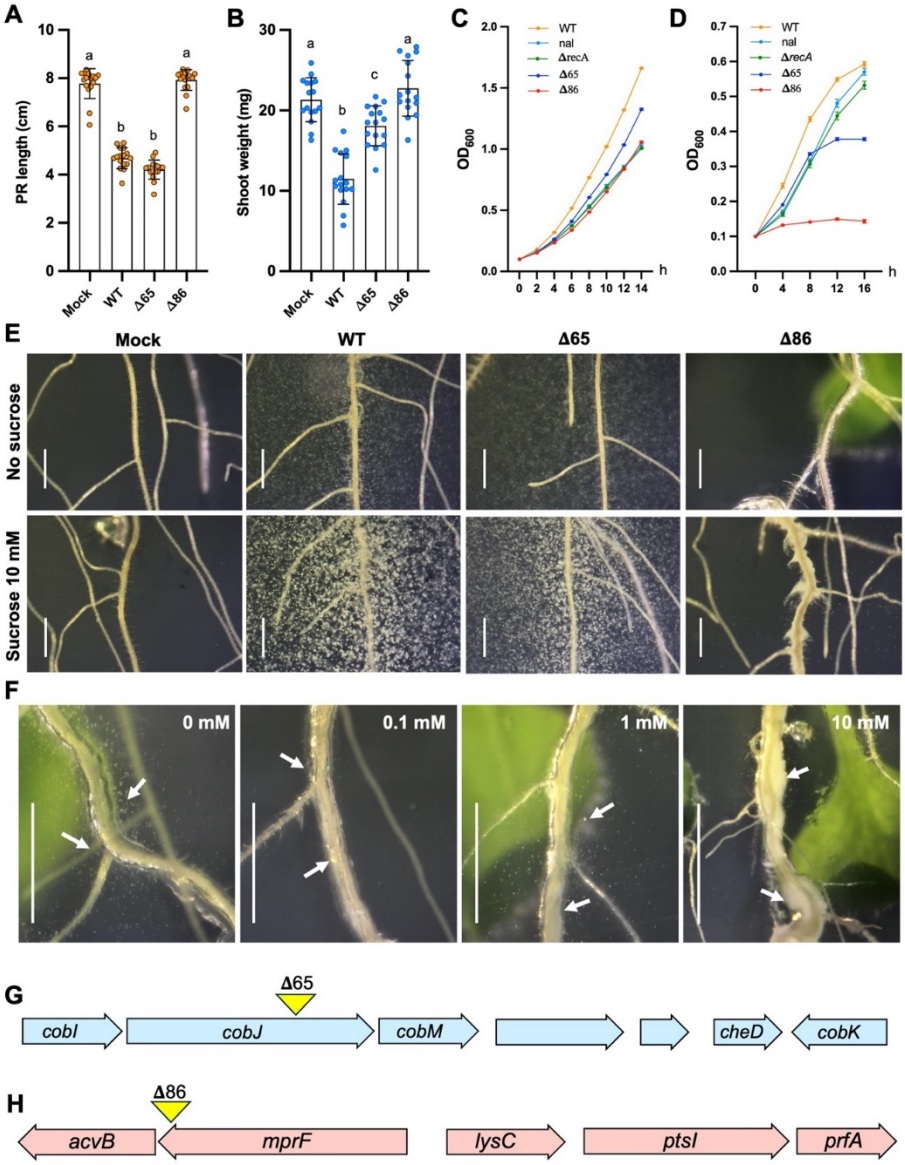

Figure S3. Transposon-insertional mutants of VAR03-1 exhibited partial and complete abolishment of growth inhibition effect on Arabidopsis.

A, B Seedlings were transferred to MS agar containing VAR03-1 strains (WT, Δ65, or Δ86, $OD_{600} = 5\times10^{-4}$ each). Primary root (PR) (**A**) and shoot weight (**B**) of Arabidopsis were measured at 8 dpi. Statistical significance was analyzed using Kruskal-Wallis test followed by Dunn's test (**A**) and ANOVA followed by Tukey's test (**B**) (n = 16). * $p < 0.05$; ** $p < 0.001$; *ns*, not significant. **C, D** Bacterial growth curve of VAR03-1 (WT, nal (nalidixic acid resistance naturally occurring mutant), *ΔrecA,* Δ65, and Δ86) in liquid KB medium (**C**) and 1% sucrose-containing liquid MS medium (**D**), respectively. Each bacterial isolate was inoculated ($OD_{600} = 0.1$) and the OD_{600} was measured at the indicated time points during culturing. **E** Colonies of VAR03-1 (WT, Δ65, and Δ86) around the upper PR regions of Arabidopsis roots (8 dpi) in MS agar medium containing with or without 10 mM sucrose. **F** Colonies of VAR03-1 (Δ86) around the upper PR regions of Arabidopsis roots (8 dpi) in MS agar medium containing sucrose at indicated concentrations (0.1, 1, and 10 mM). **G, H** Schematic representation of the transposon-insertion positions in the genome of VAR03-1 Δ65 (**G**) and Δ86 (**H**).

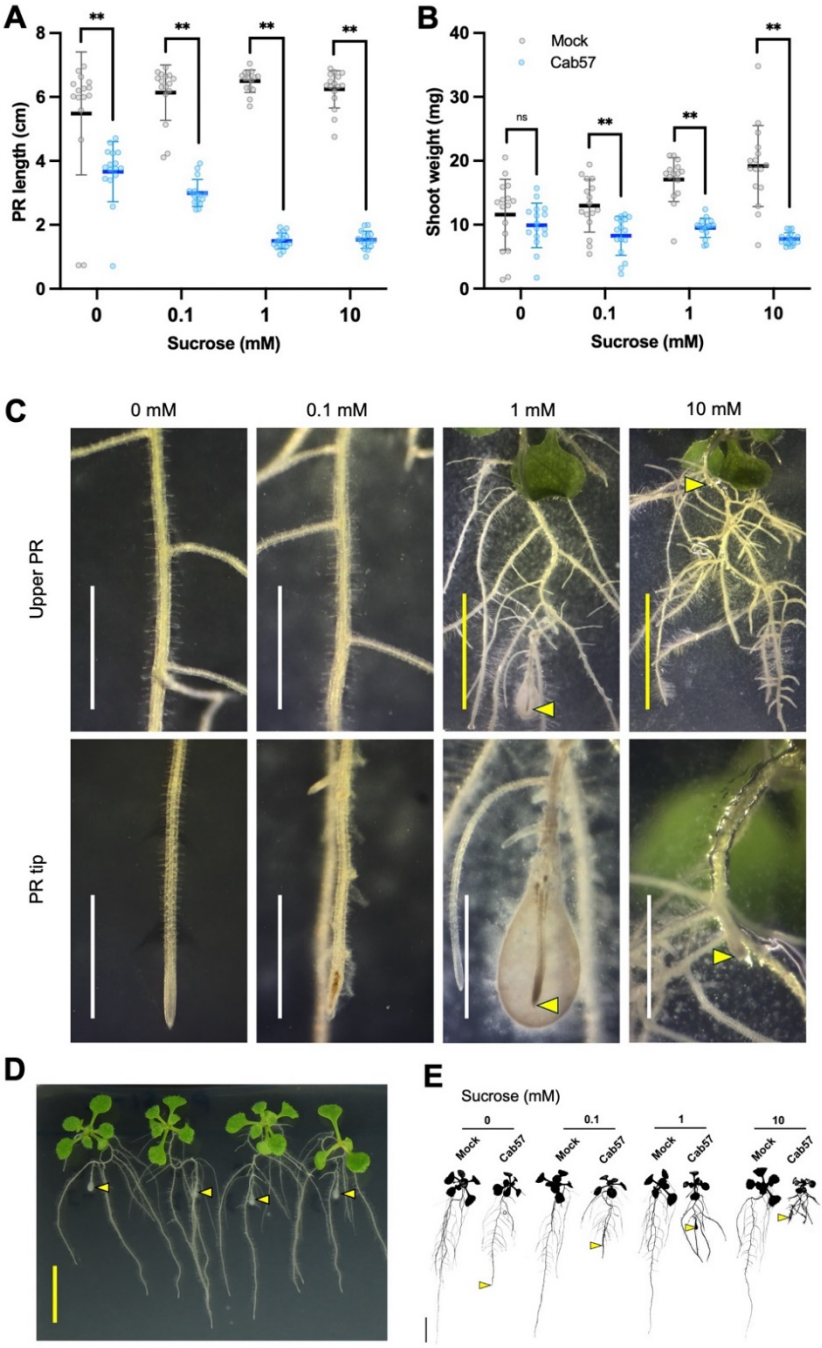

Figure S4. Effect on *P. protegens* Cab57 on Arabidopsis growth in the sucrose-containing MS agar.

A, B Primary root (PR) length (**A**) and shoot weight (**B**) of Arabidopsis plants 8 d after transplanting on MS agar medium containing different concentrations of sucrose (0.1, 1, and 10 mM) inoculated with Cab57. Statistical significance was analyzed using Mann-Whitney test (**A**) and Student's *t*-test (**B**) ($n = 16$). ** $p < 0.001$ **C** Colonies of Cab57 around the upper PR regions (*top*) and root tips (*bottom*) of Arabidopsis plants 8 d after transplanting on MS agar medium containing different concentrations of sucrose (0.1, 1, and 10 mM). **D** Morphology of Arabidopsis seedlings 8 d after transplanting on 1 mM sucrose-containing MS agar medium inoculated with Cab57. Yellow triangles indicate PR tips with swelling. **E** Morphology of Arabidopsis seedlings 8 d after transplanting on Cab57-inoculated MS agar medium containing different concentrations of sucrose (0.1, 1, and 10 mM).

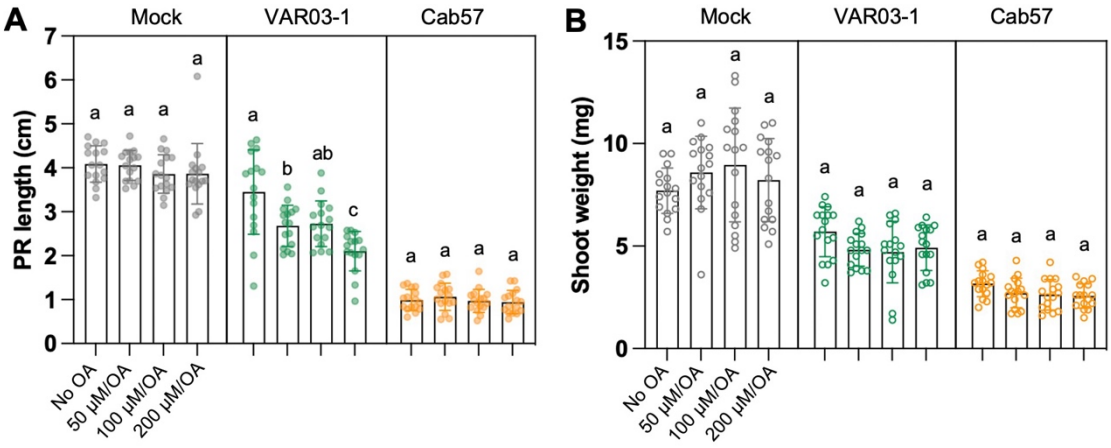

Figure S5. Growth retardation induced by VAR03-1 and Cab57 in the presence of sucrose and organic acids.

Primary root (PR) (**A**) and shoot fresh weight (**B**) of Arabidopsis seedlings (5 dpi) in MS agar containing VAR03-1 and Cab57 (OD_{600} = 5×10^{-4}) supplemented with 10 mM sucrose and a combination of organic acids (citrate, aspartic acid, *p*-hydroxybenzoate, and proline 5-oxo) at different concentrations (50, 100, and 200 µM of each). Data were obtained from the same experimental set shown in Fig. 10. Statistical analysis was performed using Brown-Forsythe ANOVA (n = 16, α = 0.05) for each bacterial treatment. Different letters indicate significant differences.

Table S1. List of bacterial strains used in this study.

Strains	Information	Reference
Allorhizobium vitis		
VAR03-1	Biocontrol, non-pathogenic	Noutoshi et al. (2020b)
VAT03-9	Tumorigenic (Ti)	Noutoshi et al., (2020c)
VAR06-30	Non-biocontrol, non-pathogenic	Noutoshi et al. (2020a)
#1	Rhizoviticn transposon random mutant, Tn5 insertion in a gene encoding a fiber protein.	Ishii et al. (2024)
Δ22980	Rhizoviticin deletion mutant of gene encoding an ssDNA-specific exonuclease,	Ishii et al. (2024)
Δ23020	Rhizoviticn deletion mutant of gene encoding a DnaB-like replicative DNA helicase.	Ishii et al. (2024)
Δ23040	Rhizoviticin deletion mutant of gene encoding a putative DNA-binding protein	Ishii et al. (2024)
*Δ*65	Growth defect transposon random mutants, Tn5 insertion in *cobJ* gene conding precorrin-3B methyltransferase.	Bao (2022)
*Δ*86	Growth defect transposon random mutants, Tn5 insertion in *mprF* gene conding multiple peptide resistance factor (MprF)	Bao (2022)
ΔrecA	VAR03-1 deletion mutant for *recA* gene.	Ishii et al. (2024)
Pseudomonas protegens		
Cab57	A biocontrol strain against *Phytium ultimum* in cucumber	Takeuchi et al. (2014)

Table S2. Arabidopsis wild-type or mutants used in this study.

Genotype	Information
Col-0	Wild-type
efr1	PTI-related mutant, impaired in functional EFR responsible for elf18 recognition
bak1-5/bkk1-1	PTI-related mutant, impaired in PTI downstream signaling mediated by BAK1 and BKK1
bik1	PTI-related mutant, impaired in functional BIK1 responsible for MAMPs recognition
npr1-5	Defense hormone mutant, impaired in salicylic biosynthesis and signaling by NPR1
sid2-2	Defense hormone mutant, impaired in salicylic biosynthesis and signaling by SID2
eds1-2	Defense hormone mutant, impaired in salicylic biosynthesis and signaling by EDS1
etr1-1	Defense hormone mutant, impaired in ethylene signaling mediated by ETR1
Atfaah ko 1	*AtFAAH* knockout mutant with Tn5 insertion in *AtFAAH* exon
Atfaah ko 2	*AtFAAH* knockout mutant with Tn5 insertion in *AtFAAH* promoter

Table S3. List of primers used in this study.

Primer name	Sequence
UBQ10-F	AGATCCAGGACAAGGAGGTATTC
UBQ10-R	CGCAGGACCAAGTGAAGAGTAG
FRK1-F	CGGTCAGATTTCAACAGTTGTC
FRK1-R	AATAGCAGGTTGGCCTGTAATC
PDF1.2_F	ACCAACAATGGTGGAAGCAC
PDF1.2_R	CACTTGTGAGCTGGGAAGAC

References

Abarca-Grau, A.M., Penyalver, R., López, M.M., Marco-Noales, E., 2011. Pathogenic and non-pathogenic *Agrobacterium tumefaciens*, *A. rhizogenes* and *A. vitis* strains form biofilms on abiotic as well as on root surfaces. *Plant Pathol*. 60, 416–425.

Agrios, G.N., 2005. Plant Pathology, 5th ed. Elsevier Academic Press, London.

Ahmed, S.A.K.S., Rudden, M., Smyth, T.J., Dooley, J.S.G., Marchant, R., Banat, I.M., 2019. Natural quorum sensing inhibitors effectively downregulate gene expression of *Pseudomonas aeruginosa* virulence factors. *Appl. Microbiol. Biotechnol*. 103, 3521–3535.

Anderson, J.C., Wan, Y., Kim, Y.M., Pasa-Tolic, L., Metz, T.O., Peck, S.C., 2014. Decreased abundance of type III secretion system inducing signals in Arabidopsis *mkp1* enhances resistance against *Pseudomonas syringae*. *Proc. Natl. Acad. Sci. USA* 111, 6846–6851.

Ankati, S., Rani, T.S., Podile, A.R., 2019. Changes in root exudates and root proteins in groundnut–*Pseudomonas* sp. interaction contribute to root colonization by bacteria and defense response of the host. *J. Plant Growth Regul.* 38, 523–538.

Aranda, J., Bardina, C., Beceiro, A., Rumbo, S., Cabral, M.P., Barbé, J., Bou, G., 2011. *Acinetobacter baumannii* RecA protein in repair of DNA damage, antimicrobial resistance, general stress response, and virulence. *J. Bacteriol*. 193, 3740–3747.

Badri, D. V., Vivanco, J.M., 2009. Regulation and function of root exudates. *Plant Cell Environ*. 32, 666–681.

Bao, J., 2022. Colonization mechanism of *Rhizobium vitis* VAR03-1, a biocontrol bacterial agent for grapevine crown gall disease in *Arabidopsis thaliana*. (Master's Thesis) Okayama University, Okayama.

Beloin, C., Valle, J., Latour-Lambert, P., Faure, P., Kzreminski, M., Balestrino, D., Haagensen, J.A.J., Molin, S., Prensier, G., Arbeille, B., Ghigo, J.M., 2004. Global impact of mature biofilm lifestyle on *Escherichia coli* K-12 gene expression. *Mol. Microbiol*. 51, 659–674.

Boller, T., Felix, G., 2009. A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* 60, 379–407.

Bolton, H., Elliott, L.F., Gurusiddaiah, S., Fredrickson, J.K., 1989. Characterization of a toxin produced by a rhizobacterial *Pseudomonas* sp. that inhibits wheat growth. *Plant Soil* 114, 279–287.

Bouffartigues, E., Duchesne, R., Bazire, A., Simon, M., Maillot, O., Dufour, A., Feuilloley, M., Orange, N., Chevalier, S., 2014. Sucrose favors *Pseudomonas aeruginosa* pellicle production through the extracytoplasmic function sigma factor SigX. *FEMS Microbiol. Lett.* 356, 193–200.

Breia, R., Conde, A., Badim, H., Fortes, A.M., Gerós, H., Granell, A., 2021. Plant Sweets: From sugar transport to plant–pathogen interaction and more unexpected physiological roles. *Plant Physiol*. 186, 836–852.

Burr, T.J., Bazzi, C., Süle, S., Otten, L., 1998. Crown gall of grape: Biology of *Agrobacterium vitis* and the development of disease control strategies. *Plant Dis.* 82, 1288–1297.

Burr, T.J., Katz, B.H., 1984. Grapevine cuttings as potential sites of survival and means of dissemination of *Agrobacterium tumefaciens*. *Plant Dis*. 68, 976–978.

Burr, T.J., Reid, C.L., Tagliati, E., Bazzi, C., Süle, S., 1997. Biological control of grape crown gall by strain F2/5 is not associated with agrocin production or competition for attachment sites on grape cells. *Phytopathol.* 87, 706–711.

Burr, T.J., Reid, C.L., Yoshimura, M., Momol, E.A., Bazzi, C., 1995. Survival and tumorigenicity of *Agrobacterium vitis* in living and decaying grape roots and canes in soil. *Plant Dis*. 79, 677–682.

Carvalhais, L.C., Dennis, P.G., Fedoseyenko, D., Hajirezaei, M.R., Borriss, R., Von Wirén, N., 2011. Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. *J. Plant Nutr. Soil Sci*. 174, 3–11.

Chaparro, J.M., Badri, D. V., Bakker, M.G., Sugiyama, A., Manter, D.K., Vivanco, J.M., 2013. Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. *PLoS One* 8, e55731.

Chen, H.Y., Huh, J.H., Yu, Y.C., Ho, L.H., Chen, L.Q., Tholl, D., Frommer, W.B., Guo, W.J., 2015. The Arabidopsis vacuolar sugar transporter SWEET2 limits carbon sequestration from roots and restricts *Pythium infection*. *Plant J*. 83, 1046–1058.

Ching, C., Brychcy, M., Nguyen, B., Muller, P., Pearson, A.R., Downs, M., Regan, S., Isley, B., Fowle, W., Chai, Y., Godoy, V.G., 2024. RecA levels modulate biofilm development in *Acinetobacter baumannii*. *Mol. Microbiol*. 121, 196–212.

Colaianni, N.R., Parys, K., Lee, H.S., Conway, J.M., Kim, N.H., Edelbacher, N., Mucyn, T.S., Madalinski, M., Law, T.F., Jones, C.D., Belkhadir, Y., Dangl, J.L., 2021. A complex immune response to flagellin epitope variation in commensal communities. *Cell Host Microbe* 29, 635-649.

Cole, B.J., Feltcher, M.E., Waters, R.J., Wetmore, K.M., Mucyn, T.S., Ryan, E.M., Wang, G., Ul-Hasan, S., McDonald, M., Yoshikuni, Y., Malmstrom, R.R., Deutschbauer, A.M., Dangl, J.L., Visel, A., 2017. Genome-wide identification of bacterial plant colonization genes. *PLoS Biol.* 15, e2002860.

Corral, J., Pérez-Varela, M., Barbé, J., Aranda, J., 2020. Direct interaction between RecA and a CheW-like protein is required for surface-associated motility, chemotaxis and the full virulence of *Acinetobacter baumannii* strain ATCC 17978. *Virulence* 11, 315–326.

Couillerot, O., Prigent-Combaret, C., Caballero-Mellado, J., Moënne-Loccoz, Y., 2009. *Pseudomonas fluorescens* and closely-related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens. *Lett. Appl. Microbiol*. 48, 505–512.

Cowles, J.R., Evans, H.J., Russell, S.A., 1969. B12 coenzyme-dependent ribonucleotide reductase in *Rhizobium* species and the effects of cobalt deficiency on the activity of the enzyme. *J. Bacteriol*. 97, 1460–1465.

Dar, D., Dar, N., Cai, L., Newman, D.K., 2021. Spatial transcriptomics of planktonic and sessile bacterial populations at single-cell resolution. *Science* 373, eabi4882.

Défago, G., Berling, C.H., Burger, U., Haas, D., Kahr, G., Keel, C., Voisard, C., Philippe Jacques, W., Wuethrich, B., 1990. Suppression of black root rot of tobacco and other root diseases by strains of *Pseudomonas fluorescens*: potential applications and mechanisms, in: Hornby, D. (Ed.), Biological Control of Soil-Borne Plant Pathogens. CAB International, Oxon, pp. 93–108.

Dorosky, R.J., Yu, J.M., Pierson, L.S., Pierson, E.A., 2017. *Pseudomonas chlororaphis* produces two distinct R-tailocins that contribute to bacterial competition in biofilms and on roots. *Appl. Environ. Microbiol.* 83, e00706-17.

Drew, G.C., Stevens, E.J., King, K.C., 2021. Microbial evolution and transitions along the parasite–mutualist continuum. *Nat. Rev. Microbiol* 19, 623–638.

Eastwell, K.C., Sholberg, P.L., Sayler, R.J., 2006. Characterizing potential bacterial biocontrol agents for suppression of *Rhizobium vitis*, causal agent of crown gall disease in grapevines. *Crop Prot*. 25, 1191–1200.

Entila, F., Han, X., Mine, A., Schulze-Lefert, P., Tsuda, K., 2024. Commensal lifestyle regulated by a negative feedback loop between Arabidopsis ROS and the bacterial T2SS. *Nat. Commun.* 15, 456.

Ernst, C.M., Peschel, A., 2011. Broad-spectrum antimicrobial peptide resistance by MprF-mediated aminoacylation and flipping of phospholipids. *Mol Microbiol* 80, 290–299.

Ferrigo, D., Causin, R., Raiola, A., 2017. Effect of potential biocontrol agents selected among grapevine endophytes and commercial products on crown gall disease. *BioControl* 62, 821–833.

Fuchs, S., Mühldorfer, I., Donohue-Rolfe, A., Kerényi, M., Emödy, L., Alexiev, R., Nenkov, P., Hacker, J., 1999. Influence of RecA on in vivo virulence and Shiga toxin 2 production in *Escherichia coli* pathogens. *Microb. Pathog*. 27, 13–23.

Gao, T., Ding, M., Wang, Q., 2020. The recA gene is crucial to mediate colonization of *Bacillus cereus* 905 on wheat roots. *Appl. Microbiol. Biotechnol*. 104, 9251–9265.

Gebhart, D., Williams, S.R., Bishop-Lilly, K.A., Govoni, G.R., Willner, K.M., Butani, A., Sozhamannan, S., Martin, D., Fortier, L.C., Scholl, D., 2012. Novel high-molecular-weight, R-type bacteriocins of *Clostridium difficile*. *J. Bacteriol*. 194, 6240–6247.

Ghequire, M.G.K., De Mot, R., 2015. The tailocin tale: Peeling off phage tails. *Trends Microbiol.* 23, 587–590.

Ghequire, M.G.K., Dillen, Y., Lambrichts, I., Proost, P., Wattiez, R., De Mot, R., 2015. Different ancestries of R tailocins in rhizospheric *Pseudomonas* isolates. *Genome Biol. Evol.* 7, 2810–2828.

Goyal, A., 2022. Horizontal gene transfer drives the evolution of dependencies in bacteria. *iScience* 25, 104312.

Gu, Y., Wei, Z., Wang, Xueqi, Friman, V.P., Huang, J., Wang, Xiaofang, Mei, X., Xu, Y., Shen, Q., Jousset, A., 2016. Pathogen invasion indirectly changes the composition of soil microbiome via shifts in root exudation profile. *Biol. Fertil. Soils* 52, 997–1005.

Gunina, A., Kuzyakov, Y., 2015. Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate. *Soil Biol. Biochem*. 90, 87–100.

Hamilton, C.D., Steidl, O.R., MacIntyre, A.M., Hendrich, C.G., Allen, C., 2021. *Ralstonia solanacearum* depends on catabolism of myo-inositol, sucrose, and trehalose for virulence in an infection stage-dependent manner. *Mol. Plant Microbe Interact*. 34, 669–679.

Hockett, K.L., Renner, T., Baltrus, D.A., 2015. Independent co-option of a tailed bacteriophage into a killing complex in *Pseudomonas*. *mBio* 6, e00452.

Hütsch, B.W., Augustin, J., Merbach, W., 2002. Plant rhizodeposition: an important source for carbon turnover in soils, *J. Plant Nutr. Soil Sci*. 165, 397–407.

Inagaki, S., Matsumoto-Nakano, M., Fujita, K., Nagayama, K., Funao, J., Ooshima, T., 2009. Effects of recombinase A deficiency on biofilm formation by *Streptococcus mutans*. *Oral Microbiol. Immunol.* 24, 104–108.

Ishii, T., Tsuchida, N., Hemelda, N.M., Saito, K., Bao, J., Watanabe, M., Toyoda, A., Matsubara, T., Sato, M., Toyooka, K., Ishihama, N., Shirasu, K., Matsui, H., Toyoda, K., Ichinose, Y., Hayashi, T., Kawaguchi, A., Noutoshi, Y., 2024. Rhizoviticin is an alphaproteobacterial tailocin that mediates biocontrol of grapevine crown gall disease. *ISME J*. 18, wrad003.

Karygianni, L., Ren, Z., Koo, H., Thurnheer, T., 2020. Biofilm matrixome: Extracellular components in structured microbial communities. *Trends Microbiol* 28, 668–681.

Kaushik, V., Tiwari, M., Tiwari, V., 2022. Interaction of RecA mediated SOS response with bacterial persistence, biofilm formation, and host response. *Int. J. Biol. Macromol*. 217, 931–943.

Kawaguchi, A., 2013. Biological control of crown gall on grapevine and root colonization by nonpathogenic *Rhizobium vitis* strain ARK-1. *Microbes Environ*. 28, 306–311.

Kawaguchi, A., Inoue, K., 2012. New antagonistic strains of non-pathogenic *Agrobacterium vitis* to control grapevine crown gall. *J. Phytopathol*. 160, 509–518.

Kawaguchi, A., Inoue, K., Ichinose, Y., 2008. Biological control of crown gall of grapevine, rose, and tomato by nonpathogenic *Agrobacterium vitis* strain VAR03-1. *Phytopathol.* 98, 1218–1225.

Kawaguchi, A., Inoue, K., Nasu, H., 2007. Biological control of grapevine crown gall by nonpathogenic *Agrobacterium vitis* strain VAR03-1. *J. Gen. Plant Pathol*. 73, 133–138.

Kawaguchi, A., Inoue, K., Nasu, H., 2005. Inhibition of crown gall formation by *Agrobacterium radiobacter* biovar 3 strains isolated from grapevine. *J. Gen. Plant Pathol*. 71, 422–430.

Kawaguchi, A., Kondo, K. ichi, Inoue, K., 2012. Biological control of apple crown gall by nonpathogenic *Rhizobium vitis* strain VAR03-1. *J. Gen. Plant Pathol*. 78, 287–293.

Kerr, A., 1972. Biological control of crown all: Seed inoculation. *J. Appl. Bacteriol*. 35, 493–497.

Kerr, A., Bullard, G., 2020. Biocontrol of crown gall by *Rhizobium rhizogenes*: Challenges in biopesticide commercialisation. *Agronomy* 10, 1126.

Kerr, A., Htay, K., 1974. Biological control of crown gall through bacteriocin production. *Physiol. Plant Pathol*. 4, 37–44.

Kinkel, L.L., Lindow, S.E., 1997. Microbial competition and plant disease biocontrol, in: Andow, D.A., Ragsdale, D.W., Nyvall, R.F. (Eds.), Ecological Interactions and Biological Control. Routledge, New York, pp. 128–138.

Köhl, J., Kolnaar, R., Ravensberg, W.J., 2019. Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. *Front. Plant Sci*. 10, 845.

Kunze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T., Felix, G., 2004. The N terminus of bacterial elongation factor Tu elicits innate immunity in Arabidopsis plants. *Plant Cell* 16, 3496–3507.

Kuzmanović, N., Puławska, J., Hao, L., Burr, T.J., 2018. The ecology of *Agrobacterium vitis* and management of crown gall disease in vineyards. *Curr. Top. Microbiol. Immunol*. 418, 15–53.

Lehoczky, J., 1971. Further evidences concerning the systemic spreading of *Agrobacterium tumefaciens* in the vascular system of grapevines. *Vitis* 10, 215–221.

Levipan, H.A., Avendaño-Herrera, R., 2017. Different phenotypes of mature biofilm in *Flavobacterium psychrophilum* share a potential for virulence that differs from planktonic state. *Front. Cell. Infect. Microbiol*. 7.

Li, E., de Jonge, R., Liu, C., Jiang, H., Friman, V.P., Pieterse, C.M.J., Bakker, P.A.H.M., Jousset, A., 2021. Rapid evolution of bacterial mutualism in the plant rhizosphere. *Nat. Commun.* 12, 3829.

Li, J.Y., Wang, J.H., Wang, H.M., 2009. Mode of action of the antibacterial compound Ar26 produced by *Agrobacterium vitis* strain E26 with activity against *A. tumefaciens*, *A. rhizogenes* and *A. vitis*. *J. Phytopathol*. 157, 159–165.

Li, Y., Gronquist, M.R., Hao, G., Holden, M.R., Eberhard, A., Scott, R.A., Savka, M.A., Szegedi, E., Sule, S., Burr, T.J., 2006. Chromosome and plasmid-encoded *N*-acyl homoserine lactones produced by *Agrobacterium vitis* wildtype and mutants that differ in their interactions with grape and tobacco. *Physiol. Mol. Plant Pathol*. 67, 284–290.

Liu, H., Zhou, Y., Xiao, W., Ji, L., Cao, X., Song, C., 2012. Shifting nutrient-mediated interactions between algae and bacteria in a microcosm: Evidence from alkaline phosphatase assay. *Microbiol Res.* 167, 292–298.

Liu, J., Chen, P., Zheng, C., Huang, Y.P., 2013. Characterization of maltocin P28, a novel phage tail-like bacteriocin from *Stenotrophomonas maltophilia*. *Appl. Environ. Microbiol.* 79, 5593–5600.

Loo, E.P.I., Durán, P., Pang, T.Y., Westhoff, P., Deng, C., Durán, C., Lercher, M., Garrido-Oter, R., Frommer, W.B., 2024. Sugar transporters spatially organize microbiota colonization along the longitudinal root axis of Arabidopsis. *Cell Host Microbe* 32, 543-556.

Lopes, L.D., Wang, P., Futrell, S.L., Schachtman, D.P., 2022. Sugars and jasmonic acid concentration in root exudates affect maize rhizosphere bacterial communities. *Appl. Environ. Microbiol.* 88, e0097122.

Lu, D., Wu, S., Gao, X., Zhang, Y., Shan, L., He, P., 2010. A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. *Proc. Natl. Acad. Sci. USA* 107, 496–501.

Maddula, V.S.R.K., Pierson, E.A., Pierson, L.S., 2008. Altering the ratio of phenazines in *Pseudomonas chlororaphis* (*aureofaciens*) strain 30-84: Effects on biofilm formation and pathogen inhibition. *J. Bacteriol.* 190, 2759–2766.

Martínez, S., Martínez-Salazar, J., Camas, A., Sánchez, R., Soberón-Chávez, G., 1997. Evaluation of the role of RecA protein in plant virulence with recA mutants of *Xanthomonas campestris* pv. *campestris*. *Mol. Plant Microbe Interact*. 10, 911–916.

McLaughlin, S., Zhalnina, K., Kosina, S., Northen, T.R., Sasse, J., 2023. The core metabolome and root exudation dynamics of three phylogenetically distinct plant species. *Nat. Commun*. 14, 1649.

Melnyk, R.A., Hossain, S.S., Haney, C.H., 2019. Convergent gain and loss of genomic islands drive lifestyle changes in plant-associated *Pseudomonas*. *ISME J.* 13, 1575–1588.

Nanda, A.M., Thormann, K., Frunzke, J., 2015. Impact of spontaneous prophage induction on the fitness of bacterial populations and host-microbe interactions. *J. Bacteriol*. 197, 410–419.

Ngou, B.P.M., Ding, P., Jones, J.D.G., 2022. Thirty years of resistance: Zig-zag through the plant immune system. *Plant Cell* 34, 1447–1478.

Nobori, T., Velásquez, A.C., Wu, J., Kvitko, B.H., Kremer, J.M., Wang, Y., He, S.Y., Tsuda, K., 2018. Transcriptome landscape of a bacterial pathogen under plant immunity. *Proc. Natl. Acad. Sci. USA* 115, E3055–E3064.

Noutoshi, Y., Toyoda, A., Ishii, T., Saito, K., Watanabe, M., Kawaguchi, A., 2020a. Complete genome sequence data of nonpathogenic strain *Rhizobium vitis* VAR03-1, a biological control agent for grapevine crown gall disease. *Mol. Plant Microbe Interact.* 33, 1451–1453.

Noutoshi, Y., Toyoda, A., Ishii, T., Saito, K., Watanabe, M., Kawaguchi, A., 2020b. Complete genome sequence data of tumorigenic *Rhizobium vitis* strain VAT03-9, a causal agent of grapevine crown gall disease. *Mol. Plant Microbe Interact*. 33, 1280–1282.

Noutoshi, Y., Toyoda, A., Ishii, T., Saito, K., Watanabe, M., Kawaguchi, A., 2020c. Complete genome sequence data of nonpathogenic and nonantagonistic strain of *Rhizobium vitis* VAR06-30 isolated from grapevine rhizosphere. *Mol. Plant Microbe Interact*. 33, 1283–1285.

Oggioni, M.R., Trappetti, C., Kadioglu, A., Cassone, M., Iannelli, F., Ricci, S., Andrew, P.W., Pozzi, G., 2006. Switch from planktonic to sessile life: A major event in pneumococcal pathogenesis. *Mol. Microbiol*. 61, 1196–1210.

Okada, K., Kubota, Y., Hirase, T., Otani, K., Goh, T., Hiruma, K., Saijo, Y., 2021. Uncoupling root hair formation and defence activation from growth inhibition in response to damage-associated Pep peptides in *Arabidopsis thaliana*. *New Phytol.* 229, 2844–2858.

Ortíz-Castro, R., Martínez-Trujillo, M., López-Bucio, J., 2008. *N*-acyl-L-homoserine lactones: A class of bacterial quorum-sensing signals alter post-embryonic root development in *Arabidopsis thaliana*. *Plant Cell Environ*. 31, 1497–1509.

Palmer, A.G., Senechal, A.C., Mukherjee, A., Ané, J.M., Blackwell, H.E., 2014. Plant responses to bacterial *N*-acyl L-homoserine lactones are dependent on enzymatic degradation to L-homoserine. *ACS Chem*. Biol. 9, 1834–1845.

Pearson, J.P., Van Delden, C., Iglewski, B.H., 1999. Active efflux and diffusion are involved in transport of *Pseudomonas aeruginosa* cell-to-cell signals. *J. Bacteriol.* 181, 1203–1210.

Pfender, W.F., Kraus, J., Loper, J.E., 1993. A genomic region from *Pseudomonas fluorescens* Pf-5 required for pyrrolnitrin production and inhibition of *Pyrenophora tritici-repentis* in wheat straw. *Phytopathol.* 83, 1223–1228.

Príncipe, A., Fernandez, M., Torasso, M., Godino, A., Fischer, S., 2018. Effectiveness of tailocins produced by *Pseudomonas fluorescens* SF4c in controlling the bacterial-spot disease in tomatoes caused by *Xanthomonas vesicatoria*. *Microbiol. Res.* 212–213, 94–102.

Ravaioli, S., Campoccia, D., Speziale, P., Pietrocola, G., Zatorska, B., Maso, A., Presterl, E., Montanaro, L., Arciola, C.R., 2020. Various biofilm matrices of the emerging pathogen *Staphylococcus lugdunensis*: Exopolysaccharides, proteins, eDNA and their correlation with biofilm mass. *Biofouling* 36, 86–100.

Recacha, E., Machuca, J., Díaz-Díaz, S., García-Duque, A., Ramos-Guelfo, M., Docobo-Pérez, F., Blázquez, J., Pascual, A., Rodríguez-Martínez, J.M., 2019. Suppression of the SOS response modifies spatiotemporal evolution, post-antibiotic effect, bacterial fitness and biofilm formation in quinolone-resistant *Escherichia coli*. *J. Antimicrob. Chemother*. 74, 66–73.

Roux, M., Schwessinger, B., Albrecht, C., Chinchilla, D., Jones, A., Holton, N., Malinovsky, F.G., Tör, M., de Vries, S., Zipfel, C., 2011. The Arabidopsis leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. *Plant Cell* 23, 2440–2455.

Russel, J., Røder, H.L., Madsen, J.S., Burmølle, M., Sørensen, S.J., 2017. Antagonism correlates with metabolic similarity in diverse bacteria. *Proc. Natl. Acad. Sci. USA* 114, 10684–10688.

Saito, K., Watanabe, M., Matsui, H., Yamamoto, M., Ichinose, Y., Toyoda, K., Kawaguchi, A., Noutoshi, Y., 2018. Characterization of the suppressive effects of the biological control strain VAR03-1 of *Rhizobium vitis* on the virulence of tumorigenic *R. vitis*. *J. Gen. Plant Pathol*. 84, 58–64.

Savka, M.A., Le, P.T., Burr, T.J., 2011. LasR receptor for detection of long-chain quorum-sensing signals: Identification of *N*-acyl-homoserine lactones encoded by the *avsI* locus of *Agrobacterium vitis*. *Curr. Microbiol*. 62, 101–110.

Scholl, D., 2017. Phage tail-like bacteriocins. *Annu, Rev. Virol.* 4, 453–467.

Schulte, R., Bonas, U., 1992. A *Xanthomonas* pathogenicity locus is induced by sucrose and sulfur-containing amino acids. *Plant Cell* 4, 79–86.

Shimasaki, T., Masuda, S., Garrido-Oter, R., Kawasaki, T., Aoki, Y., Shibata, A., Shirasu, K., Yazaki, K., Nakano, R.T., Sugiyama, A., Suda, W., 2021. Tobacco root endophytic arthrobacter harbors genomic features enabling the catabolism of host-specific plant specialized metabolites. *mBio* 12, e0084621.

Sivakumar, R., Ranjani, J., Vishnu, U.S., Jayashree, S., Lozano, G.L., Miles, J., Broderick, N.A., Guan, C., Gunasekaran, P., Handelsman, J., Rajendhran, J., 2019. Evaluation of inseq to identify genes essential for *Pseudomonas aeruginosa* PGPR2 corn root colonization. *G3* 9, 651–661.

Sohlenkamp, C., Galindo-Lagunas, K.A., Guan, Z., Vinuesa, P., Robinson, S., Thomas-Oates, J., Raetz, C.R.H., Geiger, O., 2007. The lipid lysyl-phosphatidylglycerol is present in membranes of *Rhizobium tropici* CIAT899 and confers increased resistance to polymyxin B under acidic growth conditions. *Mol. Plant Microbe Interact.* 20, 1421–1430.

Song, D., Jiao, H., Liu, Z., 2021. Phospholipid translocation captured in a bifunctional membrane protein MprF. *Nat. Commun*. 12, 2927.

Stroo, H.F., Elliott, L.F., Papendick, R.I., 1988. Growth, survival, and toxin production of root-inhibitory *Pseudomonads* on crop residues. *Soil Biol Biochem* 20, 201–207.

Süle, S., Burr, T.J., 1998. The effect of resistance of rootstocks to crown gall (*Agrobacterium* spp.) on the susceptibility of scions in grapevine cultivars. *Plant Pathol*. 47, 84–88.

Takeuchi, K., Noda, N., Someya, N., 2014. Complete genome sequence of the biocontrol strain *Pseudomonas protegens* Cab57 discovered in Japan reveals strain-specific diversity of this species. *PLoS One* 9, e93683.

Tawaraya, K., Horie, R., Saito, A., Shinano, T., Wagatsuma, T., Saito, K., Oikawa, A., 2013. Metabolite profiling of shoot extracts, root extracts, and root exudates of rice plants under phosphorous deficiency. *J. Plant Nutr.* 36, 1138–1159.

Teixeira, P.J.P.L., Colaianni, N.R., Law, T.F., Conway, J.M., Gilbert, S., Li, H., Salas-González, I., Panda, D., Risco, N.M. Del, Finkel, O.M., Castrillo, G., Mieczkowski, P., Jones, C.D., Dangl, J.L., 2021. Specific modulation of the root immune system by a community of commensal bacteria. *Proc. Natl. Acad. Sci. USA* 118, e2100678118.

Tian, T., Sun, B., Shi, H., Gao, T., He, Y., Li, Y., Liu, Y., Li, X., Zhang, L., Li, S., Wang, Q., Chai, Y., 2021. Sucrose triggers a novel signaling cascade promoting *Bacillus subtilis* rhizosphere colonization. *ISME J*. 15, 2723–2737.

Turnbull, L., Toyofuku, M., Hynen, A.L., Kurosawa, M., Pessi, G., Petty, N.K., Osvath, S.R., Cárcamo-Oyarce, G., Gloag, E.S., Shimoni, R., Omasits, U., Ito, S., Yap, X., Monahan, L.G., Cavaliere, R., Ahrens, C.H., Charles, I.G., Nomura, N., Eberl, L., Whitchurch, C.B., 2016. Explosive cell lysis as a mechanism for the biogenesis of bacterial membrane vesicles and biofilms. *Nat. Commun*. 7, 11220.

Verstraeten, N., Braeken, K., Debkumari, B., Fauvart, M., Fransaer, J., Vermant, J., Michiels, J., 2008. Living on a surface: swarming and biofilm formation. *Trends Microbiol*. 16, 496–506.

Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A., Pérez-Clemente, R.M., 2020. Root exudates: from plant to rhizosphere and beyond. *Plant Cell Rep*. 39, 3–17.

Wang, N.R., Wiesmann, C.L., Melnyk, R.A., Hossain, S.S., Chi, M.-H., Martens, K., Craven, K., Haney, C.H., Guttman, D.S., 2022. Commensal *Pseudomonas fluorescens* strains protect Arabidopsis from closely related *Pseudomonas* pathogens in a colonization-dependent manner. *mBio* 13, e0289221.

Wang, Y.H., Zhang, L.Q., Li, J.Y., Wang, J.H., Wang, H.M., 2008. The quorum-sensing system AvsR-AvsI regulates both long-chain and short-chain acyl-homoserine lactones in *Agrobacterium vitis* E26. *Anton. Leeuw. Int. J. G*. 93, 267–273.

Wei, S., Jacquiod, S., Philippot, L., Blouin, M., Sørensen, S.J., 2021. Spatial analysis of the root system coupled to microbial community inoculation shed light on rhizosphere bacterial community assembly. *Biol. Fertil. Soils* 57, 973–989.

Whitney, J.C., Howell, P.L., 2013. Synthase-dependent exopolysaccharide secretion in Gram-negative bacteria. *Trends Microbiol*. 21, 63–72.

Wippel, K., Tao, K., Niu, Y., Zgadzaj, R., Kiel, N., Guan, R., Dahms, E., Zhang, P., Jensen, D.B., Logemann, E., Radutoiu, S., Schulze-Lefert, P., Garrido-Oter, R., 2021. Host

preference and invasiveness of commensal bacteria in the Lotus and Arabidopsis root microbiota. *Nat. Microbiol*. 6, 1150–1162.

Woudstra, C., Sørensen, A.N., Sørensen, M.C.H., Brøndsted, L., 2024. Strategies for developing phages into novel antimicrobial tailocins. *Trends Microbiol*. 10.1016/j.tim.2024.03.003

Wu, M., Shan, W., Zhao, G.P., Lyu, L.D., 2022. H_2O_2 concentration-dependent kinetics of gene expression: linking the intensity of oxidative stress and mycobacterial physiological adaptation. *Emerg. Microbes Infect*. 11, 573–584.

Yahya, A.H., Harston, S.R., Colton, W.L., Cabeen, M.T., 2023. Distinct screening approaches uncover PA14_36820 and RecA as negative regulators of biofilm phenotypes in *Pseudomonas aeruginosa* PA14. *Microbiol Spectr* 11, e0377422.

Yasuda, M., Yamamoto, T., Nagakubo, T., Morinaga, K., Obana, N., Nomura, N., Toyofuku, M., 2022. Phage genes induce quorum sensing signal release through membrane vesicle formation. *Microbes Environ*. 37, ME21067.

Yu, K., Liu, Y., Tichelaar, R., Savant, N., Lagendijk, E., van Kuijk, S.J.L., Stringlis, I.A., van Dijken, A.J.H., Pieterse, C.M.J., Bakker, P.A.H.M., Haney, C.H., Berendsen, R.L., 2019. Rhizosphere-associated *Pseudomonas* suppress local root immune responses by gluconic acid-mediated lowering of environmental pH. *Curr. Biol.* 29, 3913–3920.

Zhang, C., Lv, M., Yin, W., Dong, T., Chang, C., Miao, Y., Jia, Y., Deng, Y., 2019. *Xanthomonas campestris* promotes diffusible signal factor biosynthesis and pathogenicity by utilizing glucose and sucrose from host plants. *Mol. Plant Microbe Interact*. 32, 157–166.

Zhang, S., Kan, J., Liu, X., Wu, Y., Zhang, M., Ou, J., Wang, J., An, L., Li, D., Wang, L., Wang, X.J., Fang, R., Jia, Y., 2023. Phytopathogenic bacteria utilize host glucose as a signal to stimulate virulence through LuxR homologues. *Mol. Plant Pathol*. 24, 359–373.

Zhao, M., Zhao, J., Yuan, J., Hale, L., Wen, T., Huang, Q., Vivanco, J.M., Zhou, J., Kowalchuk, G.A., Shen, Q., 2021. Root exudates drive soil-microbe-nutrient feedbacks in response to plant growth. *Plant Cell Environ*. 44, 613–628.

Zhou, Y., Zhao, D., Duan, Y., Chen, L., Fan, H., Wang, Y., Liu, X., Chen, L.Q., Xuan, Y., Zhu, X., 2023. AtSWEET1 negatively regulates plant susceptibility to root-knot nematode disease. *Front. Plant Sci*. 14, 1010438.

Zipfel, C., 2009. Early molecular events in PAMP-triggered immunity. *Curr. Opini. Plant Biol*. 12, 414–420.