applied microbiology

MDPI

Review

Biocontrol of Grapevine Crown Gall Performed Using *Allorhizobium vitis* Strain ARK-1

Akira Kawaguchi

Western Region Agricultural Research Center (WARC) (Kinki, Chugoku and Shikoku Regions), National Agriculture and Food Research Organization (NARO), 6-12-1 Nishifukatsu-cho, Fukuyama 721-8514, Hiroshima, Japan; kawaguchia240@affrc.go.jp; Tel.: +81-84-923-5336

Abstract: Grapevine crown gall (GCG), which is caused by tumorigenic *Allorhizobium vitis* (=*Rhizobium vitis*), is the most important bacterial disease in grapevine, and its economic impact on grapevine is very high. When young vines develop GCG, they often die, whereas older vines may show stress and poor growth depending on the severity of GCG, because GCG interferes with the vascular system of the grapevine trunk and prevents nutrient flow, leading to inferior growth and death. Viticultural practices and chemical control designed to inhibit GCG are only partially effective presently; thus, a biocontrol procedure could be a desirable and effective approach for GCG prevention. This article reviews the practical use of biocontrol options for GCG inhibition that involve using nonpathogenic and antagonistic *A. vitis* strains. In these studies, screening tests of biocontrol agents discovered nonpathogenic *A. vitis* strains VAR03-1, ARK-1, ARK-2, and ARK-3. After dipping grapevine roots in a suspension of candidate strains prior to planting in the field, treatment using ARK-1 was shown to significantly reduce the number of plants with GCG. A meta-analysis indicated that ARK-1 is very useful for controlling crown gall in various plant species, including grapevine. It was reported that when a mixture of ARK-1 and a tumorigenic strain was examined in grapevines, the expression levels of several virulence genes of the virulent strain were significantly lower. ARK-1 can reduce the pathogen population in grapevines and gall incidence. Moreover, ARK-1 can prime the induction of certain defense genes of grapevine. These results indicate that ARK-1 has a unique biocontrol mechanism and that it is a promising new biocontrol agent to control GCG.

Keywords: *Allorhizobium vitis*; grapevine crown gall; biocontrol; meta-analysis; suppression of virulence genes

Citation: Kawaguchi, A. Biocontrol of Grapevine Crown Gall Performed Using *Allorhizobium vitis* Strain ARK-1. *Appl. Microbiol.* **2022**, *2*, 981–991. https://doi.org/10.3390/applmicrobiol2040075

Academic Editor: Francesco Vinale

Received: 21 October 2022
Accepted: 18 November 2022
Published: 19 November 2022

1. Introduction

1.1. What Is Crown Gall?

Crown gall (CG) is one of the most important soil-borne diseases [1–4]. Symptoms of CG are identified as overgrowths appearing as the formation of "galls" (tumors) on roots and/or at the base of plants [1–5]. In addition, grapevine crown gall (GCG) is one of the most important and economically destructive diseases in viticulture around the world [2–5]. GCG is mainly caused by plant pathogenic bacteria *Allorhizobium vitis* (Ti) (=*Rhizobium vitis* (Ti), *Agrobacterium vitis* (Ti), and *A. tumefaciens* biovar 3), where "Ti" indicates "tumorigenic" [6]. Countries with GCG include China, Japan, Chile, South Africa, Hungary, Israel, Italy, Spain, and many countries in Europe, North and South America, and the Middle East [3,4]. CGs usually form on the trunks and cordons of old and young grapevines, including even 1-year-old nursery stocks (Figure 1A) [4]. Infected grapevines often grow poorly, and GCG causes partial or complete grapevine death [7] (Figure 1B,C). In plants with gall symptoms, the risks of inferior growth and death become 14.8-fold and 18.0-fold greater than in plants with no gall symptoms, respectively, indicating that poor growth and death are highly increased by GCG incidence [7]. In addition, in grapevines, wounding events (e.g., mechanical damages or freezing injuries) are often associated with

the occurrence of GCG, because wounded plant tissues are susceptible to infection by *A. vitis* [2–4].

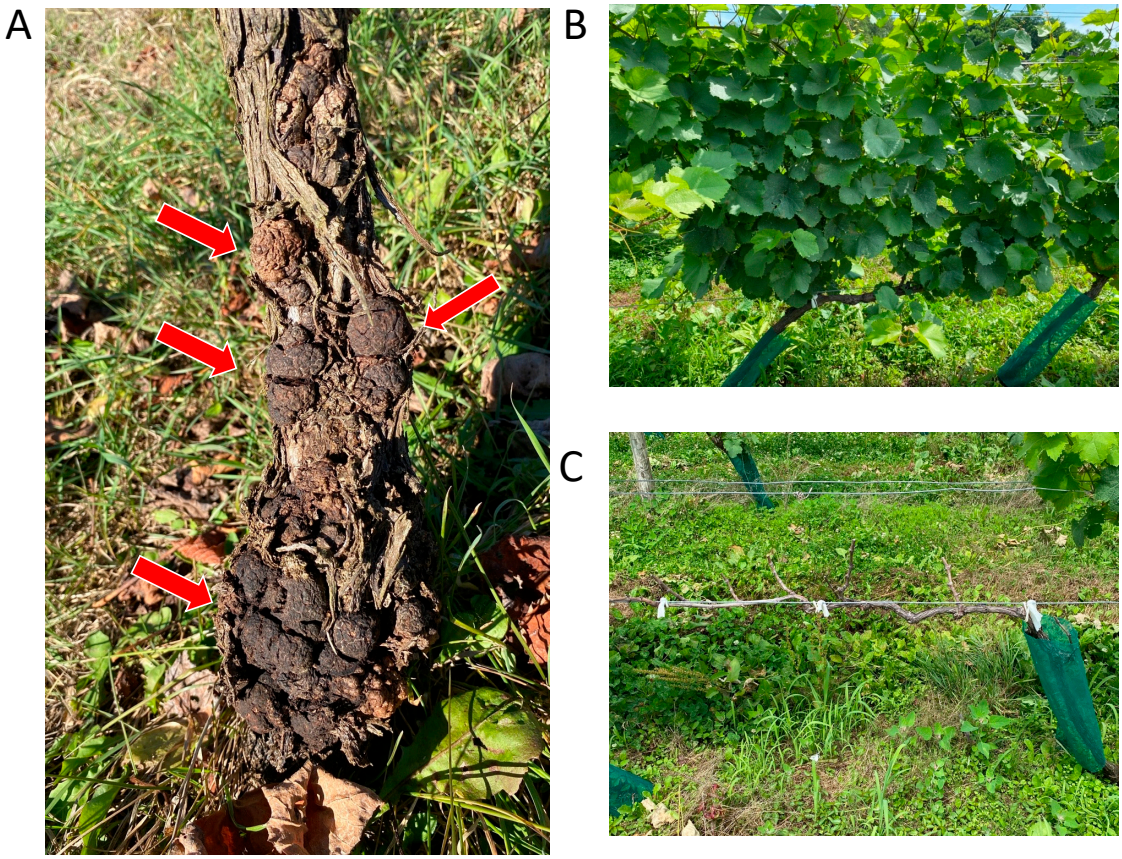

Figure 1. Grapevine crown gall (GCG): (**A**) Galls formed on grapevine stems. Red arrows indicate galls. (**B**) Healthy grapevine without GCG. (**C**) Complete grapevine death by GCG.

1.2. Mechanism of GCG Development

Abnormal cell development is due to the transfer of DNA from Ti strains as the CG pathogen into the plant [8–11]. The bacterial DNA is transferred, incorporated into, and expressed in the plant genomic DNA (Figure 2A) [8–11]. The infection of plants by *A. vitis* (Ti) is a multistage process [8–11]. The first step is the chemotactic attraction to plant cells wounded due to a variety of causes, such as grafting and/or some mechanical damages (Figure 2A) [2–5]. *A. vitis* moves to the underground portion of the grapevine and attaches itself to cells (Figure 2A). Transfer DNA (T-DNA) and virulence (*vir*)-related genes are located mostly on tumor-inducing plasmids (pTi). *A. vitis* (Ti) strains transfer T-DNA in the single-strand form and several virulence effector proteins into plant host cells through a bacterial type IV secretion system [8–11]. The plant molecule acetosyringone (AS) induces the whole *vir* regulon in *A. vitis* (Ti) (Figure 2A) [7–10]. In nature, AS specifically occurs in the exudates from injured and metabolically active plant cells and allows Ti strains to identify susceptible plant cells [8–11]. The transfer of T-DNA and processing need products of the *vir* genes (*virA-E*, and *G*) (Figure 2A) [8–11]. T-DNA is transferred and injected into the plant nuclear DNA (Figure 2A) [8–11]. The posterior expression of T-DNA results in the overproduction of cytokinins and auxins [8–11]. Eventually, an abnormal gall forms in the host plant (Figure 2A) [8–11]. Then, T-DNA genes in gall tissues produce gall-specific compounds called opines, which serve as nutrients for *A. vitis* [3].

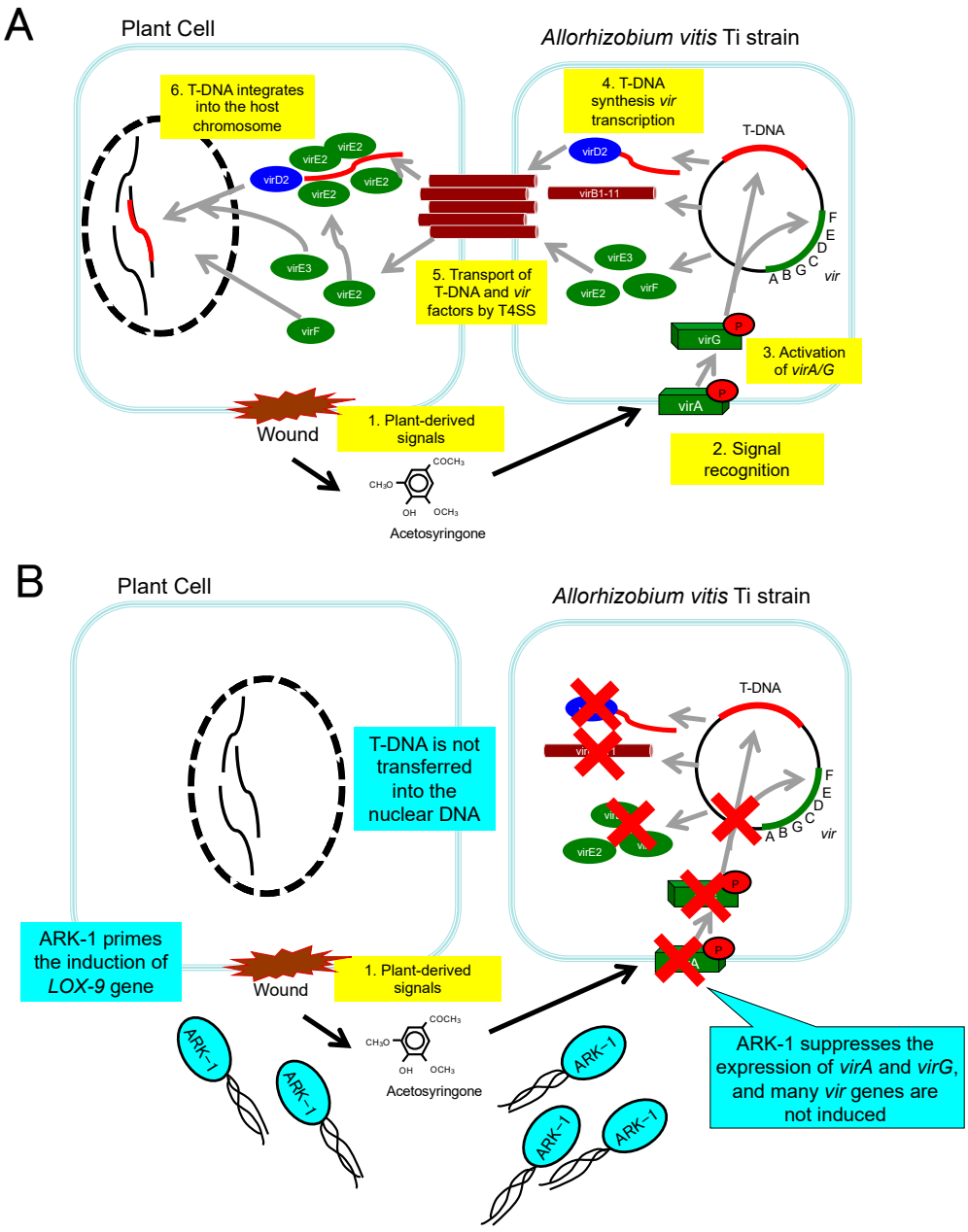

Figure 2. Mechanism of grapevine crown gall (GCG) in a plant cell: (**A**) The infection of plants by tumorigenic *Allorhizobium vitis* strain is a multistage process. Tumorigenic *A. vitis* strains transfer T-DNA and effector proteins. (**B**) The hypothesis of ARK-1′s effect mechanism: ARK-1 suppresses the expression of *virA* and *virG* and then inhibits the pathway of T-DNA transfer into the nuclear DNA of the plant. The ISR priming phenomenon induced by ARK-1 might be a related to its mechanism as a subordinate factor.

1.3. Necessity of GCG Management

The most serious problem is the lack of effective control methods against GCG. Viticultural practices and chemical control designed to inhibit GCG are only partially effective presently. Although copper bactericides and antibiotics are able to kill the bacterium upon contact, they do not penetrate the plants and come into contact with the Ti strains residing inside systemically. Thus, a biocontrol procedure could be a desirable and effective approach to GCG management [3]. The history of the search for practical biocontrol agents for CG goes back to the early 1970s [12–14]. *R. rhizogenes* (=*A. rhizogenes* and *A. radiobacter* biovar 2) strain K84 prevents the growth of *Rhizobium* strains and decreases CG forma-

tion [12–16]. K84 produces an antibacterial molecule, agrocin 84, that is antagonistic to certain Ti strains of *Agrobacterium* and *Rhizobium* [12–17]. Strain K84 has been used successfully to prevent CG incidence in various plant species [12–17]. A new biocontrol strain, K1026, has been constructed using recombinant DNA techniques [18]. K1026 is unable to transfer its mutant agrocin 84 plasmid, designated pAgK1026, to other agrobacteria [18,19]. However, K84 does not inhibit GCG caused by tumorigenic *A. vitis* [2–4].

Several laboratories have attempted to identify other biocontrol agents for GCG (Table 1) [2,4,20–46]. Staphorst et al. [20] evaluated nonpathogenic *A. vitis* strain F2/5, which suppressed the growth of *A. vitis* (Ti) strains in culture plates and inhibited GCG in wounded stems in greenhouse experiments. F2/5 was reported to produce a bacteriocin that inhibits CG at the wound sites on plant stems inoculated with an *A. vitis* (Ti) strain and to have a mechanism associated with quorum sensing (QS) and polyketide synthesis based on caseinolytic protease component (*clp*) genes [21–24]. However, F2/5 was shown to have very low antibiotic activity against tumorigenic *A. radiobacter* (=*R. radiobacter* (Ti), *A. tumefaciens* (Ti), and *A. tumefaciens* biovar 1) and *R. rhizogenes* (=*A. rhizogenes* (Ti) and *A. tumefaciens* biovar 2) and not to inhibit CG caused by other Ti strains of *A. vitis* [22–24]. Chen and Xiang [26] reported that *A. radiobacter* strain HLB-2 isolated from CGs from hop produced an agrocin-like compound and inhibited *A. vitis* (Ti) strains in medium plates. Moreover, HLB-2 was also reported to prevent gall formation in grapevine and sunflower seedlings when co-inoculated with *A. vitis* (Ti) in a greenhouse experiment [26]. Wang et al. [27] reported that an antimicrobial compound, "Ar26", produced by nonpathogenic *A. vitis* strain E26 suppressed the growth of *A. radiobacter* and *A. vitis* in vitro. Chen et al. [29] reported that *Rahnella aquatilis* strain HX2 inhibited GCG incidence.

Table 1. Bacterial strains that have been evaluated for biocontrol of grapevine crown gall (GCG).

Bacterium	Strain	Origin	Reference
Allorhizobium vitis (nonpathogenic)	VAR03-1	Grapevine, Japan	[2,4,33,34,36,37,42–44]
Allorhizobium vitis (nonpathogenic)	ARK-1	Grapevine, Japan	[4,35,40,41,45,46]
Allorhizobium vitis (nonpathogenic)	ARK-2	Grapevine, Japan	[4,35]
Allorhizobium vitis (nonpathogenic)	ARK-3	Grapevine, Japan	[4,35]
Allorhizobium vitis (nonpathogenic)	F2/5	Grapevine, South Africa	[20–24]
Rhizobium rhizogenes (tumorigenic)	J73	Plum, South Africa	[25]
Allorhizobium vitis (nonpathogenic)	E26	Grapevine, China	[27,28]
Agrobacterium radiobacter (nonpathogenic)	HLB-2	Hop, China	[26]
Rahnella aquatilis	HX2	Grapevine, China	[29]
Agrobacterium radiobacter (nonpathogenic)	MI15	Grapevine, China	[30]

As described above, several researchers have tried to develop other biocontrol agents for GCG and reported potential bacterial and fungal strains, but no practical development has been achieved to date due to the lack of successful evidence from field trials. This review focuses on nonpathogenic *A. vitis* strain ARK-1 as a new antagonistic strain, which was identified to strongly inhibit GCG in vineyards and has a unique biocontrol mechanism (Figures 2B and 3).

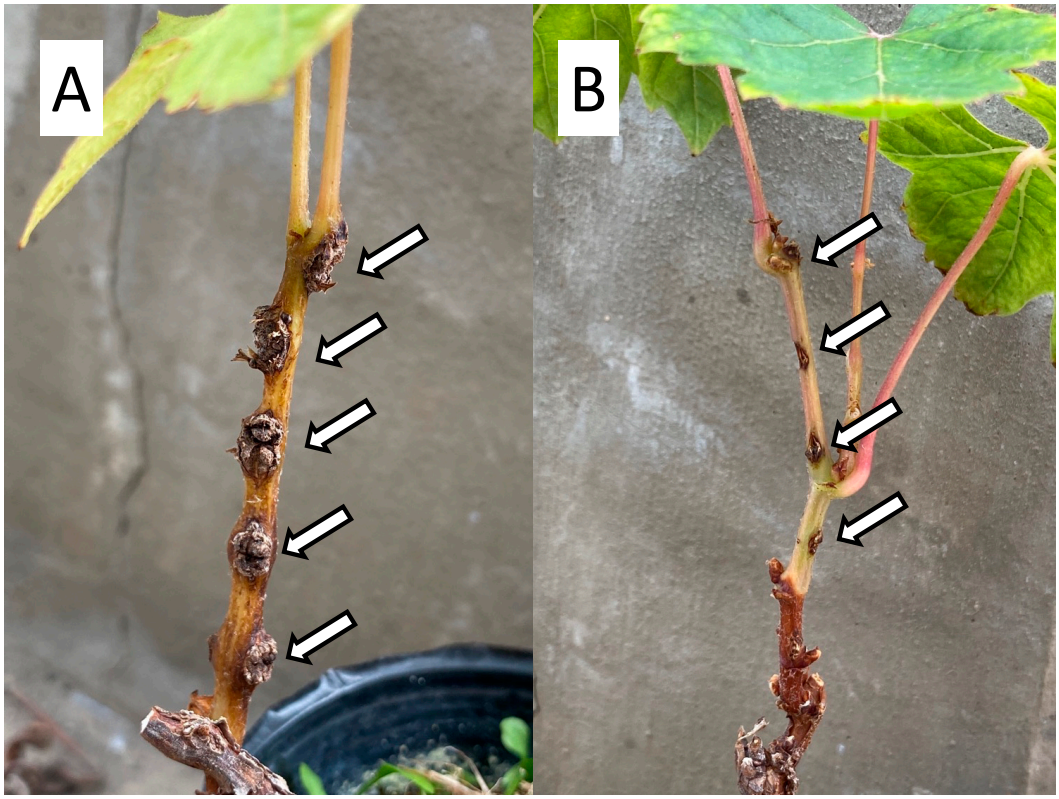

Figure 3. Effect of nonpathogenic *A. vitis* (Ti) strain ARK-1 on gall formation: (**A**) A stem of grapevine seedlings was inoculated with the *A. vitis* (Ti) strain alone. White arrows indicate galls forming at the inoculated site. (**B**) A stem of grapevine was inoculated with mixtures of Ti and ARK-1 strains in a 1:1 cell ratio at the same time. White arrows indicate no gall formation at the inoculation wound site.

2. Screening Tests for Biocontrol Agents

A field survey of potential *A. vitis* (Ti) infections of grapevine in Okayama Prefecture, Japan, isolated numerous nonpathogenic *A. vitis* strains [32,33]. Screening tests for nonpathogenic *A. vitis* strains as biocontrol agents using the needle-prick method were carried out [33–35] on over 500 candidate strains to evaluate their activities against a Ti strain. In a test employing a 1:1 cell ratio of Ti and nonpathogenic strains applied to the stems of seedings of grapevine, sunflower, and tomato, some strains strongly inhibited gall formation and its size compared with plants inoculated with a Ti strain alone (Figure 3). Then, nonpathogenic *A. vitis* strains VAR03-1, ARK-1, ARK-2, and ARK-3 were chosen as biocontrol agents [33–35]. In seedlings of grapevine, each strain (VAR03-1 and ARK-1 to -3) added to the mixture (1:1 cell ratio) of seven different *A. vitis* (Ti) strains, which had been isolated in three different countries (Australia, Greece, and Japan) significantly inhibited gall development in grapevine stems [33–38]. The ARK-1 strain was the strongest among the tested strains, and ARK-1 reduced gall development by 90.6% compared with grapevine seedlings inoculated with the Ti strain alone (Figure 3) [34]. Moreover, ARK-1 co-inoculation significantly reduced gall formation and gall size caused by *A. vitis* Ti strains isolated in several vineyards in Virginia, which is in the mid-Atlantic region of the USA [39]. These results suggest that the ARK-1 strain could be a promising new agent to control GCG in several grape-growing regions in the world.

Moreover, the migration of ARK-1 is very fast, and ARK-1 was reported to have migrated at least 20 cm upward within 5 days through the stems of grapevine after inoculation [40]. Additionally, a Ti strain did not induce gall formation after it moved to the site pre-inoculated with ARK-1 in a previous study [38]. This result supports the effectiveness of the pre-treatment of plants roots with the ARK-1 strain before transplantation to inhibit GCG.

3. Root-Dipping Inoculation for Practical Use

3.1. Field Trials

Field trials are an essential part of the development of new agricultural technologies and are especially important for developing biocontrol agents. Even if positive results may be obtained in laboratory and/or greenhouse experiments, field experiments often show unexpected results. Therefore, field trials were conducted to confirm the control effect of strains ARK-1 and VAR03-1 using the root-dipping method with 1- or 2-year-old grapevine nursery stocks in some experimental and commercial fields [4,34,35,41–43]. From 2009 to 2019, nine field trials for the biocontrol of GCG were carried out in three different fields [4,42]. The roots of plants (nursery stocks of grapevine) were soaked for 1 h in ARK-1 cell suspension (10^7 to 10^8 cells/mL) or water, and they were planted in Ti-contaminated vineyards [4,44]. The results in several field trials were subjected to a meta-analysis, which is one of the statistical techniques for combining findings from independent experiments [4,44,45]. The effect size of antagonist treatment was demonstrated as an integrated risk ratio (IRR) [4,44,45].

The IRR was 0.18 (95% confidence interval, 0.10–0.32), indicating that ARK-1 treatment significantly reduced the GCG incidence regardless of the differences in grape cultivars, fields, and year [4,44]. The IRR value of 0.18 suggested that GCG incidence during ARK-1 treatment was reduced to 18% of that without ARK-1, indicating that the control effect was extremely high in the vineyards. In addition to grapevine, ARK-1 was reported to be able to effectively control CG in rose, tomato, Japanese pear, peach, and apple trees caused by *A. radiobacter* (Ti) (=*R. radiobacter* (Ti)) and *R. rhizogenes* (Ti) strains in greenhouse and field trials [45]. Therefore, ARK-1 effectively protects six different species of host plants, including grapevine, against three different genera and species of *Agrobacterium*, *Rhizobium*, and *Allorhizobium* Ti strains, and this represents the first achievement in the biocontrol of CG in various plant species [4,44,45].

3.2. Population Dynamics of ARK-1 in Roots of Grapevine

When plant diseases are controlled using biocontrol, antagonistic microorganisms need to colonize the host plants well during cultivation. It was reported that the ARK-1 strain was inoculated into roots using the root-dipping method, and ARK-1 was isolated from the roots [4,44]. The result suggested that ARK-1 established populations in the rhizosphere and persisted inside the roots for up to 36 months [4,44]. Although the microbial diversity in grapevine is rich [46], ARK-1 can survive inside grapevine for at least 36 months [4,44]. Thus, ARK-1 is one of the endophytic bacteria. The ability of ARK-1, which is able to colonize grapevine roots, could stably affect the persistence of GCG control.

4. Unique Mechanism of ARK-1 Strain

4.1. Live Strain of ARK-1 Is Needed to Control GCG

Although the live ARK-1 strain in a cell suspension can significantly suppress gall incidence, dead cells and culture filtrate suspension cannot [34]. Generally, the mechanism of antagonistic microorganisms as biocontrol agents involves bacteriocins or antibacterial compounds. However, the lack of control activity of dead ARK-1 cells and culture filtrate of ARK-1 suspension indicates a different mechanism for ARK-1 [34]. Thus, ARK-1 may have cellular functions to manage GCG [32]. An antimicrobial compound, Ar26, which is produced by *A. vitis* strain E26, was noted to prevent the growth of Ti strains in vitro [27]. The antibiotic activity of *A. vitis* strain VAR03-1 may be one of main the factors for the control of CG in grapevine, apple, and sunflower [43,47]. On the other hand, Burr and Reid [21,22] reported that the biocontrol of GCG performed using *A. vitis* F2/5 was not associated with the production of agrocin or the competition for attachment sites on cells of grapevine seedlings. Although the antibiotic activity of ARK-1 in culture plates is unstable due to a difference in the kind of medium [48], the suppressing activity against CG of ARK-1 was reported to be stable and overwhelmingly powerful in all greenhouse and field experiments [4,34,39,40,44,45]. Additionally, ARK-1 does not inhibit the growth of

Ti strains in media containing grapevine extracts alone, which indicates that ARK-1 may not show antibiotic effects on plants [48]. In another study, the *A. radiobacter* (Ti) strain named AtC1 was insensitive to the antibiotic activity of ARK-1, and ARK-1 did not inhibit its growth in culture media [48]. However, ARK-1 was able to control GCG caused by AtC1 in grapevine seedlings [48]. Thus, this body of evidence indicates that the antibiosis of ARK-1 may not be the main mechanism responsible for biocontrol [48].

4.2. Reducing Pathogen Population in Plants

ARK-1 cannot inhibit the growth of Ti strains in culture media, but it can inside plants. In previous studies, when grapevine seedlings were co-inoculated with ARK-1 and *A. vitis* Ti strains (1:1 cell ratio), the population of ARK-1 in the plants resulted in a significantly higher number than that of the Ti strains 7 and 9 days after inoculation (dai) [48,49]. On the other hand, populations of the non-antagonistic *A. vitis* strain and Ti strains did not significantly differ in numbers up to 9 dai [48,49]. Although ARK-1 did not reduce the pathogen population in grapevines during the early period after infection, it suppressed the population of the Ti strains after 7 dai [48,49]. Colonization by ARK-1 remained approximately constant at 10^7 CFU/g in grapevine shoots for up to 3 months after inoculation (mai), but colonization by the Ti strains declined to 10^6 CFU/g 3 mai [48]. In the co-inoculation assay, the populations of the Ti strains were about 10^7 CFU/g in grapevine shoots with galls and 10^6 CFU/g in shoots without galls, suggesting that ARK-1 reduced the populations of the Ti strains significantly, by ten times [48]. Thus, one of the mechanisms of the biocontrol activity of ARK-1 was noted as a noteworthy suppression of population enlargement of the Ti strains after 7 dai [48,49]. The transformation of the host DNA of plants caused by Ti strains most likely occurs before 5 dai [50]. In the above studies, the suppression of CG development caused by ARK-1 occurred even with the lack of inhibition of Ti strain growth caused by ARK-1 until 5 dai [48,49]. These results indicate that the mechanism of biocontrol activity by which ARK-1 suppresses the development of CG symptoms might not involve the suppression of Ti strain populations in grapevine.

4.3. Suppressing Expression of vir Genes in Ti Strains

Two different biocontrol mechanisms of CG using antagonistic microorganisms have been reported. The first one relates to antibacterial materials produced by bacterial strains [18,19,26,27,33,36,37]. The second relates to a unique mechanism associated with QS and polyketide synthesis based on the *clp* genes of strain F2/5 [24]. The biocontrol mechanism of ARK-1 is still unclear regardless of any evidence obtained by the previous studies described above [34,48,49]. To provide insights into the biocontrol mechanism of ARK-1, the effects of ARK-1 of suppressing the expression of five *vir* genes (*virA*, *virD2*, *virD3*, *virE2*, and *virG*) of Ti strains were investigated [49,51]. This suppression appeared to be responsible for the biocontrol performed using ARK-1 [49,51]. The amounts of mRNA expressing the *vir* genes of an *A. vitis* (Ti) strain were analyzed using RT-qPCR [49,51]. In a test employing a 1:1 cell ratio of ARK-1 and the Ti strain in the stems of grapevine, the expression levels of all five *vir* genes were significantly suppressed in comparison with inoculation with the Ti strain alone [49,51]. However, the expression levels of all five *vir* genes were not significantly suppressed by co-inoculation with the non-antagonistic *A. vitis* strain or culture filtrate of ARK-1 and the Ti strain [49,51]. Moreover, acetosyringone (AS), which induces the entire *vir* regulon in *A. vitis* (Ti) [7–10], is not catabolized by ARK-1, suggesting that ARK-1 is not able to metabolize AS to interfere with the *vir* genes of Ti strains and that ARK-1 could directly affect the induction of a broad range of *vir* genes (Figure 2A,B) [51]. The evidence suggests that the mRNA suppressing induced by ARK-1 is one of the main biocontrol mechanisms for controlling GCG in plant tissues.

4.4. Inducing Disease Resistance with ARK-1

Plants are able to protect themselves from pathogens by inducing an organized defense system activated by immunity-related phytohormones such as salicylic acid (SA), jasmonic

acid (JA), and ethylene (ET) [52]. These actions are known as systemic acquired resistance (SAR) [53]. For example, *Pseudomonas fluorescens* can boost plant immunity in above-ground parts with induced systemic resistance (ISR) [54]. Interestingly, ARK-1 shows ISR priming [55]. The *LOX-9* gene, which is used as a marker of JA signaling, was induced 24 and 48 h after *A. vitis* (Ti) inoculation alone in ARK-1-pre-treated grapevine plants, but *PR-1* (SA signaling), *PR-4* (SA signaling), *PDF1.2* (JA signaling), and *ERF* (ET signaling) were not [55]. LOX is known to be involved in the production of oxidized fatty acids in plants, which makes it the precursor of signal molecules such as oxylipins [56]. The activation of SAR and/or ISR by *P. putida*, *Bacillus* spp., and nonpathogenic rhizobacteria was associated with the inducing of the *LOX-9* gene and LOX activity in tomato and bean [57]. In the case of pre-treatment with the ARK-1 strain, therefore, priming the *LOX-9* gene might be part of the biocontrol activity, together with reducing Ti strain populations and suppressing the expression of *vir* genes of pTi in grapevine tissues [44,46,53]. However, the biological role of the *LOX-9* gene is still unknown. Further research, such as transcriptome analyses, is needed and could help to reveal the role of this unique *LOX-9* gene priming activity in the suppressive activity against GCG.

5. Conclusions

GCG is a devastating bacterial disease in grapevines and is still severe problem in agriculture that continuously causes a huge, negative economic impact [2,3]. The economic impact of GCG varies significantly depending upon the region. For example, in the state of Pennsylvania, losses over a 6-year period were estimated at 46,500 USD per 0.4 ha of vineyard [58,59]. Thus, the management of GCG is a top priority in commercial vineyards, but GCG is one of the most difficult plant diseases to control. Viticultural practices and chemical control designed to inhibit GCG are only partially effective presently [3,59]. Therefore, a biocontrol method could hopefully be an effective approach to GCG management. This review article shows that nonpathogenic and antagonistic *A. vitis* ARK-1, which was isolated in Japan, can effectively inhibit GCG in fields. A hypothesis about the biocontrol effect mechanism of ARK-1 based on our research results is shown in Figure 2B. Antibiosis is not the main mechanism of biocontrol when using ARK-1. ARK-1 also reduces the population of the Ti strain at the wound site. It seems that suppressing the expression of *vir* genes is the key mechanism to inhibit GCG. Moreover, ISR priming might be related as a subordinate factor. Thus, ARK-1 can suppress the development of GCG via what appears to be a previously unreported mechanism. Recently, the results of a genomic analysis of *A. vitis* using draft whole-genome sequencing were reported [60]. In addition, whole-genome sequences of Japanese *A. vitis* strains were reported [61–63]. ARK-1 isolated in Japan should be analyzed using whole-genome information to clarify what kinds of genes are key in the biocontrol mechanism. Additional research is required to determine whether either of these mechanisms is correct.

This study may contribute to controlling CG in diverse crop species, including grapevine, around the world in the near future. Presently, our research group is developing a new biopesticide made from ARK-1 and obtaining positive results indicating that the new biopesticide treatment is effective in the management of CG in grapevines and other plant species as shown in several field trials. The new ARK-1 biopesticide could contribute to managing CG in agriculture around the world.

Funding: This research was funded by Grant-in-Aid for Scientific Research (KAKENHI) from Japanese Society for the Promotion of Science, grant numbers 25850038 and 17H03778.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: The author expresses their sincere thanks to the following people for their useful comments: K. Inoue, H. Nasu, N. Kirino, M. Takagaki, K. Ozaki, N. Myojo, H. Sawada, K. Kondo, Y. Ichinose, T. Shiraishi, K. Suzaki, T. Burr, A. Kerr, M. Nita, and Y. Noutoshi.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Kado, C.I. Crown gall. *The Plant Health Instructor* **2002**. [CrossRef]
2. Kawaguchi, A. Biological control for grapevine crown gall. In *Grapevines: Varieties, Cultivation and Management*; Szabo, P.V., Shojania, J., Eds.; Nova Science Publishers: New York, NY, USA, 2012; pp. 153–167.
3. Burr, T.J.; Bazzi, C.; Süle, S.; Otten, L. Crown gall of grape: Biology of *Agrobacterium vitis* and the development of disease control strategies. *Plant Dis.* **1998**, *82*, 1288–1297. [CrossRef]
4. Kawaguchi, A.; Inoue, K.; Tanina, K.; Nita, M. Biological control for grapevine crown gall using nonpathogenic *Rhizobium vitis* strain ARK-1. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* **2017**, *93*, 547–560. [CrossRef]
5. Kawaguchi, A.; Inoue, K. Grapevine crown gall caused by *Rhizobium radiobacter* (Ti) in Japan. *J. Gen. Plant Pathol.* **2009**, *75*, 205–212. [CrossRef]
6. Mousavi, S.A.; Willems, A.; Nesme, X.; de Lajudie, P.; Lindstrom, K. Revised phylogeny of Rhizobiaceae: Proposal of the delineation of *Pararhizobium* gen. nov., and 13 new species combinations. *Syst. Appl. Microbiol.* **2015**, *38*, 84–90. [CrossRef] [PubMed]
7. Kawaguchi, A. Risk assessment of inferior growth and death of grapevines due to crown gall. *Euro. J. Plant Pathol.* **2022**, *162*. *in press*. [CrossRef]
8. Chilton, M.D.; Drummond, M.H.; Merlo, D.J.; Sciaky, D.; Montoya, A.L.; Gordon, M.P.; Nester, E.W. Stable incorporation of plasmid DNA into higher plant cells: The moleculer basis of crown gall tumorigenesis. *Cell* **1977**, *11*, 263–271. [CrossRef]
9. Zupan, J.R.; Zambryski, P. The *Agrobacterium* DNA transfer complex. *Crit. Rev. Plant Sci.* **1997**, *16*, 279–295. [CrossRef]
10. Gelvin, S.B. Traversing the cell: *Agrobacterium* T-DNA's journey to the host genome. *Front. Plant Sci.* **2012**, *3*, 52. [CrossRef] [PubMed]
11. Nester, E.W. *Agrobacterium*: Nature's genetic engineer. *Front. Plant Sci.* **2015**, *5*, 730. [CrossRef]
12. New, P.B.; Kerr, A. Biological control of grown gall: Field measurements and glasshouse experiments. *J. Appl. Bacteriol.* **1972**, *35*, 279–287. [CrossRef]
13. Kerr, A.; Htay, K. Biological control of crown gall through bacteriocin production. *Physiol. Plant Pathol.* **1974**, *4*, 37–44. [CrossRef]
14. Moore, L.W.; Warren, G. *Agrobacterium radiobacter* strain 84 and biological control of crown gall. *Ann. Rev. Phytopathol.* **1979**, *17*, 163–179. [CrossRef]
15. Kerr, A. Biological control of crown gall through production of agrocin 84. *Plant Dis.* **1980**, *64*, 24–30.
16. Kerr, A.; Bullard, G. Biocontrol of crown gall by *Rhizobium rhizogenes*: Challenges in biopesticide commercialization. *Agronomy* **2020**, *20*, 1126. [CrossRef]
17. Reader, J.S.; Ordoukhanian, P.T.; Kim, J.G.; de Crécy-Lagard, V.; Hwang, I.; Farrand, S.; Schimmel, P. Major biocontrol of plant tumors targets tRNA synthetase. *Science* **2005**, *309*, 1533. [CrossRef] [PubMed]
18. Jones, D.A.; Kerr, A. *Agrobacterium radiobacter* strain K1026, a genetically engineered derivative of strain K84, for biological control of crown gall. *Plant Dis.* **1989**, *73*, 15–18. [CrossRef]
19. Penyalver, R.; Vicedo, B.; López, M.M. Use of the genetically engineered *Agrobacterium* strain K1026 for biological control of crown gall. *Euro. J. Plant Pathol.* **2000**, *106*, 801–810. [CrossRef]
20. Staphorst, J.L.; van Zyl, F.G.H.; Strijdom, B.W.; Groenewold, Z.E. Agrocin-producing pathogenic and nonpathogenic biotype-3 strains of *Agrobacterium tumefaciens* active against biotype-3 pathogens. *Curr. Microbiol.* **1985**, *12*, 45–52. [CrossRef]
21. Burr, T.J.; Reid, C.L. Biological control of grape crown gall with nontumorigenic *Agrobacterium vitis* F2/5. *Am. J. Enol. Viticul.* **1994**, *45*, 213–219.
22. Burr, T.J.; Reid, C.L.; Taglicti, E.; Bazzi, C.; Süle, S. Biological control of grape crown gall by strain F2/5 is not associated with agrocin production or competition for attachment site on grape cells. *Phytopathology* **1997**, *87*, 706–711. [CrossRef]
23. Burr, T.J.; Otten, L. Crown gall of grape: Biology and disease management. *Annu. Rev. Phytopathol.* **1999**, *37*, 53–80. [CrossRef]
24. Kaewnum, S.; Zheng, D.; Reid, C.L.; Johnson, K.L.; Gee, J.C.; Burr, T.J. A host-specific biological control of grape crown gall by *Agrobacterium vitis* strain F2/5; its regulation and population dynamics. *Phytopathology* **2013**, *103*, 427–435. [CrossRef]
25. Webster, J.; Thomson, J.A. Agrocin-producing *Agrobacterium tumefaciens* strain active against grapevine isolates. *Appl. Environ. Microbiol.* **1986**, *52*, 217–219. [CrossRef]
26. Chen, X.Y.; Xiang, W.N. A strain of *Agrobacterium radiobacter* inhibits growth and gall formation by biotype III strains of *Agrobacterium tumefaciens* from grapevine. *Acta. Microbiol. Sin.* **1986**, *26*, 193–199. [CrossRef]
27. Wang, H.M.; Wang, H.X.; Ng, T.B.; Li, J.Y. Purification and characterization of an antibacterial compound produced by *Agrobacterium vitis* strain E26 with activity against *A. tumefaciens*. *Plant Pathol.* **2003**, *52*, 134–139. [CrossRef]
28. Liang, Y.; Di, Y.; Zhao, J.; Ma, D. A biotype 3 strain of *Agrobacterium radiobacter* inhibits crown gall formation on grapevine. *Acta Microbiol. Sin.* **1990**, *30*, 165–171.
29. Chen, F.; Guo, Y.B.; Wang, J.H.; Li, J.Y.; Wang, H.M. Biological control of grape crown gall by *Rahnella aquatilis* HX2. *Plant Dis.* **2007**, *91*, 957–963. [CrossRef]
30. Xie, X.M.; You, J.F.; Chen, P.M.; Guo, J.M. On a strain MI15 of *Agrobacterium radiobacter* for biological control of grapevine crown gall. *Acta Phytopathol. Sin.* **1993**, *23*, 137–141.

31. Ferrigo, D.; Causin, R.; Raiola, A. Effect of potential biocontrol agents selected among grapevine endophytes on crown gall disease. *Bio Control.* **2017**, *62*, 821–833. [CrossRef]
32. Kawaguchi, A.; Sawada, H.; Inoue, K.; Nasu, H. Multiplex PCR for the identification of *Agrobacterium* biover 3 strains. *J. Gen. Plant Pathol.* **2005**, *71*, 54–59. [CrossRef]
33. Kawaguchi, A.; Inoue, K.; Nasu, H. Inhibition of crown gall formation by *Agrobacterium radiobacter* biovar 3 strains isolated from grapevine. *J. Gen. Plant Pathol.* **2005**, *71*, 422–430. [CrossRef]
34. Kawaguchi, A.; Inoue, K. New antagonistic strains of non-pathogenic *Agrobacterium vitis* to control grapevine crown gall. *J. Phytopathol.* **2012**, *160*, 509–518. [CrossRef]
35. Kawaguchi, A. Studies on the diagnosis and biological control of grapevine crown gall and phylogenetic analysis of tumorigenic *Rhizobium vitis*. *J. Gen. Plant Pathol.* **2009**, *75*, 462–463. [CrossRef]
36. Kawaguchi, A.; Sawada, H.; Ichinose, Y. Phylogenetic and serological analyses reveal genetic diversity of *Agrobacterium vitis* strains in Japan. *Plant Pathol.* **2008**, *57*, 747–753. [CrossRef]
37. Kawaguchi, A. Genetic diversity of *Rhizobium vitis* strains in Japan based on multilocus sequence analysis of *pyrG*, *recA* and *rpoD*. *J. Gen. Plant Pathol.* **2011**, *77*, 299–303. [CrossRef]
38. Kawaguchi, A.; Sone, T.; Ochi, S.; Matsushita, Y.; Noutoshi, Y.; Nita, M. Origin of pathogens of grapevine crown gall disease in Hokkaido in Japan as characterized by molecular epidemiology of *Allorhizobium vitis* strains. *Life* **2021**, *11*, 1265. [CrossRef]
39. Wong, A.T.; Kawaguchi, A.; Nita, M. Efficacy of a biological control agent *Rhizobium vitis* ARK-1 against Virginia *R. vitis* isolates, and relative relationship among Japanese and Virginia *R. vitis* isolates. *Crop Prot.* **2021**, *146*, 105685. [CrossRef]
40. Kawaguchi, A.; Noutoshi, Y. Migration of biological control agent *Rhizobium vitis* strain ARK-1 in grapevine stems and inhibition of galls caused by tumorigenic strain of *R. vitis*. *J. Gen. Plant Pathol.* **2022**, *88*, 63–68. [CrossRef]
41. Kawaguchi, A.; Inoue, K.; Nasu, H. Biological control of grapevine crown gall by nonpathogenic *Agrobacterium vitis* strain VAR03-1. *J. Gen. Plant Pathol.* **2007**, *73*, 133–138. [CrossRef]
42. Kawaguchi, A.; Inoue, K.; Ichinose, Y. Biological control of crown gall of grapevine, rose, and tomato by nonpathogenic *Agrobacterium vitis* strain VAR03-1. *Phytopathology* **2008**, *98*, 1218–1225. [CrossRef]
43. Kawaguchi, A.; Kondo, K.; Inoue, K. Biological control of apple crown gall by nonpathogenic *Rhizobium vitis* strain VAR03-1. *J. Gen. Plant Pathol.* **2012**, *78*, 287–293. [CrossRef]
44. Kawaguchi, A. Biological control of crown gall on grapevine and root colonization by nonpathogenic *Rhizobium vitis* strain ARK-1. *Microbes Environ.* **2013**, *28*, 306–311. [CrossRef]
45. Kawaguchi, A.; Inoue, K.; Tanina, K. Evaluation of the nonpathogenic *Agrobacterium vitis* strain ARK-1 for crown gall control in diverse plant species. *Plant Dis.* **2015**, *99*, 409–414. [CrossRef] [PubMed]
46. Gan, H.M.; Szegedi, E.; Fersi, R.; Chebil, S.; Kovács, L.; Kawaguchi, A.; Hudson, A.O.; Burr, T.J.; Michael, A.; Savka, M.A. Insight into the microbial co-occurrence and diversity of 73 grapevine (*Vitis vinifera*) crown galls collected across the northern hemisphere. *Front. Microbiol.* **2019**, *10*, 1896. [CrossRef] [PubMed]
47. Saito, K.; Watanabe, M.; Matsui, H.; Yamamoto, M.; Ichinose, Y.; Toyoda, K.; Kawaguchi, A.; Noutoshi, Y. 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.* **2018**, *84*, 58–64. [CrossRef]
48. Kawaguchi, A. Reduction in pathogen populations at grapevine wound sites is associated with the mechanism underlying the biological control of crown gall by *Rhizobium vitis* strain ARK-1. *Microbes Environ.* **2014**, *29*, 296–302. [CrossRef]
49. Kawaguchi, A. Biological control agent *Agrobacterium vitis* strain ARK-1 suppresses expression of the *virD2* and *virE2* genes in tumorigenic *A. vitis*. *Eur. J. Plant Pathol.* **2015**, *143*, 789–799. [CrossRef]
50. Jones, H.D.; Doherty, A.; Wu, H. Review of methodologies and a protocol for the *Agrobacterium*-mediated transformation of wheat. *Plant Methods* **2005**, *1*, 5–11. [CrossRef]
51. Kawaguchi, A.; Nita, M.; Ishii, T.; Watanabe, M.; Noutoshi, Y. Biological control agent *Rhizobium* (=*Agrobacterium*) vitis strain ARK-1 suppresses expression of the essential and non-essential *vir* genes of tumorigenic *R. vitis*. *BMC Res. Notes* **2019**, *12*, 1–6. [CrossRef]
52. Thomma, B.P.; Eggermont, K.; Penninckx, I.A.; Mauch-Mani, B.; Vogelsang, R.; Cammue, B.; Broekaert, W.F. Separate jasmonate-dependent and salicylate- dependent defense response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 15107–15111. [CrossRef] [PubMed]
53. Goellner, K.; Conrath, U. Priming: It's all the world to induced disease resistance. *Eur. J. Plant Pathol.* **2008**, *121*, 233–242. [CrossRef]
54. Pieterse, C.M.J.; Leon-Reyes, A.; Van der Ent, S.; Van Wees, S.C.M. Networking by small-molecule hormones in plant immunity. *Nat. Chem. Biol.* **2009**, *5*, 308–316. [CrossRef]
55. Kawaguchi, A.; Noutoshi, Y. Insight into inducing disease resistance with *Allorhizobium vitis* strain ARK-1, a biological control agent against grapevine crown gall disease. *Eur. J. Plant Pathol.* **2022**, *162*, 981–987. [CrossRef]
56. Perazzolli, M.; Roatti, B.; Bozza, E.; Pertot, I. *Trichoderma harzianum* T39 induces resistance against downy mildew by priming for defense without costs for grapevine. *Biol. Cont.* **2011**, *58*, 74–82. [CrossRef]
57. Akram, A.; Ongena, M.; Duby, F.; Dommes, J.; Thonart, P. Systemic resistance and lipoxygenase-related defense response induced in tomato by *Pseudomonas putida* strain BTP1. *BMC Plant Biol.* **2008**, *8*, 113. [CrossRef] [PubMed]

58. Filo, A.; Sabbatini, P.; Sundin, G.W.; Zabadal, T.J.; Safir, G.R.; Cousins, P.S. Grapevine crown gall suppression using biological control and genetic engineering: A review of recent research. *Am. J. Enol. Vitic.* **2013**, *64*, 1–14. [CrossRef]
59. Stewart, E.L.; Wenner, N.G. Grapevine decline in Pennsylvania and New York. *Wine East.* **2004**, *32*, 12–53.
60. Kuzmanović, N.; Biondi, E.; Overmann, J.; Puławska, J.; Verbarg, S.; Kornelia Smalla, K.; Lassalle, F. Genomic analysis provides novel insights into diversification and taxonomy of *Allorhizobium vitis* (i.e. *Agrobacterium vitis). BMC Genomic.* **2022**, *23*, 462. [CrossRef]
61. Noutoshi, Y.; Toyoda, A.; Ishii, T.; Saito, K.; Watanabe, M.; Kawaguchi, A. Complete genome sequence data of tumorigenic *Rhizobium vitis* strain VAT03-9, a causal agent of grapevine crown gall disease. *Mol. Plant Microbe Interact.* **2020**, *33*, 1280–1282. [CrossRef]
62. Noutoshi, Y.; Toyoda, A.; Ishii, T.; Saito, K.; Watanabe, M.; Kawaguchi, A. Complete genome sequence data of nonpathogenic and nonantagonistic strain of *Rhizobium vitis* VAR06-30 isolated from grapevine rhizosphere. *Mol. Plant Microbe Interact.* **2020**, *33*, 1283–1285. [CrossRef] [PubMed]
63. Noutoshi, Y.; Toyoda, A.; Ishii, T.; Saito, K.; Watanabe, M.; Kawaguchi, A. Complete genome sequence data of nonpathogenic *Rhizobium vitis* strain VAT03-1, a biological control agent for grapevine crown gall disease. *Mol. Plant Microbe Interact.* **2020**, *33*, 1451–1453. [CrossRef] [PubMed]