Article

Sustainable Wireworm Control in Wheat via Selected *Bacillus thuringiensis* Strains: A Biocontrol Perspective

Marina Dervišević Milenković [1], Magdalena Knežević [2], Marina Jovković [2], Jelena Maksimović [2], Uroš Buzurović [2], Jelena Pavlović [2,*] and Aneta Buntić [2]

[1] Institute of Pesticides and Environmental Protection, Banatska 31b, 11080 Belgrade, Serbia; dervisevic.marina1989@gmail.com

[2] Institute of Soil Science, Teodora Drajzera 7, 11000 Belgrade, Serbia; knez.magdalena@gmail.com (M.K.); soils.jovkovic@gmail.com (M.J.); jelena.maks@yahoo.com (J.M.); soilsbuzurovic@gmail.com (U.B.); anetabuntic@gmail.com (A.B.)

* Correspondence: soils.pavlovic@gmail.com

Abstract

Wireworms are often referred as a hardly manageable group of pests due to their unstable lifestyle and uneven distribution in soils. The current strategy of wireworm control involves the heavy use of chemical pesticides. To find an effective and eco-friendly biological control agent against wireworms, evaluation of bacterial properties and insecticidal effects of six *Bacillus thuringiensis* (*Bt*) strains against *Agriotes lineatus* was performed under laboratory conditions. The presence of *cry11*, *cyt2* and *krsA* gene was detected in *Bt* strain BHC 2.4, while the same strain had the ability to produce siderophores, protease, amylase and cellulase. Single inoculums of *Bt* strains (BHC 2.4; BHC 4.5; BHC 4.7; 1.5; 4.3; 6.1) showed mortality against *Agriotes lineatus* larvae in the range of 6.67–72.22%. However, the compatible *Bt* dual cultures showed significantly higher efficiency in comparison with the single inoculums, with the highest efficiency of 79.63% recorded for *Bt* strain BHC 2.4 + *Bt* strain 1.5. The efficiency of applied *Bt* strains might be associated with the presence of genes coding for antibiotics and toxins. Therefore, the use of selected *Bt* strains applied in a form of compatible mixes could offer a sustainable solution for wireworm management in wheat.

Keywords: *Agriotes lineatus*; entomopathogenic bacteria; biocontrol; microbial inoculant; wheat protection

check for updates

Academic Editor: Aocheng Cao

Received: 30 July 2025

Revised: 26 September 2025

Accepted: 28 September 2025

Published: 29 September 2025

Citation: Dervišević Milenković, M.; Knežević, M.; Jovković, M.; Maksimović, J.; Buzurović, U.; Pavlović, J.; Buntić, A. Sustainable Wireworm Control in Wheat via Selected *Bacillus thuringiensis* Strains: A Biocontrol Perspective. *Agriculture* **2025**, *15*, 2049. https://doi.org/10.3390/agriculture15192049

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Wireworms are a significant group of soil pests that feed on underground parts of a wide range of crops, including vegetables, cereals, ornamental and wild plant species [1]. Among wireworms, *Agriotes lineatus* is one of the most economically important pest species, which can lead to reduced growth of crops, while the yield losses can reach up to 70% [2]. Control of wireworms is a highly challenging process, due to their complex and variable life cycle and uneven distribution in soils [2]. Additionally, an increase in the *Agriotes lineatus* problem has been partly attributed to the discontinuation of previously used insecticides, such as organochlorines, carbamates and organophosphates [3,4]. In addition to the insufficient efficacy and the risk of resistance development in target pests, the excessive use of insecticides results in environmental contamination through leaching into soil and water, adverse effects on non-target organisms (including pollinators and

natural enemies) and accumulation of toxic residues, which together amplify the negative ecological footprint of conventional pest management [5].

Although most farmers still use soil insecticides or insecticide-treated seeds when planting their crops, sustainable agricultural practices, such as a reduction in chemical use for wireworm control, have begun to be more commonly adopted. Some species of entomopathogenic fungi (*Beauveria* spp. and *Metarhizium* spp.) are being reported to infect both larval and adult stages of *Agriotes lineatus* and have been used as biocontrol agents against these pests [6–8]. Nematodes have also been tested against *Agriotes lineatus*, but their effectiveness can be limited by many factors, such as species properties, soil type and agro-ecological properties of the location [9,10]. The use of bacteria appears to be the least studied alternative in wireworm control, although they have been known to successfully control other harmful insects [11]. Danismazoglu et al. [12] indicated that some wireworm-associated bacteria, including *B. thuringiensis*, seemed to be good candidates as possible microbial agents against *A. lineatus* in potato.

Bacillus thuringiensis (*Bt*) has been previously known for its ability to produce a variety of proteins with insecticidal activity (Cry, Vip, Cyt) [13,14]. In general, insecticidal proteins become toxic for the insect after ingestion. Namely, Cry proteins bind to epithelial cells in the insects' midgut, resulting in pore formation, cell lysis and ultimately the death of the larvae [15,16]. Cry toxins produced by *Bt* are highly specific, and are harmless to animals, humans and plants, while their property to completely degrade indicates that no toxic products are left behind. In addition, *Bt* strains produce numerous extracellular compounds, such as exotoxins and vegetative insecticidal proteins, which may contribute to virulence [17]. In crops such as cereals, the germinating seeds need to be protected against *Agriotes lineatus*, as the early stage of plant development represents the most critical phase for susceptibility to wireworm damage [18]. Among *Bacillus* species, *Bt* strains have been the most studied, and their activity is almost exclusively aimed against larval stages of different insects [11,19]. However, the use of *B. thuringiensis* in the biocontrol of *Agriotes lineatus* larvae has not been comprehensively studied yet. In addition to the insecticidal activity of *Bt*, certain bacterial traits associated with biocontrol potential against phytopathogenic fungi and plant growth promotion—such as the ability to solubilize inorganic phosphates, produce siderophores or synthesize other bioactive metabolites—are highly relevant when designing effective inoculants for agricultural applications [20,21]. These traits not only support plant nutrition and growth but also enhance the persistence and competitiveness of the bacteria in the complex soil environment, enabling them to better establish in the rhizosphere and maintain efficacy under natural conditions. Hence, consideration of both biocontrol potential and plant growth-promoting characteristics is essential for selecting strains that can provide dual benefits in crop protection and soil health. Therefore, the aim of this study was to evaluate the insecticidal effect of *Bacillus thuringiensis* strains (BHC 2.4, BHC 4.5, BHC 4.7, 1.5, 4.3 and 6.1) against agricultural pest (*Agriotes lineatus* larvae). The presence of genes coding for antibiotics (*fenD*, *bmyB*, *ituC*, *srfAA*, *bacA*, *spaS* and *krsA*) and toxins (*cry11*, *cry1B*, *vip1*, *vip2*, *cyt1* and *cyt2*) was tested in *Bt* strains, together with enzyme production and other bacterial properties significant for inoculant formulation (siderophore production and phosphate solubilization). The mortality of *Agriotes lineatus* larvae influenced by bacterial inoculation was evaluated under laboratory conditions.

2. Materials and Methods

2.1. Insect Sampling

Agriotes lineatus larvae were collected from a commercial cereal field located in Smederevska Palanka, Serbia (42°21′17″, 20°56′55″) where an increased abundance of *A. lineatus*

had been observed in previous years. Insect collection was conducted using a modified method described by Morales-Rodriguez et al. [22]. Traps were constructed by filling socks with 200 g of a seed mixture, consisting of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) in a 1:1 v/v ratio. Filled socks were then soaked in water for 24 h prior to field placement to initiate seed germination (Figure S1). A total of 20 traps were randomly distributed across the plot, maintaining a distance of at least 5 m between each trap, and were buried so that the top was 5–10 cm below the soil surface. Traps were deployed in early April when soil temperatures reached approximately 10 °C and larvae were feeding near the soil surface due to adequate moisture and temperature. After 14 days, traps were recovered and transported to the laboratory, where their contents were carefully sieved to extract wireworm larvae. When sieving did not allow the complete wireworm isolation, larvae were collected by hand sorting. In addition, isolated larvae were confirmed as *A. lineatus* based on the identification protocol described by the literature [23]. Larvae were then placed in 3 L jars containing finely sieved soil and wheat germ to simulate natural conditions. Potato slices were also added to provide an easily accessible food source. The larvae were stored under these conditions at room temperature in a dark place, until the moment of use in bioassays.

2.2. Bioagents and Preparation of Working Cultures

The isolation of new bacterial isolates from two soil samples obtained in Smederevska Palanka, Serbia (42°21′17″, 20°56′55″) and Lazarevac, Serbia (44°22′28″, 20°15′30″) was carried out based on the serial dilution method, where the isolation was performed by inoculating 1 mL of 10^{-5} of soil suspension dilution to Petri dishes with nutrient agar (NA) (BioLife, Milan, Italy). To obtain the pure cultures of *B. thuringiensis* isolates, colonies with characteristic morphology (large, white to creamy colonies with irregular margins) were re-streaked several times on nutrient agar (NA) and maintained on inclined NA at 4 °C until further use. From a total of 129 bacterial isolates obtained from two rhizosphere soil samples, only those with *Bt*-like morphology were selected for this study (BHC 2.4, BHC 4.5 and BHC 4.7). In addition, three previously characterized *Bt* strains (1.5, 4.3 and 6.1) [24] were included, since the aim of this research was specifically focused on *B. thuringiensis*. For the detection of bacterial properties and compatibility tests, working cultures of bacterial strains were freshly prepared in nutrient broth (BioLife, Milan, Italy) in an orbital shaker (24 h, 130 rpm, 25 °C) (BioBase, Jinan, China) until the concentration of approximate 10^9 CFU mL^{-1} was reached, or in an incubator (BioBase, Jinan, China) (24 h, 28 °C).

2.3. Molecular Identification of Bacterial Isolates

Isolation of the total bacterial DNA was performed as described by Dimkić et al. [25]. Identification of isolates was performed based on the 16S RNA gene sequence by using P_0 (5′-GAGAGTTTGATCCTGGCTCAG-3′) and P_6 (5′-CTACGGCTACCTTGTTACGA-3′) primers (Metabion, Planegg, Bavaria, Germany), following a method previously described by Oro et al. [26]. After the sequence amplification, PCR products were purified and sequenced by using a commercial service (Macrogen Europe). The obtained 16S rRNA gene sequences were manually checked for quality based on the obtained chromatograms and deposited to the National Center for Biotechnology Information database (NCBI) to obtain accession numbers. The Neighbour-Joining (NJ) phylogenetic tree was constructed in the Mega V.11 program. Prior to phylogenetic tree construction, DNA sequences were aligned by the ClustalW function, and the Kimura two-parameter nucleotide substitution model was used to calculate genetic distances. The phylogenetic position of the three tested isolates from this study was determined in relation to the other *Bacillus* species

(NR_041455.1, NR_113991.1, NR_113945.1, NR_112629.1, NR_112630.1, NR_114581.1, NR_043403.1, NR_112780.1, NR_117473.1). The tree was rooted with *Methanobacterium espanolae* (NR_104983.1).

2.4. Detection of Antibiotic- and Toxin-Producing Bacterial Genes

Bacterial isolates were screened for the presence of genes associated with the biosynthesis of different antibiotics, such as *fenD* (fengycin), *bmyB* (bacyllomicin), *ituC* (iturin), *srfAA* (surfactin), *bacA* (bacylisin), *spaS* (subtilin) and *krsA* (kurstakin), by using previously described PCR protocols [27,28]. Also, the presence of *cry11* and *cry1B* (crystal protein) genes was tested by using the protocol described by Jain et al. [29] and Thammasittirong and Attathom [30], while the presence of *vip1* and *vip2* (vegetative insecticidal protein synthetase) was tested as described in Senthilkumar et al. [31]. The presence of *cyt1* and *cyt2* (cytolytic toxin synthetase) was evaluated based on Ibarra et al. [32].

All PCR (Eppendorf, Hamburg, Germany) amplifications were performed in a total reaction volume of 25 mL consisting of the following PCR components: 12.5 mL of PCR TaqNova-RED Master Mix (2X) (Qiagen, Hilden, Germany), 9.5 mL of PCR-grade water, 1 mL of primers (forward and reverse) and 1 mL of bacterial DNA. The obtained PCR amplicons were checked on the transilluminator (Hoefer, Holliston, MA, USA) for the presence of a band at the expected position in relation to the 100–10,000 bp DNA ladder (Thermo Fisher Scientific, Waltham, MA, USA), and the presence/absence of the band was considered a positive/negative reaction. The list of all DNA primers used in this study, as well the expected lengths of obtained PCR products, is presented in Table 1.

Table 1. DNA primers used for the screening of bacterial antibiotic- and toxin-producing genes.

Gene	Primer	Sequence (5′ → 3′)	Product Size (bp)	Reference
fenD	FEND-F	GGCCCGTTCTCTAAATCCAT	269	[25]
	FEND-R	GTCATGCTGACGAGAGCAAA		
bmyB	BMYB-F	GAATCCCGTTGTTCTCCAAA	370	
	BMYB-R	GCGGGTATTGAATGCTTGTT		
ituC	ITUC-F	GGCTGCTGCAGATGCTTTAT	423	
	ITUC-R	TCGCAGATAATCGCAGTGAG		
srfAA	SRFA-F	TCGGGACAGGAAGACATCAT	201	
	SRFA-R	CCACTCAAACGGATAATCCTGA		
bacA	BAC-F	CAGCTCATGGGAATGCTTTT	498	
	BAC-R	CTCGGTCCTGAAGGGACAAG		
spaS	SPAS-F	GGTTTGTTGGATGGAGCTGT	375	
	SPAS-R	GCAAGGAGTCAGAGCAAGGT		
krsA	AKS-F	TCHACWGGRAATCCAAAGGG	1125, 1152, 1161, 1167, 1173	[26]
	TKS-R	CCACCDKTCAAAKAARKWATC		
cry11	Un11-F	TTCCAACCCAACTTTCAAGC	305	[27]
	Un11-R	AGCTATGGCCTAAGGGGAAA		
cry1B	c1B-F	CAGAAACAACAGAACGACC	921	[28]
	c1B-R	CACTTCCCCACCATCCAT		
vip1	Vip1-sc.fw	TATTAGATAAACAACAACAAGAATATCAATCTATTMGNTGGATHGG	585	[29]
	Vip1-sc.rev	GATCTATATCTCTAGCTGCTTTTTCATAATCTSARTANGGRTC		

Table 1. *Cont.*

Gene	Primer	Sequence (5′ → 3′)	Product Size (bp)	Reference
vip2	Vip2-sc.fw	GATAAAGAAAAAGCAAAAGAATGGGRNAARRA	845	[29]
	Vip2-sc.rev	CCACACCATCTATATACAGTAATATTTTCTGGDATNGG		
cyt1	cyt1gral-F	CCTCAATCAACAGCAGGGTTATT	477–480	[30]
	cyt1gral-R	TGCAAACAGGACATTGTATGTGTAATT		
cyt2	cyt2gral-F	ATTACAAATTGCAAATGGTATTCC	355–356	
	cyt2gral-R	TTTCAACATCCACAGTAATTTCAAATGC		

2.5. Detection of Bacterial Properties

2.5.1. Phosphate Solubilization and Siderophore Production

To evaluate additional traits potentially relevant for bacterial survival and efficacy in the soil, *Bt* isolates were tested for their ability to solubilize inorganic phosphates and produce siderophores. Although these traits are not directly linked to insecticidal activity of bacteria, they may enhance bacterial persistence and colonization in the rhizosphere, thereby supporting the long-term effectiveness of *Bt* strains as inoculants in agricultural soils. Phosphate solubilization capacity of bacterial strains was investigated by using the methodology described in Rokhbakhsh-Zamin et al. [33]. Strains were grown on Pikovskaya medium, and the appearance of a halo zone around the bacterial growth (7 days, 28 °C) was considered a positive result. Siderophore production was determined by growing bacterial strains on Chrome Azurol S (CAS) (Fluka, Buchs, Switzerland) Blue agar (5 days, 28 °C) [34]. A change in the color of the CAS agar from blue to yellow was considered a positive result.

2.5.2. Enzymatic Activity

The production of extracellular protease, lipase, cellulase and amylase was evaluated qualitatively. Bacterial strains were spot inoculated on skim milk agar (SMA, 3%), on NA medium amended with Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) [35] and on a medium supplemented with 1 g L^{-1} of carboxymethyl cellulose (Thermo Fisher Scientific, Waltham, MA, USA) or starch (Lach:Ner, Brno, Czech Republic) [36] for the production of protease, lipase, cellulase and amylase activity, respectively. After an incubation period (3–5 days, 28 °C), the development of a clear zone around the bacterial growth indicates the production of protease, cellulase and amylase, while the presence of calcium complex precipitate around the inoculation site was considered as a positive result for lipolytic activity of isolates.

For the tested bacterial properties (phosphate solubilization and enzymatic activity), results (halo zone radius) were expressed by using the following designations: no production (no halo zone radius); + low production (halo zone radius up to 5 mm); ++ moderate production (halo zone radius up to 10 mm); +++ high production (halo zone radius above 10 mm).

2.6. Compatibility of Bacterial Strains

The compatibility of bacterial strains and their potential to be applied in a form of mixed cultures was evaluated by a modified well diffusion method. Sterile molds for the wells were placed on the solid NA, and 7 mL of NA soft agar inoculated with 100 μL (10^9 CFU mL^{-1}) of the appropriate strain was added. Plates were kept at room temperature for 2 h in order for agar to solidify, and 20 μL of different bacterial cultures was applied in the wells. Plates were then incubated at 28 °C for 24 h. The strains were

considered compatible if no halo zone (growth inhibition) appeared around the wells, and mixtures of compatible strains were selected for the following bioassay.

2.7. Bioassays

For the seed inoculation, the single bacterial cultures were applied, as well as the mixes (dual bacterial inoculum) of different strains in a proportion of 1:1. For the bioassays, bacterial cultures were grown in nutrient broth for 96 h at 28 °C, on a rotary shaker. Wheat seeds were soaked for 5 min in the prepared bacterial suspension (30 mL, 10^9 CFU mL^{-1}) and then dried at room temperature. After that, 100 seeds were placed into Petri dishes including sterile soil (approximately 200 g) in order to mimic natural conditions, and they were left for 72 h to initiate germination. The *A. lineatus* larvae (approximately 15–20 mm in length), visually inspected and confirmed to be healthy and active, were then added. To reduce variability, larvae that were found to have fed on potato slices at the time of selection were used for the bioassays, and in order to increase their sensitivity on bacterial isolates in bioassays, the larvae were left without food for 48 h [37]. Each treatment was replicated three times, and in the control treatment, wheat seeds were soaked in sterile distilled water. The Petri dishes were stored at room temperature in a dark place, and they were checked for mortality daily until 10 days after treatment. The mortality was determined by touching the larvae with a soft brush and observing their response, such as leg movement, body contractions or convulsions. In order to confirm the presence of the bacterial isolates in the cadavers of treated *A. lineatus* larvae, isolation of bacteria was conducted based on the procedure previously described in the literature [12], followed by morphological identification of isolated bacteria.

2.8. Data Analysis

Mortality data were corrected according to Abbott's formula [38]. One-way ANOVA (post hoc: Duncan) was applied to determine the significance of the differences among the average efficacy of individual treatments. Data were expressed as mean ± SD.

3. Results

3.1. Molecular Identification of Bacterial Isolates

Based on the NJ tree, three tested isolates were identified as *Bacillus thuringiensis* (Figure 1). The obtained sequences were deposited to the NCBI database under the following accession numbers: *B. thuringiensis* BHC 2.4—PV018794; *B. thuringiensis* BHC 4.5—PV018795; and *B. thuringiensis* BHC 4.7—PV018839.

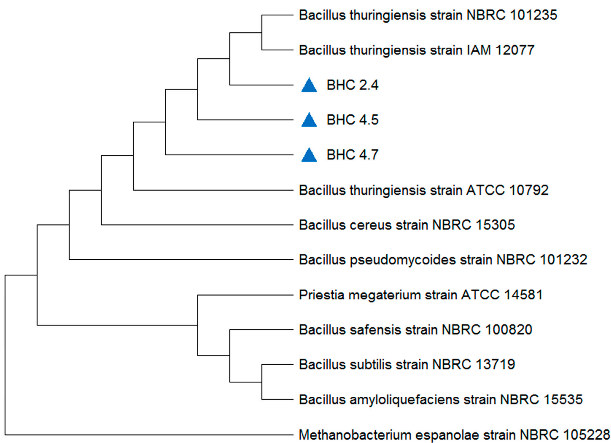

Figure 1. Neighbor-Joining phylogenetic tree based on 16S rRNA sequences. Three *Bacillus* spp. isolates (BHC 2.4; BHC 4.5 and BHC 4.7) characterized in this study are marked with a blue triangle.

3.2. Detection of Antibiotic- and Toxin-Producing Bacterial Genes

The results of PCR testing showed that *Bt* strains BHC 2.4, BHC 4.5 and BHC 4.7 do not have the ability to produce antibiotics such as surfactin, bacylisin, fengycin, bacillomycin and iturin C (Table S1). Out of the previously identified strains, the gene for bacylisin was detected for strain *Bt* 4.3 in our previous research [24]. *Bacillus thuringiensis* strain BHC 2.4 was the only strain positive for the presence of kurstakin. In addition, the presence of *cry11* and *cyt2* genes was confirmed only for the *Bt* strain BHC 2.4, while *B. thuringiensis* strains BHC 4.5, 4.7, 1.5 and 4.3 were positive for the presence of *cry1B* gene (Table 2). On the other hand, the presence of *vip1* and *vip2* genes was not detected in tested strains.

Table 2. The presence of toxin-producing genes in 6 tested bacterial strains.

Toxin-Producing Gene	Bacterial Strain					
	***B. thuringiensis* BHC 2.4**	***B. thuringiensis* BHC 4.5**	***B. thuringiensis* BHC 4.7**	***B. thuringiensis* 1.5**	***B. thuringiensis* 4.3**	***B. thuringiensis* 6.1**
cry11	+	−	−	−	−	−
cry1B	−	+	+	+	+	−
vip1	−	−	−	−	−	−
vip2	−	−	−	−	−	−
cyt1	−	−	−	−	−	−
cyt2	+	−	−	−	−	−

3.3. Bacterial Properties of Tested Bacillus thuringiensis Strains

The results of the qualitative and quantitative tests for the PGP and biocontrol traits of six *B. thuringiensis* strains are presented in Table 3. Out of the six tested strains, only two *Bt* strains (BHC 4.5 and BHC 4.7) were able to solubilize phosphates. Among the new *Bt* strains, BHC 2.4 was the highest siderophore producer, followed by *Bt* strains BHC 4.5 and BHC 4.7. The ability to produce amylase, cellulase and protease was confirmed for all tested strains, where *Bt* strain BHC 2.4 showed the highest potential for the production of extracellular enzymes. Lipase activity was confirmed only for *Bt* strain BHC 4.5.

Table 3. Bacterial properties of the tested bacterial strains.

Bacterial Strains	Amylase	CMCase	Protease	Lipase	Phosphate Solubilization	Siderophore (mm)
B. thuringiensis BHC 2.4	++	+++	+++	−	−	17 ± 0.58
B. thuringiensis BHC 4.5	++	++	++	+	+	9 ± 0.29
B. thuringiensis BHC 4.7	+++	++	+	−	+	7 ± 0.50
B. thuringiensis 1.5	++	++ *	+ *	−	− *	1.83 ± 0.58 *
B. thuringiensis 4.3	++	+++ *	++ *	−	− *	− *
B. thuringiensis 6.1	++	+ *	++ *	−	− *	− *

– no production (no halo zone); + low production (halo zone radius up to 5 mm); ++ moderate production (halo zone radius up to 10 mm); +++ high production (halo zone radius above 10 mm); * previously published in [24].

3.4. Compatibility of Bacillus thuringiensis Strains

The compatibility of the six tested *Bt* strains for their potential application in a form of mixed culture is presented in Table 4.

According to the well diffusion test, *Bt* strains 1.5 and 4.3 showed compatibility with two strains. On the other hand, *Bt* strains BHC 2.4, BHC 4.5 and BHC 4.7 were compatible with only one strain. *Bacillus thuringiensis* strain 6.1 was not compatible with other tested strains. Besides single-strain inoculums, the selection of strains to be used in a form of mixture (dual bacterial inoculum) in the wireworm bioassay was determined: mix 1: BHC 2.4 + 1.5; mix 2: BHC 2.4 + 4.3; mix 3: BHC 4.7 + BHC 4.5; mix 4: BHC 4.7 + 4.3; mix 5: 1.5 + 4.3.

Table 4. Compatibility of *Bacillus thuringiensis* strains.

Bacterial Strain	BHC 2.4	BHC 4.5	BHC 4.7	1.5	4.3	6.1
B. thuringiensis BHC 2.4		–	–	+	+	–
B. thuringiensis BHC 4.5	–		+	–	–	–
B. thuringiensis BHC 4.7	–	+		–	+	–
B. thuringiensis 1.5	+	–	–		+	–
B. thuringiensis 4.3	+	–	+	+		–
B. thuringiensis 6.1	–	–	–	–	–	

+ compatible strains; – incompatible strains.

3.5. Biological Control of Agriotes lineatus Larvae

In bioassays, the activity of six bacterial strains against *A. lineatus* larvae was individually evaluated. All tested strains exhibited higher mortality rates than the control group. Based on the mortality data in the treatments with individual isolates (Figure 2), it can be concluded that the highest average efficiency of 72.22% was recorded in the treatment with the *Bt* BHC 2.4. It was followed by *Bt* strains 1.5 and 4.3, with an efficiency of 61.85% and 58.89%, respectively. The lowest average value for individual mortality was recorded for *Bt* strain BHC 4.5 (6.67%). The isolation of bacteria from treated *A. lineatus* cadavers showed that the bacterial strains with specific *Bt* morphology (Gram-positive cells with round, white colonies with raised elevation) were represented in all treatments.

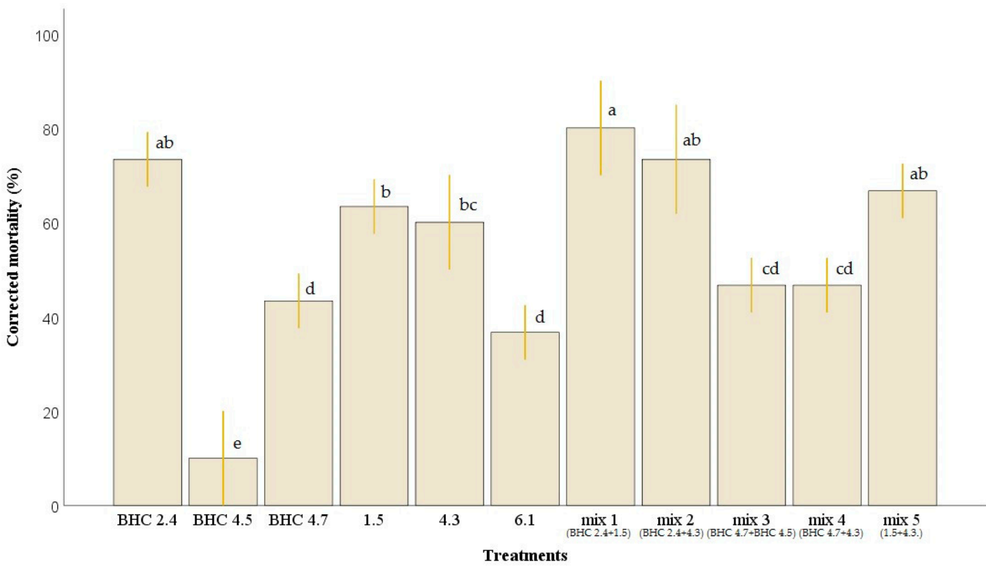

Figure 2. Mortality (%) of *Agriotes lineatus* larvae induced by *B. thiringiensis* strains. a–e: Means followed by the same superscript letters are not significantly different according to Duncan's multiple range test ($p \leq 0.05$). Bars above means represent standard deviation. Mix 1: BHC 2.4 + 1.5; mix 2: BHC 2.4 + 4.3; mix 3: BHC 4.7 + BHC 4.5; mix 4: BHC 4.7 + 4.3; mix 5: 1.5 + 4.3.

In treatments with dual cultures (mixes), it was determined that the highest average efficiency of 79.63% was achieved with mix 1 (BHC 2.4 + 1.5). After that, mix 2 (BHC 2.4 + 4.3) showed an efficiency of 72.60% followed by mix 5 (1.5 + 4.3—65.56%), which were different from mix 3 (BHC 4.7 + BHC 4.5) and mix 4 (BHC 4.7 + 4.3—44.81%) (Figure 2).

4. Discussion

The mode of bacterial action (including plant-growth-promoting bacteria) in the process of pest suppression is complex and has not been fully clarified yet. The most common antagonistic action of bacteria against pests is related to the production of insecticidal

toxins (crystal proteins, vegetative insecticidal proteins and cytolytic toxins) and specific antimicrobial compounds such as antibiotics [39,40]. The biocontrol of *A. lineatus* by using entomopathogenic bacteria has not been comprehensively studied [41]. However, the most widely accepted primary mode of bacterial entomopathogenic action is due to the involvement of toxins produced by bacteria in the lysis of an insect's digestive system.

Among *Bacillus* species, *Bt* strains have been the most studied, and their activity is almost exclusively aimed against larval stages of different insects [11,19]. This bacterial species has been widely reported as an insecticidal agent due to the production of toxins such as crystal protein (Cry), vegetative insecticidal protein (Vip) and secreted insecticidal protein (Sip) [11,42,43]. The main mechanism of Cyt and Cry toxins' activity is similar in regard to their hemolytic activity, while the action mode of Vip proteins remains partly unclear. In addition, Cry proteins target a wide range of insects, including caterpillars (Lepidoptera), beetles (Coleoptera), black flies and mosquitoes (Diptera), as well as soil nematodes such as *Caenorhabditis elegans* (Rhabditida: Rhabditidae) [44]. Among *Bt* Cry toxins, Cry1 is the most prevalent one with demonstrated activity against Coleopterans, Dipterans and Lepidopterans [42]. The presence of the *cry1B* gene was detected for *Bt* strains BHC 4.5, BHC 4.7, 1.5 and 4.3, and it might be associated with the insecticidal activity of the studied bacteria against *A. lineatus* larvae. On the other hand, different forms of Cry3 proteins have shown activity against some of the major Coleopteran families (Tenebrionidae, Chrysomelidae, Scarabaeidae and Curculionidae) [45]. Cyt proteins express their dipteran specificity through cytolytic (hemolytic) activity, while some of them also have toxic activity against Coleopteran pests [46]. Biocontrol activity of *Bt* strain BHC 2.4 against *A. lineatus* larvae could also be due to the Cyt2 toxin, according to the detection of the *cyt2* gene. Federici et al. [47] noted a biocontrol activity of the Cyt1Aa toxin against the cottonwood leaf beetle, while Mahmoud et al. [48] linked the activity of Cyt2Ca1 toxin to the *Diaprepes abbreviates*.

Antibiosis is a natural process where microorganisms produce antimicrobial substances, including lipopeptides, which have the ability to disrupt critical pathogen processes, such as cell membrane synthesis or metabolic pathways [49,50]. The amphiphilic nature of the most abundant lipopeptides families, such as iturins, surfactins and fengycins, allows them to interact with the target organisms cell membranes [49]. Recently, another family (kurstakins) has been recognized as a crucial component in biocontrol strategies. The kurstakin gene cluster contains three genes (*krsA*, *krsB* and *krsC*), which encode three large multifunctional proteins as the parts of complete synthetase [50]. Interestingly, it has been demonstrated that the transcription of the *krs* locus is probably activated by the quorum sensor NprR, which is a major regulator required for the necrotrophic stage during the life cycle of *Bt*, suggesting that the activity of kurstakin could be regulated by environmental factors [51,52]. In addition, activation of this quorum sensor could also be linked to the activity of Cry toxins. Namely, after ingestion, Cry toxins induce cell lysis, subsequently activating the NprR regulator [53]. Additionally, an intriguing property of kurstakin is its ability to enhance the survival of *Bt* within insect cadavers [54]. By facilitating the spread of *Bt* throughout the cadaver, kurstakin may improve access to new substrates and promote environmental dissemination. As the *Bt* strain BHC 2.4 had both *cry11* and *krsA* genes, its effectiveness against wireworm could be associated with the activity of these proteins, as this strain induced the highest mortality of wireworm applied both as a single culture as well as in the mix (mix 5).

Soil microorganisms most commonly infect insects through the mouth and digestive tract, where various bacterial extracellular lytic enzymes may contribute to increased insect mortality. The increased mortality occurs due to the degradation of external barriers or the peritrophic matrix, which subsequently allow the bacteria to enter the insects' hemo-

coel [40,55]. As glycoprotein, proteoglycan and chitin are the constituents of the insect peritrophic membrane, protease-producing bacteria and other lytic enzyme-producing bacteria can induce severe damage to the insect membrane [43,55]. Besides their role in biocontrol of other phytopathogenes, such as fungi, several papers reported that proteases and lipase could have a synergistic function in the entomopathogenic effect of *Bacillus* species, including *B. thuringiensis* [43,55–57]. Knežević et al. [58] reported *B. velezensis* with PGP traits as effective against *A. lineatus* and fungal plant pathogen *Fusarium poae*. Rocha et al. [59] found that *B. subtilis* strains induced mortality of Coleoptera for up to 33%, indicating that the diversity of bacterial metabolites could contribute to their overall insecticidal potential. Similarly, Rakshiya et al. [57] suggested that the production of siderophores, along with the synthesis of toxins and HCN, could be responsible for insecticidal effects of bacteria against termites. Thus, the highest biocontrol activity against *A. lineatus* was demonstrated for the *Bt* strain BHC 2.4, the best siderophore producer. Although there is no direct evidence that the production of bacterial metabolites such as siderophores and the ability of bacteria to solubilize inorganic phosphates afford them insecticidal activity, these traits could contribute to bacterial survival in the soil environment, making these bacteria a suitable choice to be used as inoculants with insecticidal activity in contemporary agricultural practices.

5. Conclusions

This study indicates that native strains of *Bacillus thuringiensis* could have potential in the biocontrol of *A. lineatus* larvae—an important wheat pest. The highest insecticidal effect was recorded for mix 1 (BHC 2.4 + 1.5), suggesting that the activity of a single *Bt* strain could be enhanced in a mixture with another compatible strain. Further research is needed to evaluate the potential of single and combined *Bt* strains to suppress *A. lineatus* under field conditions. Ultimately, a properly formulated *Bt*-based inoculant could offer an ecologically sound solution for integrated *A. lineatus* management.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agriculture15192049/s1, Figure S1: Traps for wireworms; Table S1: The presence of antibiotic-producing genes in tested bacterial strains.

Author Contributions: Conceptualization, A.B. and M.K.; methodology, M.D.M., M.K. and A.B.; software, M.D.M.; validation, M.K. and A.B.; formal analysis, M.D.M., M.K., M.J., J.M., J.P., U.B. and A.B.; investigation, M.D.M., M.K., M.J., J.M. and A.B.; resources, M.D.M., M.K. and A.B.; data curation, M.D.M., M.K. and A.B.; writing—original draft preparation, M.D.M.; writing—review and editing, M.K., U.B. and A.B.; visualization, M.J., U.B. and J.P.; supervision, M.K. and A.B.; project administration, A.B.; funding acquisition, M.K. and A.B. 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 Innovations of the Republic of Serbia, contract Nos. 451-03-136/2025-03/200011, 451-03-136/2025-03/200214, and by the Science Fund of the Republic of Serbia, Grant No. 10815, The necessity of healthy crops: Development of a multifunctional bacterial inoculant for the biological protection of cereals—BioHealCrop.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Sequence data have been deposited in the NCBI under the following accession numbers: *B. thuringiensis* BHC 2.4—PV018794, *B. thuringiensis* BHC 4.5—PV018795 and *B. thuringiensis* BHC 4.7—PV018839.

Conflicts of Interest: The authors declare no competing interests.

Abbreviations

The following abbreviations are used in this manuscript:

Bt	*Bacillus thuringiensis*
bacA	Bacylisin
bmyB	Bacyllomicin
CAS	Chrome Azurol S
Cry	Crystal protein
CYT	Cytolytic toxin
fenD	Fengycin
ituC	Iturin
krsA	Kurstakin
NA	Nutrient agar
NCBI	National Center for Biotechnology Information
NJ	Neighbor-Joining phylogenetic tree
PGP	Plant growth promotion
Sip	Secreted insecticidal protein
SMA	Skim milk agar
spaS	Subtilin
srfAA	Surfactin
Vip	Vegetative insecticidal protein

References

1. Hokkanen, H.M.T.; Menzler-Hokkanen, I. Implications for management of wireworms: Insect pest suppressiveness of soils with plant functional diversity. *Arthropod-Plant Interact.* **2023**, *17*, 441–453. [CrossRef]
2. Parker, W.E.; Howard, J.J. The biology and management of wireworms (*Agriotes* spp.) on potato with particular reference to the U.K. *Agric. For. Entomol.* **2001**, *3*, 85–98. [CrossRef]
3. Vernon, B.; Lagasa, E.; Philip, H. Geographic and temporal distribution of *Agriotes obscurus* and *A. lineatus* (Coleoptera: Elateridae) in British Columbia and Washington as determined by pheromone trap surveys. *J. Entomol. Soc. Brit. Columbia* **2001**, *98*, 257–266.
4. Horton, D.; Landolt, P. Orientation response of Pacific coast wireworm (Coleoptera: Elateridae) to food baits in laboratory and effectiveness of baits in field. *Can. Entomol.* **2002**, *134*, 357–367. [CrossRef]
5. Furlan, L. An IPM approach targeted against wireworms: What has been done and what still has to be done. *IOBC/WPRS Bull.* **2005**, *28*, 91–100.
6. Kleespies, R.G.; Ritter, C.; Zimmermann, G.; Burghause, F.; Feiertag, S.; Leclerque, A. A survey of microbial antagonists of *Agriotes* wireworms from Germany and Italy. *J. Pest Sci.* **2013**, *86*, 99–106. [CrossRef]
7. Kabaluk, T. Targeting the click beetle *Agriotes obscurus* with entomopathogens as a concept for wireworm biocontrol. *BioControl* **2014**, *59*, 607–616. [CrossRef]
8. Brandl, M.A.; Schumann, M.; Przyklenk, M.; Patel, A.; Vidal, S. Wireworm damage reduction in potatoes with an attract-and-kill strategy using *Metarhizium brunneum*. *J. Pest. Sci.* **2017**, *90*, 479–493. [CrossRef]
9. Ansari, M.A.; Evans, M.; Butt, T.M. Identification of pathogenic strains of entomopathogenic nematodes and fungi for wireworm control. *Crop. Prot.* **2009**, *28*, 269–272. [CrossRef]
10. Campos-Herrera, R.; Gutiérrez, C. Screening Spanish isolates of steinernematid nematodes for use as biological control agents through laboratory and greenhouse microcosm studies. *J. Invertebr. Pathol.* **2009**, *100*, 100–105. [CrossRef] [PubMed]
11. Kumar, P.; Kamle, M.; Borah, R.; Mahato, D.K.; Sharma, B. *Bacillus thuringiensis* as microbial biopesticide: Uses and application for sustainable agriculture. *Egypt. J. Biol. Pest Control* **2021**, *31*, 95. [CrossRef]
12. Danismazoglu, M.; Demir, İ.; Sevim, A.; Demirbag, Z.; Nalcacioglu, R. An investigation on the bacterial flora of *Agrioteslineatus* (Coleoptera: Elateridae) and pathogenicity of the flora members. *Crop Prot.* **2012**, *40*, 1–7. [CrossRef]
13. Palma, L.; Muñoz, D.; Berry, C.; Murillo, J.; Caballero, P. *Bacillus thuringiensis* toxins: An overview of their biocidal activity. *Toxins* **2014**, *6*, 3296–3325. [CrossRef]
14. Noran, E.M.; Amin, M.K.; Abdelsalam, A.; Elashtokhy, M.M. Isolation and characterization of halophilic *Bacillus thuringiensis* from local Egyptian sites and their potential against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). *Egypt. J. Biol. Pest Control* **2024**, *34*, 62. [CrossRef]
15. Bravo, A.; Gill, S.S.; Soberón, M. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon* **2007**, *49*, 423–435. [CrossRef]

16. Vachon, V.; Laprade, R.; Schwartz, J.-L. Current models of the mode of action of *Bacillus thuringiensis* insecticidal crystal proteins: A critical review. *J. Invertebr. Pathol.* **2012**, *111*, 1–12. [CrossRef]
17. Schnepf, E.; Crickmore, N.; Van Rie, J.; Lereclus, D.; Baum, J.; Feitelson, J.; Zeigler, D.R.; Dean, D.H. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* **1998**, *62*, 775–806. [CrossRef]
18. van Herk, W.G.; Vernon, R.S.; Roitberg, B.D. Repellency of a wireworm, *Agriotes obscurus* (Coleoptera: Elateridae), on exposure to synthetic insecticides in a soil-less bioassay. *Environ. Entomol.* **2008**, *37*, 534–545. [CrossRef]
19. Mampallil, L.J.; Faizal, M.H.; Anith, K.N. Bacterial bioagents for insect pest management. *J. Entomol. Zool. Stud.* **2017**, *5*, 2237–2244.
20. Wang, M.; Geng, L.; Sun, X.; Shu, C.; Song, F.; Zhang, J. Screening of *Bacillus thuringiensis* strains to identify new potential biocontrol agents against *Sclerotinia sclerotiorum* and *Plutellaxylostella* in *Brassica campestris* L. *Biol. Control* **2020**, *145*, 104262. [CrossRef]
21. Gupta, R.; Keppanan, R.; Leibman-Markus, M.; Elbaz, M.; Bar, M.; Ment, D.; Yedidia, I. *Bacillus thuringiensis* promotes systemic immunity in tomato, controlling pests and pathogens and promoting yield. *Food Sec.* **2024**, *16*, 675–690. [CrossRef]
22. Morales-Rodriguez, A.; Ospina, A.; Wanner, K.W. Evaluation of four bait traps for sampling wireworm (Coleoptera: Elateridae) infesting cereal crops in Montana. *Int. J. Insect Sci.* **2017**, *9*, 1179543317709275. [CrossRef] [PubMed]
23. Furlan, L.; Benvegnù, I.; Bilò, M.F.; Lehmhus, J.; Ruzzier, E. Species Identification of Wireworms (*Agriotes* spp.; Coleoptera: Elateridae) of Agricultural Importance in Europe: A New "Horizontal Identification Table". *Insects* **2021**, *12*, 534. [CrossRef] [PubMed]
24. Knežević, M.; Berić, T.; Buntić, A.; Delić, D.; Nikolić, I.; Stanković, S.; Stajković-Srbinović, O. Potential of root nodule nonrhizobial endophytic bacteria for growth promotion of *Lotus corniculatus* L. and *Dactylis glomerata* L. *J. Appl. Microbiol.* **2021**, *131*, 2929–2940. [CrossRef]
25. Dimkić, I.; Živković, S.; Berić, T.; Ivanović, Ž.; Gavrilović, V.; Stanković, S.; Fira, D. Characterization and evaluation of two *Bacillus* strains, SS-12.6 and SS-13.1, as potential agents for the control of phytopathogenic bacteria and fungi. *Biol. Control* **2013**, *65*, 312–321. [CrossRef]
26. Oro, V.; Knežević, M.; Dinić, Z.; Delić, D. Bacterial microbiota isolated from cysts of *Globodera rostochiensis* (Nematoda: Heteroderidae). *Plants* **2020**, *9*, 1146. [CrossRef]
27. Mora, I.M.R.; Cabrefiga, J.; Montesinos, E. Antimicrobial peptide genes in *Bacillus* strains from plant environments. *Int. Microbiol.* **2011**, *14*, 213–223. [CrossRef]
28. Jelušić, A.; Popović, T.; Dimkić, I.; Mitrović, P.; Peeters, K.; Miklavčič Višnjevec, A.; Tavzes, Č.; Stanković, S.; Berić, T. Changes in the winter oilseed rape microbiome affected by *Xanthomonas campestris* pv. *campestris* and biocontrol potential of the indigenous *Bacillus* and *Pseudomonas* isolates. *Biol. Control* **2021**, *160*, 104695. [CrossRef]
29. Jain, D.; Sunda, S.D.; Sanadhya, S.; Nath, D.J.; Khandelwal, S.K. Molecular characterization and PCR-based screening of cry genes from *Bacillus thuringiensis* strains. *3 Biotech* **2017**, *7*, 4. [CrossRef] [PubMed]
30. Thammasittirong, A.; Attathom, T. PCR-based method for the detection of cry genes in local isolates of *Bacillus thuringiensis* from Thailand. *J. Invertebr. Pathol.* **2008**, *98*, 121–126. [CrossRef] [PubMed]
31. Senthilkumar, M.; Amaresan, N.; Sankaranarayanan, A. *Plant-Microbe Interactions*; Springer Protocols Handbooks; Springer: Berlin/Heidelberg, Germany, 2021; pp. 249–251.
32. Ibarra, J.E.; Rincon, M.C.; Orduz, S. Diversity of *Bacillus thuringiensis* strains from Latin America. *Appl. Environ. Microbiol.* **2003**, *69*, 5269–5274. [CrossRef] [PubMed]
33. Rokhbakhsh-Zamin, F.; Sachdev, D.; Kazemi-Pour, N.; Engineer, A.; Pardesi, K.R.; Zinjarde, S.; Dhakephalkar, P.K.; Chopade, B.A. Characterization of plant-growth-promoting traits of *Acinetobacter* species isolated from rhizosphere of *Pennisetum glaucum*. *J. Microbiol. Biotechnol.* **2011**, *21*, 556–566. [CrossRef]
34. Milagres, A.M.; Machuca, A.; Napoleão, D. Detection of siderophore production from several fungi and bacteria by a modification of chrome azurol S (CAS) agar plate assay. *J. Microbiol. Methods* **1999**, *37*, 1–6. [CrossRef] [PubMed]
35. Figueira, C.; Ferreira, M.J.; Silva, H.; Cunha, A. Improved germination efficiency of *Salicornia ramosissima* seeds inoculated with *Bacillus aryabhattai* SP101620. *Ann. Appl. Biol.* **2019**, *174*, 319–328. [CrossRef]
36. Mihajlovski, K.R.; Radovanović, N.R.; Miljković, M.G.; Šiler-Marinković, S.; Rajilić-Stojanović, M.D.; Dimitrijević-Branković, S.I. β-Amylase production from packaging-industry wastewater using a novel strain *Paenibacillus chitinolyticus* CKS 1. *RSC Adv.* **2015**, *5*, 90895–90903. [CrossRef]
37. Vernon, R.S.; Van Herk, W.; Tolman, J.; Ortiz Saavedra, H.; Clodius, M.; Gage, B. Transitional sublethal and lethal effects of insecticides after dermal exposures to five economic species of wireworms (Coleoptera: Elateridae). *J. Econ. Entomol.* **2008**, *101*, 365–374. [CrossRef]
38. Abbott, W.S. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **1925**, *18*, 265–267. [CrossRef]
39. Bajsa, N.; Fabiano, E.; Rivas-Franco, F. Biological control of phytopathogens and insect pests in agriculture: An overview of 25 years of research in Uruguay. *Environ. Sustain.* **2023**, *6*, 121–133. [CrossRef]

40. Sindhu, S.S.; Sehrawat, A.; Sharma, R.; Khandelwal, A. Biological control of insect pests for sustainable agriculture. In *Advances in Soil Microbiology: Recent Trends and Future Prospects*; Adhya, T., Mishra, B., Annapurna, K., Verma, D., Kumar, U., Eds.; Microorganisms for Sustainability; Springer: Singapore, 2017; Volume 4, pp. 189–218. [CrossRef]
41. Poggi, S.; Le Cointe, R.; Lehmhus, J.; Plantegenest, M.; Furlan, L. Alternative strategies for controlling wireworms in field crops: A review. *Agriculture* **2021**, *11*, 436. [CrossRef]
42. Khan, A.R.; Mustafa, A.; Hyder, S.; Valipour, M.; Rizvi, Z.F.; Gondal, A.S.; Yousuf, Z.; Iqbal, R.; Daraz, U. *Bacillus* spp. as bioagents: Uses and application for sustainable agriculture. *Biology* **2022**, *11*, 1763. [CrossRef] [PubMed]
43. Ajuna, H.B.; Lim, H.I.; Moon, J.H.; Won, S.J.; Choub, V.; Choi, S.I.; Yun, J.Y.; Ahn, Y.S. The prospect of hydrolytic enzymes from *Bacillus* species in the biological control of pests and diseases in forest and fruit tree production. *Int. J. Mol. Sci.* **2023**, *24*, 16889. [CrossRef]
44. Wei, J.Z.; Hale, K.; Carta, L.; Platzer, E.; Wong, C.; Fang, S.C.; Aroian, R.V. *Bacillus thuringiensis* crystal proteins that target nematodes. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 2760–2765. [CrossRef] [PubMed]
45. Domínguez-Arrizabalaga, M.; Villanueva, M.; Escriche, B.; Ancín-Azpilicueta, C.; Caballero, P. Insecticidal activity of *Bacillus thuringiensis* proteins against Coleopteran pests. *Toxins* **2020**, *12*, 430. [CrossRef] [PubMed]
46. de Maagd, R.A.; Bravo, A.; Berry, C.; Crickmore, N.; Schnepf, H.E. Structure, diversity, and evolution of protein toxins from spore-forming entomopathogenic bacteria. *Annu. Rev. Genet.* **2003**, *37*, 409–433. [CrossRef] [PubMed]
47. Federici, B.A.; Bauer, L.S. Cyt1Aa protein of *Bacillus thuringiensis* is toxic to the cottonwood leaf beetle, *Chrysomela scripta*, and suppresses high levels of resistance to Cry3Aa. *Appl. Environ. Microbiol.* **1998**, *64*, 4368–4371. [CrossRef]
48. Mahmoud, S.B.; Ramos, J.E.; Shatters, R.G.; Hall, D.G.; Lapointe, S.L.; Niedz, R.P.; Rougé, P.; Cave, D.; Borovsky, D. Expression of *Bacillus thuringiensis* cytolytic toxin (Cyt2Ca1) in citrus roots to control *Diaprepesabbreviatus* larvae. *Pestic. Biochem. Physiol.* **2017**, *136*, 1–11. [CrossRef]
49. Fira, D.; Dimkić, I.; Berić, T.; Lozo, J.; Stanković, S. Biological control of plant pathogens by *Bacillus* species. *J. Biotechnol.* **2018**, *285*, 44–55. [CrossRef]
50. Nihorimbere, G.; Korangi-Alleluya, V.; Nimbeshaho, F.; Nihorimbere, V.; Legrève, A.; Ongena, M. *Bacillus*-based biocontrol beyond chemical control in Central Africa: The challenge of turning myth into reality. *Front. Plant Sci.* **2024**, *15*, 1349357. [CrossRef]
51. Béchet, M.; Caradec, T.; Hussein, W.; Abderrahmani, A.; Chollet, M.; Leclère, V.; Dubois, T.; Lereclus, D.; Pupin, M.; Jacques, P. Structure, biosynthesis, and properties of kurstakins, nonribosomal lipopeptides from *Bacillus* spp. *Appl. Microbiol. Biot.* **2012**, *95*, 593–600. [CrossRef]
52. Gélis-Jeanvoine, S.; Canette, A.; Gohar, M.; Caradec, T.; Lemy, C.; Gominet, M.; Jacques, P.; Lereclus, D.; Slamti, L. Genetic and functional analyses of krs, a locus encoding Kurstakin, a lipopeptide produced by Bacillus thuringiensis. *Res. Microbiol.* **2017**, *168*, 356–368. [CrossRef] [PubMed]
53. Raymond, B.; Johnston, P.R.; Nielsen-LeRoux, C.; Lereclus, D.; Crickmore, N. *Bacillus thuringiensis*: An impotent pathogen? *Trends Microbiol.* **2010**, *18*, 189–194. [CrossRef] [PubMed]
54. Dubois, T.; Faegri, K.; Perchat, S.; Lemy, C.; Buisson, C.; Nielsen-LeRoux, C.; Gohar, M.; Jacques, P.; Ramarao, N.; Kolsto, A.B.; et al. Necrotrophism is a quorum-sensing-regulated lifestyle in *Bacillus thuringiensis*. *PLoS Pathog.* **2012**, *8*, e1002629. [CrossRef]
55. Raddadi, N.; Cherif, A.; Ouzari, H.; Marzorati, M.; Brusetti, L.; Boudabous, A.; Daffonchio, D. *Bacillus thuringiensis* beyond insect biocontrol: Plant growth promotion and biosafety of polyvalent strains. *Ann. Microbiol.* **2007**, *57*, 481–494. [CrossRef]
56. Ruiu, L. Plant-growth-promoting bacteria (PGPB) against insects and other agricultural pests. *Agronomy* **2020**, *10*, 861. [CrossRef]
57. Rakshiya, Y.S.; Verma, M.K.; Sindhu, S.S. Efficacy of antagonistic soil bacteria in management of subterranean termites (Isoptera). *Res. Environ. Life Sci.* **2016**, *9*, 949–955.
58. Knežević, M.; Dervišević, M.; Jovković, M.; Jevđenović, G.; Maksimović, J.; Buntić, A. Versatile role of *Bacillus velezensis*: Biocontrol of *Fusarium poae* and wireworms and barley plant growth promotion. *Biol. Control* **2025**, *145*, 105789. [CrossRef]
59. Rocha, G.T.; Queiroz, P.R.M.; Grynberg, P.; Togawa, R.C.; de Lima Ferreira, A.D.C.; Do Nascimento, I.N.; Gomes, A.C.M.M.; Monnerat, R. Biocontrol potential of bacteria belonging to the *Bacillus subtilis* group against pests and diseases of agricultural interest through genome exploration. *Antonie Leeuwenhoek* **2023**, *116*, 599–614. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.