

Article

The Role of Root Endophyte *Pseudomonas putida* A32 in the Protection of Two Pepper Genotypes from *Pseudomonas syringae* pv. *aptata*

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Abstract: Endophytes, as an integral part of plants, form unique relationships with their hosts that go beyond classical definitions of symbiosis and influence plant development, immunity, and stress responses. The pepper endophyte strain *Pseudomonas putida* A32 has several plant growth-promoting properties and increases the tolerance of pepper to drought, but its biocontrol potential is unknown. In this study, we investigated the protective role of *P. putida* A32 against infection with the pathogenic bacterium *P. syringae* pv. *aptata* P21 in two pepper genotypes in laboratory experiments. The percentage of lesion reduction in genotype 26 treated with *P. putida* A32 was 46.62%. The results showed a significant reduction in hydrogen peroxide and malondialdehyde levels by 29.45 and 20.22%, respectively, in infected genotype 26. The treated but uninfected controls showed a significant increase in superoxide dismutase activity in genotype 26 by 41.26% and ascorbate peroxidase activity in genotype 19 by 40.28% in the treated infected plants. The tolerant genotype 19 was much less dependent on the bacterial treatment under stress conditions than the susceptible genotype 26. Future research will investigate the role of *P. putida* A32 in the induced systemic resistance of different pepper genotypes to protect against pathogens.

Keywords: biotic stress; biocontrol; phytopathogen; sustainable agriculture



Academic Editor: Francesco Lops

Received: 11 April 2025

Revised: 8 May 2025

Accepted: 13 May 2025

Published: 15 May 2025

Citation: Mesaroš, A.; Nedeljković, M.; Atanasković, I.; Anđelković, M.; Danojević, D.; Stanković, S.; Lozo, J. The Role of Root Endophyte

Pseudomonas putida A32 in the Protection of Two Pepper Genotypes from *Pseudomonas syringae* pv. *aptata*. *Horticulturae* **2025**, *11*, 536.

<https://doi.org/10.3390/horticulturae11050536>

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1. Introduction

The demands placed on agricultural production are growing and are increasingly challenging to meet. The growth of the world's population increases the need for food production. Agriculture currently still relies heavily on chemical fertilizers and pesticides, although there are numerous environmental concerns associated with their use [1]. The losses imposed by plant pathogens are very high, and finding a substitution for the intensive use of pesticides is a burning issue. The annual loss of food production due to infection with plant pathogens is in second place, immediately after the losses that could be caused by drought. Various strategies are being used to address this problem, from traditional breeding methods to transgenic technologies or priming methods [2]. However, an alternative that is increasingly being considered to overcome the effects of biotic and abiotic

stressors is the use of microorganisms that promote plant growth and stress resilience as bioinoculants (biofertilizers) [3]. Sustainable agriculture is based on these complex, mostly unexplored relationships between plants and microbes that are crucial for plant growth, health, development, productivity, and disease suppression [4]. An effective method of improving soil microbial communities is reductive soil disinfestation (RSD) as an environmentally friendly method of controlling soil-borne diseases and ensuring crop productivity. This treatment can reduce bacterial and fungal diversity, but also alter the nutrient capacity of the soil, reduce pathogen abundance, and increase interactions within the microbial community of the plant rhizosphere [5]. It has been shown that there is a synergistic effect between RSD, biochar, and antagonistic microbes in the control of *Fusarium* wilt in cucumber by altering the structure of the microbial community in the rhizosphere [6]. The interaction between plants and soil microorganisms that colonize the rhizosphere and roots, and then the entire plant, is considered one of the key factors in the rapid adaptation of the plant to environmental stress [7]. Consequently, this interaction leads to the formation of the holobiont [8], a structure that influences the stability, adaptability, and evolution of the organisms it contains. This bacterial action, called plant growth promotion (PGP), is reflected in the synthesis of phytohormones, enzymes, siderophores, nitrogen fixation, and phosphate solubilization as a means of direct PGP mechanisms [9]. Plants also benefit from these bacteria through indirect PGP mechanisms that protect them from pathogens and induce systemic resistance to diseases. These beneficial microbes can act as biological control agents, suppressing pathogens either directly—through competition, antibiosis, or parasitism—or indirectly, by priming the plant's immune system and enhancing its natural defenses [10].

Members of the *Pseudomonas* genus can grow under a variety of environmental conditions, which affects their wide distribution and the numerous roles they play in living organisms and the environment. They are well-known PGPs, widely used in sustainable agriculture to control disease and promote plant growth [11,12]. However, bacteria from the species *Pseudomonas syringae* are the most widespread plant pathogens on the planet due to their epiphytic and endophytic lifestyle [13]. Their pathogenicity is based on a number of virulence factors, including the translocation of effector proteins into plant cells via the type III secretion system (T3SS). This is their main weapon in plant infection and has been found in all pathogenic strains of this species, while other virulence factors may or may not be present [13]. The strain *Pseudomonas syringae* pv. *aptata* P21, which was originally isolated as a pathogen from sugar beet [14], showed its broad pathogenic potential for numerous plant species such as pepper, tomato, cucumber, zucchini, pea, watermelon, melon, cabbage, lettuce, parsley, onion, beetroot, and Swiss chard (unpublished data). Given its highly virulent potential, supported by the presence of the T3SS and the T6SS, as well as the fine-tuning of the secretion of the effector repertoire and the tuning of virulence [15], it is necessary to identify biological control agents for its control. Natural *Bacillus* spp. isolates and their lipopeptides were proposed as candidates for biological control of this strain [16]. However, the use of this type of secondary metabolites in the environment frequently could not be implemented due to their cytotoxicity and/or genotoxicity. The plant microbiome represents a rich source of potential biocontrol agents capable of protecting the host through both direct antagonistic activity and indirect mechanisms such as induced resistance. For example, sugar beet—identified as the primary site of isolation for *P. syringae* pv. *aptata* P21—also harbors beneficial *Pseudomonas* strains, which enhance resistance to infection with *P. syringae* pv. *aptata* P21 [17]. We have previously shown that the pepper-root endophyte bacterium *P. putida* A32, characterized as a strain with many PGP characteristics, is a good candidate for the protection of pepper against abiotic drought stress. The strain synthesizes hydrogen cyanide and has a broad spectrum of antimicrobial

activity in vitro against various pepper phytopathogenic bacteria [18]. These properties make the strain *P. putida* A32 a promising agent for the biological control of plant pathogens by direct biocontrol mechanisms.

The primary objective of the study was to investigate the protective role of *P. putida* A32 against infection with the pathogenic bacterium *P. syringae* pv. *aptata* P21 in two pepper genotypes, 26 and 19. We also aimed to test the hypothesis that genotype 19, which exhibits tolerance to *Xanthomonas euvesicatoria* and greater drought resilience compared to genotype 26, would also display an enhanced response to infection with *Pseudomonas syringae* pv. *aptata* P21. Moreover, we hypothesize that the enhanced baseline tolerance of genotype 19 to both biotic and abiotic stressors will impact its reliance on microbial assistance, resulting in a comparatively weaker response to beneficial treatments such as *P. putida* A32. In contrast, stress-sensitive genotypes like 26 may exhibit a more pronounced response. Therefore, we aimed to compare these two genotypes but also to look into the mechanisms underlying the induction of infection resistance. This study provides valuable insights into genotype-dependent responses to both pathogenic challenge and beneficial bacterial treatment, highlighting the complex interplay between inherent stress tolerance and microbe-mediated protection in pepper.

2. Materials and Methods

2.1. Bacterial Strains and Growth Conditions

The strain *Pseudomonas putida* A32 used in this study is an endophyte isolated from the roots of pepper genotype 26, as described previously [18]. Plant pathogen *P. syringae* pv. *aptata* P21, previously isolated from sugar beet [14], was used for the infection tests. Both bacterial strains were routinely cultured on Luria–Bertani agar (LA) (all components from Torlak, Belgrade, Serbia) and King’s B agar (KB) (TM Media, Titan Biotech LTD, Rajasthan, India) plates at 30 °C. Prior to experimental use, the strains were grown in Luria–Bertani (LB) broth for 16 h at 30 °C with shaking (IKA KS 3000 i control, IKA-Werke GmbH & Co. KG, Staufen, Germany) to obtain fresh bacterial cultures.

2.2. Antibacterial Activity In Vitro Assay

The antibacterial activity of *P. putida* A32 against *P. syringae* pv. *aptata* P21 was tested using the well-diffusion assay described by Krstić Tomić et al. [19]. To evaluate the antibacterial activity of previously detected volatile antimicrobial compounds produced by *P. putida* A32 [18], split Petri dishes were used. One half of this Petri dish was filled with the LA medium and the other with the KB medium. An overnight culture of *P. syringae* pv. *aptata* P21 adjusted to an OD₆₀₀ of 1 was spotted onto the LA medium as 10 µL drops of 10^{−1} to 10^{−4} serial dilutions, while *P. putida* A32 was inoculated onto the KB agar medium. As a control, plates were prepared in which only the pathogen was inoculated on the LA side, while the opposite side of the plate with KB medium remained uninoculated. The Petri dish was sealed with parafilm and incubated at 30 °C. After 24 h of growth and exposure, colonies growing on serial dilutions spotted on plates were quantified. Each spot was carefully punched out from the agar plate, transferred to a tube containing 1 mL of sterile distilled water, and vortexed for 1 min. The resulting suspension was then transferred to a cuvette, and the optical density at 600 nm was measured (Orion Aquamate 8000, UV-VIS Spectrophotometer, Thermo Scientific, Waltham, MA, USA) [20].

2.3. Bacterial Treatment and Infection Susceptibility Assay

This study included two different genotypes of pepper plants (*Capsicum annuum* L.), the genotype 26 (obtained by self-pollination of a plant of the variety Amfora), which is susceptible to phytopathogenic bacteria *Xanthomonas euvesicatoria*, and the genotype 19,

crossed between the Amfora variety and the pepper line with tolerance to *X. euvesicatoria* [21]. Pepper seeds were sterilized with sodium hypochlorite and ethanol and treated overnight with a culture of *P. putida* A32 strain adjusted to a concentration of 10^8 CFU/mL, as previously established by Hyder et al. (2021) [22]. The seeds of the control groups were treated with sterile water. Seeds were then sown in plastic pots (10 cm × 10 cm × 10 cm) filled with commercial soilless substrate (Plagron, Weert, The Netherlands), placed in a growth box, and watered regularly. The temperature was maintained at 20 ± 2 °C, the relative humidity was 20%, and a 16/8 h photoperiod was applied. The experiment was set up in a completely randomized design and the pots were rotated weakly across the bench to minimize the effects of position on the variability of temperature and light conditions. After six weeks, the plants were infected with *P. syringae* pv. *aptata* P21. The bacterial suspension was adjusted to a concentration of 10^5 CFU/mL and 100 µL of the suspension was injected into the abaxial side of the first true leaf using a sterile syringe. Leaves in the control group were injected with sterile distilled water. Five days after the infection, the leaves were harvested and scanned with a computer scanner. Necrotic lesions were quantified using ImageJ software, version 1.54m (December 2024). [23]. Leaf samples were ground in liquid nitrogen and stored at -80 °C until further use.

2.4. Quantification of Lipid Peroxidation and Hydrogen Peroxide in Pepper Leaves

The determination of the level of lipid peroxidation and H_2O_2 content as a parameter of the stress response was determined using previously established methods [24]. The degree of lipid peroxidation was determined indirectly by measuring the malondialdehyde (MDA) content, using the extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ and expressed as nmol MDA/g dry weight (DW). The hydrogen peroxide content was determined using a spectrophotometric assay based on the oxidation of iodide by H_2O_2 to iodine in an acidic medium. The amount of H_2O_2 was determined using the standard curve and expressed as nmol/g DW.

2.5. Antioxidant Enzyme Activity

The activity of antioxidant enzymes, total soluble peroxidases (POD), ascorbate peroxidase (APX) and superoxide dismutase (SOD) was determined as previously described [24]. In brief, previously prepared protein extracts were used for all spectrophotometric measurements of enzyme activity. For the activity of POD, absorbance was measured at 470 nm for colored products formed in the reaction with guaiacol. The activity of APX was measured at 290 nm, and ascorbate was used as a reactive reagent. SOD activity was measured at 560 nm as an inhibition of the photochemical reduction in nitro blue tetrazolium.

2.6. Determination of Phenylalanine Ammonia Lyase Activity

Phenylalanine ammonia lyase (PAL) activity was assessed by quantifying the production of trans-cinnamic acid, as previously described [25]. The reaction mixture consisted of 100 µL of enzyme extract, 0.5 mL of 50 mM Tris-Cl buffer (pH 8.8), and 0.6 mL of 1 mM L-phenylalanine. The mixture was incubated in water bath at 40 °C for 60 min, and the reaction was stopped by adding 1 mL of 2 N HCl. Absorbance was measured at 290 nm, and enzyme activity was expressed as µg of trans-cinnamic acid per gram of leaf tissue.

2.7. Statistical Analysis

All experiments were performed with three biological replicates. All data were analyzed with an unpaired *t*-test with Welch's correction at the 0.05 probability level using GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

In Vitro and In Planta Antagonistic Activity of Pseudomonas Putida A32 Against Phytopathogen Bacteria Pseudomonas syringae pv. aptata P21

In a well-diffusion essay, the strain *P. putida* A32 showed no inhibitory effect on *P. syringae* pv. *aptata* P21. To confirm the result obtained, we tested different concentrations of the indicator strain P21 and the growth time for the A32 overnight culture. None of these approaches gave a positive result. To determine the antagonistic activity of volatile metabolites produced by this endophyte strain against *P. syringae* pv. *aptata* P21, split Petri dishes were used (Figure 1). The results showed that in the presence of the antagonistic strain, although there was no contact between them, the number of pathogenic bacteria decreased significantly, making this strain a potential candidate as a biological control agent.

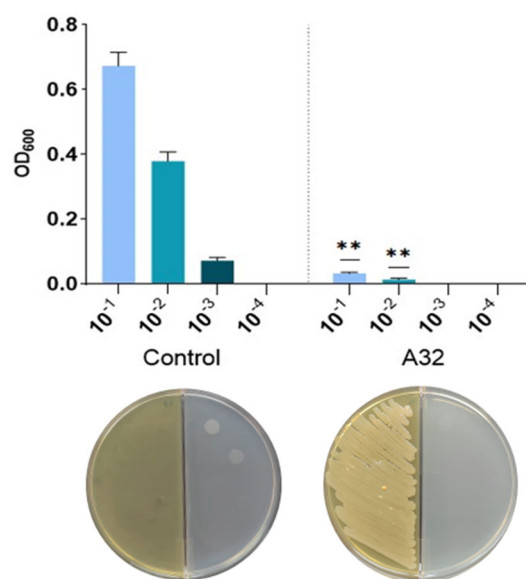


Figure 1. The quantification of the effect of exposure to volatile metabolites of *P. putida* on the growth of *P. syringae* pv. *aptata* P21 on LB agar medium in split Petri dishes. Pathogenic bacteria were grown on 10⁻¹ to 10⁻⁴ dilution spots and exposed to *P. putida* volatiles (A32) or not (control). Images were taken after 24 h of incubation at 30 °C. Each spot was punched out of the LB agar plate and resuspended in water, and the corresponding OD₆₀₀ was determined. Statistics correspond to a two-tailed, unpaired *t*-test with Welch correction. ** *p* ≤ 0.01.

To verify the potential biocontrol activity of *P. putida* A32, we performed experiments in plants. The protective effect of seed pretreatment with the strain *P. putida* A32 was determined by the reduction in the development of lesions due to infection with the phytopathogenic bacterium *P. syringae* pv. *aptata* P21 in both pepper genotypes (Figure 2). This reduction was significant in genotype 26, where lesions were 46.62% smaller than in the control group, and 45% smaller than in genotype 19 plants (Figure 2A,B).

An increased concentration of reactive oxygen species (ROS) is the first stress response, followed by lipid peroxidation among other changes in plant tissue. One of the most important types of ROS is hydrogen peroxide (H₂O₂). After infection, the H₂O₂ concentration decreased significantly in genotype 26, whose seeds had been pretreated with *P. putida* A32. This genotype was more sensitive to the bacterial treatment, as the H₂O₂ concentration increased significantly by 82.16% in uninfected but *P. putida* A32-treated plants compared to the control (Figure 3A). Both genotypes showed a reduction in the concentration of malondialdehyde (MDA), a marker for lipid peroxidation, in the uninfected plants after treatment

with *P. putida* A32 (Figure 3B). It is important to point out that the MDA concentration in the infected plants was also significantly reduced in genotype 26 treated with *P. putida* A32.

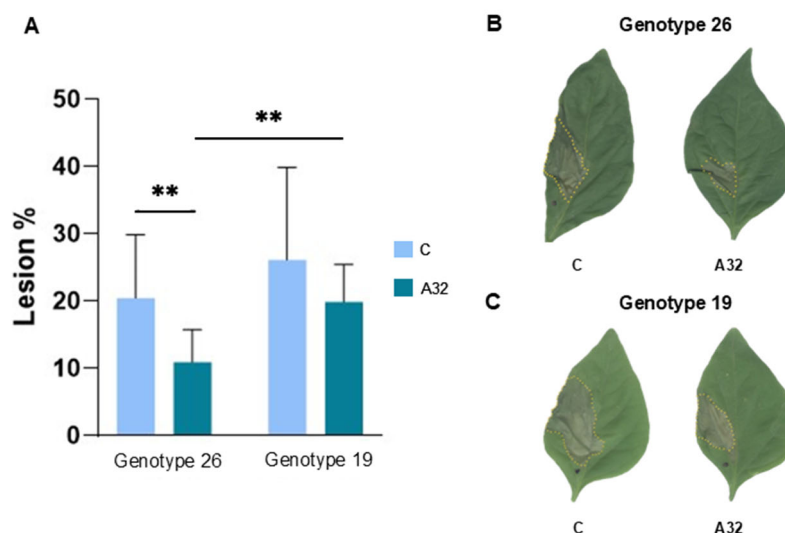


Figure 2. The effect of treatment with *P. putida* A32 on the susceptibility of two pepper genotypes to infection with *P. syringae* pv. *aptata* P21. Pepper seeds were pretreated with *P. putida* A32, and the control group was not exposed to the bacteria. The leaves were infected with *P. syringae* pv. *aptata* P21, and the susceptibility was evaluated by the size of the necrotic lesions at the infection sites. (A) Percentage of lesions due to *P. syringae* pv. *aptata* P21 infection in genotypes 26 and 19 treated with A32 compared to the untreated control group (C). The statistics correspond to a two-tailed unpaired *t*-test with Welch correction. ** $p \leq 0.01$. (B,C) Representative necrotic lesions in genotype 26 (B) and genotype 19 (C) in the control and the A32-treated group.

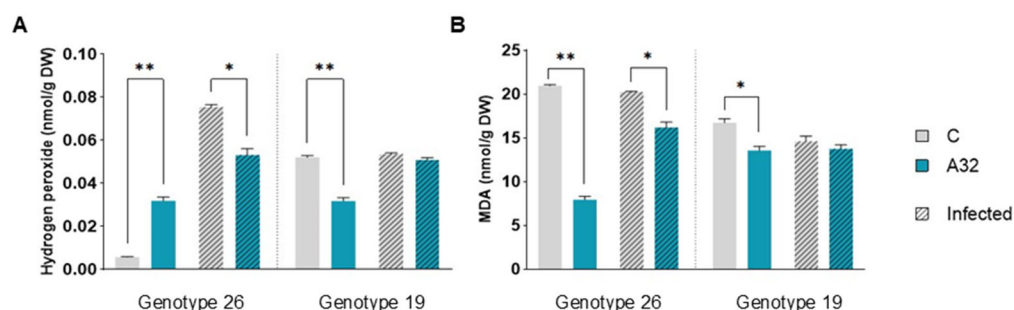


Figure 3. Changes in H₂O₂ and MDA content in pepper plants uninfected and infected with *P. syringae* pv. *aptata* P21. Two pepper genotypes treated with *P. putida* A32 (A32) were compared with the control group (C), which was not exposed to the bacteria. The leaves were harvested 5 days after infection, and the biochemical parameters were analyzed in comparison to the uninfected leaves. (A) Changes in H₂O₂ content. (B) Changes in MDA content. The statistics correspond to a two-tailed unpaired *t*-test with Welch correction. Significant differences are indicated as follows: $p < 0.05$ (*), $p < 0.01$ (**).

The activation of antioxidant enzymes in plants exposed to biotic and abiotic stress is one of the first lines of defense. In genotype 26, uninfected plants treated with *P. putida* A32 exhibited increased superoxide dismutase (SOD) activity (Figure 4A). In contrast, *P. putida* A32-treated plants of genotype 19 showed a significant activation of ascorbate peroxidase (APX) in the infected plants (Figure 4B), while a significant decrease in APX activity was observed in the uninfected plants. Both genotypes showed a significant decrease in the activity of total soluble peroxidases in the infected plants after treatment with *P. putida* A32 (Figure 4C). The activity of phenylalanine ammonia lyase (PAL), an enzyme of phenylpropanoid metabolism related to the biosynthesis of phytoalexins and

other compounds that contribute to pathogen resistance, was determined. However, the activity of PAL was reduced in both uninfected and infected plants after treatment with *P. putida* A32 (Figure 4D).

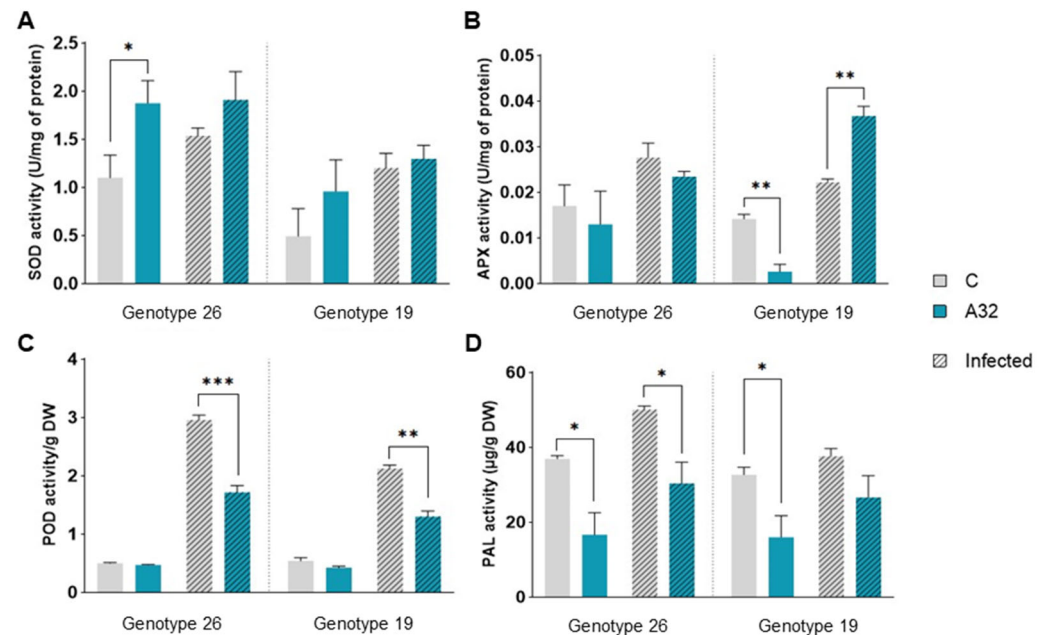


Figure 4. Changes in protective enzyme activity in pepper plants not infected and infected with *P. syringae* pv. aptata P21. Two pepper genotypes treated with *P. putida* A32 (A32) were compared with the control group (C), which was not exposed to bacteria. The leaves were harvested 5 days after infection, and the biochemical parameters were analyzed in comparison to the uninfected leaves. (A) Superoxide dismutase (SOD) activity. (B) Ascorbate peroxidase (APX) activity. (C) Peroxidase (POD) activity. (D) Phenylalanine ammonia lyase (PAL) activity. The statistics correspond to a two-tailed unpaired *t*-test with Welch correction. Significant differences are indicated as follows: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

4. Discussion

Plants, like all other organisms, have a microbiome that serves as a partner in their growth, development, protection, and maintenance of plant health [26,27]. The coevolution of the microbiome with its host plant leads to the development of an even more complex relationship [28]. A unique and very specific part of this microbiome consists of endophytes, microorganisms that inhabit the inner plant tissue and form very close and specific relationships with the plant itself [29]. In this study, we investigated the biocontrol potential of the endophytic pepper-root bacterium *P. putida* A32 against the highly virulent phytopathogenic bacterium *P. syringae* pv. aptata P21. Although the strain did not inhibit the growth of the pathogen in a well-diffusion assay, a split Petri dish test showed an antagonistic effect of the volatile compounds produced, most likely due to HCN synthesis, which has been previously demonstrated [18]. The antagonistic effect of strains synthesizing HCN against various bacteria, fungi, and nematodes, especially those belonging to the group of pathogenic microorganisms, has been previously demonstrated [30]. The results of this study point to previous research that has shown that HCN plays more than just an antagonistic role, as it is involved in the regulation of gene expression in the producer and the host [31]. We were able to demonstrate a protective effect in plants treated with *P. putida* A32 by reducing the size of lesions caused by *P. syringae* pv. aptata P21 infection compared to untreated plants. Since direct inhibition did not yield results, we hypothesize that secondary metabolites are involved in modulating the plant response to pathogen infection. Recent research highlights the role of HCN as a signaling molecule that mod-

ulates plant immunity by inducing the expression of defense-related genes, promoting the production of reactive oxygen species (ROS), and mediating the function of proteins through S-cyanlation, thereby increasing plant resistance not by directly inhibiting the pathogen but by activating its defense mechanisms [31]. Our results emphasize this finding by indicating that the protective effect of the strain is not due to a direct effect on the pathogen, but most likely to a modulation of the response of the plant itself, making it more resistant to the pathogen's action. The seed treatment led to changes in the plant that were most likely caused by the colonization of the plant by *P. putida* A32 that we assume had a greater potential for this due to its endophytic origin, which has already been shown previously [32]. The ability of *Pseudomonas* species to efficiently colonize plants has been demonstrated for *Pseudomonas aurantiaca* JD37, which efficiently colonizes maize roots and protects the plants by inducing resistance to *Bipolaris maydis* [33]. The endophytic nature of the strain *Pseudomonas aeruginosa* FG106 enables it to effectively colonize not only the tomatoes from which it was isolated, but also potatoes, taro, and strawberries, providing effective protection against various pathogenic bacteria and fungi [34].

The genotype of the plant is an important determinant for the development of a positive plant–endophyte association. Several techniques must be used to minimize the effects of the host genotype in plant–bacteria interactions, like micropropagation and vegetative growth [35]. In our study, both pepper genotypes had in common that the plants treated with *P. putida* A32 showed a lower concentration of H₂O₂ and malondialdehyde (MDA) after infection. The measured parameters are interdependent, as the accumulation of reactive oxygen species (ROS) causes lipid peroxidation. This effect of bacterial treatment can be explained by their ability to neutralize the effects of the ROS produced and possibly reduce their production [36]. Our study showed that the genotype 26 plants treated with *P. putida* A32 had significantly increased levels of H₂O₂ under uninfected conditions, which can be explained by the fact that H₂O₂ is not only a reactive oxygen species but also a signaling molecule [37]. This increase could reflect the interactions between this strain and the plant under stress conditions, especially the colonization of the host plant by bacteria. The endophytic nature of *P. putida* A32 allows it to interact with and colonize plants more rapidly, which has already been confirmed for endophytic bacteria [32]. Contrasting results were obtained when duckweed was co-cultivated with *P. gessardii* C31-106/3, with a decrease in H₂O₂ concentration, while the same treatment under salt-stress conditions led to an increase [38,39]. The treatment of duckweed with *P. oryzae* D1-104/3 decreased the H₂O₂ content under salt stress [39]. However, duckweed rhizobacteria *P. oryzae* D1-104/3 and *P. gessardii* C31-106/3 decreased MDA in treated duckweed exposed to salt stress [39]. The treatment of rapeseed growing under water stress with the strain *P. protegens* ML15 also had a similar effect [40]. In addition to abiotic stress, the reduction in MDA after treatment with PGP strains was also demonstrated under biotic stress conditions. The strain *Bacillus subtilis* BS-2301 with biocontrol potential against *Sclerotinia sclerotiorum* reduced the MDA concentration in infected soybean plants [41].

An increase in the activity of antioxidant enzymes and, thereby, an increase in the resistance of plants to stress as a consequence of treatment with PGP bacteria, was reported previously [42]. Our study confirmed a plant genotype-specific response in the activity of enzymes of antioxidant protection. Genotype 19, which is tolerant to *Xanthomonas euvesicatoria*, a biotic stressor, activates ascorbate peroxidase (APX), which is directly involved in the elimination of H₂O₂ as a first line of defense, as we have previously shown for drought as an abiotic stress [18]. Moreover, a comparison of these two genotypes under different growth conditions has shown that both increase APX activity in the field when multiple stressors are present [21]. One explanation for this is the fact that plant peroxidases not only serve to remove excess hydrogen peroxide but are also the first line of defense against

pathogens and toxic compounds in many plants [43]. Thus, APX in genotype 19 is the first antioxidant enzyme to be activated during the defense response. Although we observed a significant decrease in lipid peroxidation in *P. putida* A32-treated and infected plants, indicating a possible increase in superoxide dismutase (SOD) activity, SOD activity was significantly increased only in uninfected plants of genotype 26. This suggests that superoxide anions were removed with bacterial SOD or that *P. putida* A32 produces protective substances that protect the electron transport chains in the mitochondria and chloroplasts, where most superoxide anions are produced [36]. Our previous study has also shown that SOD was one of the enzymes activated in genotype 26 under certain growth conditions in which the plant was isolated with a plastic cover [21]. This renewed response to the bacterial treatment explains the high H_2O_2 concentration even in uninfected plants.

The genotype-specific response was expected, but from all the studies we conducted on these two pepper genotypes, we were able to draw conclusions and propose some hypotheses for future research. It is interesting to note that the tolerant genotype 19 showed a weaker response to treatment with strain *P. putida* A32 in our earlier study [18] and this study. We hypothesize that the reason for this could be that since less decay occurred under the given conditions, the plant was able to conserve energy to initiate various biochemical processes and respond to stress and treatment. On the other hand, the more sensitive genotype 26 responded much better to treatment with the *P. putida* A32 strain. This not only highlights the protective effect of the strain, which is evident in both genotypes, but also demonstrates how the plant responds under the given conditions. Drought and high temperatures lead to changes in the plant that make it more susceptible to biotic factors and eventually lead to its complete collapse. Formulating bioinoculants with strains that can perform more than one protective function makes them more effective and cheaper to produce, and therefore more accessible to the end user in food production. This ability of the proposed candidate strains is rarely analyzed, but its importance, particularly concerning abrupt climate change, is highlighted in a review paper by Anuar and coauthors. [44]. Strain *P. putida* A32 is an excellent candidate that has been shown to be effective in protecting peppers against drought [18] and infection with phytopathogenic bacteria, as demonstrated in this study. In addition, this isolate is an endophyte, which better prepares it for close interaction with the plant. Furthermore, we believe that this classifies it as a strain that is safe for use in biological preparations for sustainable agriculture, as it was already an integral part of the plant tissue. In the future, it will be necessary to validate its effect on induced systemic resistance in plants and protection against disease, as well as the possible role of HCN in this activity. Another future research direction will be to test the efficacy of *P. putida* A32 in the simultaneous application of both stressors, not only in pepper as a model organism, but also in other crops.

Author Contributions: Conceptualization, J.L.; methodology, A.M.; formal analysis, A.M., M.N. and M.A.; resources, D.D.; data curation, I.A.; writing—original draft preparation, J.L.; writing—review and editing, S.S. and I.A.; supervision, J.L.; funding acquisition, S.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant numbers 451-03-137/2025-03/200178, 451-03-136/2025-03/200178, and 451-03-136/2025-03/200032 and the International Centre for Genetic Engineering and Biotechnology grant CRP/SRB23-04_EC.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the corresponding author upon request.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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