horticulturae

MDPI

Article

Genomic Insight into a Potential Biological Control Agent for *Fusarium*-Related Diseases in Potatoes: *Bacillus cabrialesii* Subsp. *cabrialesii* Strain PE1

Brenda Valenzuela-Aragon [1], Amelia C. Montoya-Martínez [1], Fannie Isela Parra-Cota [2] and Sergio de los Santos-Villalobos [1,*]

[1] Instituto Tecnológico de Sonora, 5 de Febrero 818 sur, Ciudad Obregon 85000, Sonora, Mexico; brendavalenzuelaaragon@gmail.com (B.V.-A.)
[2] Campo Experimental Norman E. Borlaug, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Ciudad Obregon 85000, Sonora, Mexico
* Correspondence: sergio.delossantos@itson.edu.mx

Abstract: *Bacillus* strain PE1, which was isolated from potatoes harvested in the Yaqui Valley, Mexico, was evaluated as a potential biological control agent against *Fusarium languescens*. The draft genome sequence was obtained through Illumina NovaSeq sequencing, revealing a genomic size of 4,071,293 bp, with a G + C content of 44.13%, an N50 value of 357,305 bp, and 27 contigs. The taxonomic affiliation was confirmed by analyzing the 16S rRNA gene and overall genome relatedness indices (OGRIs) and constructing a phylogenomic tree based on the whole genome, which showed a close relationship to *Bacillus cabrialesii* subsp. *cabrialesii*. Genomic annotation using RAST and Prokka identified 4261 coding DNA sequences (CDSs) distributed across 331 subsystems, highlighting genes associated with biocontrol, stress response, and iron acquisition. AntiSMASH 7.1 was used for genome mining, revealing seven biosynthetic gene clusters that potentially produce biocontrol-related metabolites. In vitro assays confirmed the antagonistic activity of strain PE1 against *Fusarium languescens* CE2, demonstrating its potential to inhibit mycelial growth. The study provides a genomic basis for investigating *B. cabrialesii* subsp. *cabrialesii* PE1 as a potential biological control agent in potato production.

Keywords: *Bacillus cabrialesii*; biological control; genomic analysis; *Fusarium*; *Solanum tuberosum*; biosynthetic gene cluster; secondary metabolites

Citation: Valenzuela-Aragon, B.; Montoya-Martínez, A.C.; Parra-Cota, F.I.; de los Santos-Villalobos, S. Genomic Insight into a Potential Biological Control Agent for *Fusarium*-Related Diseases in Potatoes: *Bacillus cabrialesii* Subsp. *cabrialesii* Strain PE1. *Horticulturae* **2024**, *10*, 357. https://doi.org/10.3390/horticulturae10040357

Academic Editors: Rafael José Carvalho Mendes, Leandro Pereira Dias, Renato Lopes Gil and Fernando Tavares

Received: 2 March 2024
Revised: 31 March 2024
Accepted: 3 April 2024
Published: 4 April 2024

1. Introduction

The potato (*Solanum tuberosum* L.) is a widely cultivated crop and is one of the top five most produced crops worldwide [1]. However, unfavorable conditions such as diseases can make potato production unsustainable, negatively impacting productivity and yield [2]. *Fusarium* is one of the most severe plant pathogens that attack potatoes, following late blight. Fusarium-related diseases are serious soil-borne diseases that cause economic losses worldwide. They can affect potatoes at any growth stage by inducing *Fusarium* wilt on plants and *Fusarium* dry rot on tubers [3]. Several *Fusarium* species have been associated with potatoes, mainly *Fusarium sambucinum*, *F. solani*, *F. graminearum*, *F. oxysporum*, *F. verticillioides*, and *F. oxysporum* f. sp. *tuberosi* [4,5]. *Fusarium* species cause different symptoms in potatoes. *Fusarium oxysporum* f. sp. *tuberosi* causes vascular wilt, and *F. solani* and *F. sambucinum* lead to *Fusarium* dry rot in tubers [3–6]. *Fusarium* diseases are caused by individual *Fusarium* or co-occurring species [7]. The relative importance of the *Fusarium* species varies depending on factors such as local climate, agricultural practices, and host susceptibility [8]. For instance, according to Montoya-Martinez and Cota-Barreras [9,10], *Fusarium languescens* is a significant regional phytopathogen in Sonora, Mexico, that could impact potato production.

Currently, new alternative control methods to synthetic fungicides are being considered for sustainable agricultural systems, due to the development of fungicide resistance by *Fusarium* strains [11–13], as well as the negative impact of these synthetic compounds on the environment and health [14]. Thus, a promising approach that has gained attention is the use of biological control agents (BCAs) [15,16]. Reports suggest that bacterial antagonists can be used to combat phytopathogenic *Fusarium* species, for example, the inoculation of *Pseudomonas fluorescens* to potato tuber seeds [5]. Similarly, postharvest studies using *P. fluorescens* and *Bacillus subtilis* have shown antagonistic properties against dry rot caused by *F. sambucinum* and *F. solani*, respectively [17].

Bacillus is a predominant bacterial genus, and numerous *Bacillus* species have been reported as biocontrol agents [18,19]. *Bacillus* uses various direct and indirect mechanisms to promote plant growth and control pathogen proliferation. These mechanisms include solubilization and mineralization of nutrients such as phosphorus and potassium, nitrogen fixation, production of 1-aminocyclopropne-1-carboxlyic acid (ACC), phytohormones, antimicrobial compounds, hydrolytic enzymes, and siderophores, as well as abiotic-stress tolerance [18,20]. However, identifying appropriate strains of potential biocontrol agents and characterizing associated metabolites remains a complex and arduous task [21–23]. To address this issue, bioinformatics tools such as genome mining for biosynthetic genes can be used to rapidly predict the secondary metabolites produced by a BCA strain [22–24]. Thus, the identification of genetic blueprints is essential for recognizing bioactive secondary metabolites and enzymes that mediate most biocontrol mechanisms [22].

In this context, there is a need to reduce the heavy reliance on the chemical control of *Fusarium* diseases and explore the use of BCAs as a sustainable alternative by effectively searching for beneficial strains [25]. This study aims to explore the ability of *Bacillus cabrialesii* subsp. *cabrialesii* strain PE1—isolated from potatoes—as a promising biological control agent against *Fusarium languescens* CE2, a regional phytopathogen. Both microorganisms were isolated from a commercial field in the Yaqui Valley, Mexico, the birthplace of the Green Revolution in the 1950s. This strategy was carried out by sequencing, annotation, and mining of the strain PE1 genome to identify biosynthetic gene clusters associated with its biocontrol ability; in addition, this genomic insight was supported by testing the antagonistic activity of extracellular metabolites produced by strain PE1 against *F. languescens* CE2.

2. Materials and Methods

2.1. Bacteria Isolation and Culture Conditions

The bacterial strain PE1 was isolated from potatoes harvested from a commercial field in the Yaqui Valley, Mexico (27°17′43.7″ N 109°51′44.1″ W). The site was selected because the plants exhibited symptoms of disease. Potatoes without symptoms were selected for sampling. The isolation process involved superficial disinfection of potatoes using commercial sodium hypochlorite at a concentration of 1.5% for 15 min. Then, disinfected potatoes were thrice washed with sterilized water before being cut and macerated. The serial dilution method (1:10) was subsequently employed up to 10^{-6}. Thus, 1 mL of each dilution was evenly spread on nutrient agar (NA) Petri dishes and incubated at 28 °C for 2 days. After incubation, strain PE1 was isolated, purified, and characterized based on its morphological traits, such as cell and colony shape, color, elevation, and opacity. Following these assessments, the strain was cryopreserved at −80 °C, using a nutrient broth (NB) culture medium containing glycerol (30%), at the Colección de Microorganismos Edáficos y Endófitos Nativos (COLMENA, itson.edu.mx/micrositios/COLMENA, accessed on 18 February 2024).

2.2. Genomic Analysis

High-quality genomic DNA was extracted from a fresh culture of strain PE1 grown in nutrient broth (NB), under growth conditions set at 30 °C for 24 h and at 121 rpm, obtaining 1×10^6 Colony Forming Units (CFU/mL). Thus, 40 µL of the cell suspension

was lysed with 120 µL of TE buffer containing lysozyme (final concentration 0.1 mg/mL) and RNase A (final concentration 0.1 mg/mL), incubated for 25 min at 37 °C. Proteinase K (final concentration 0.1 mg/mL) and SDS (final concentration 0.5% *v*/*v*) were added and incubated for 5 min at 65 °C. Genomic DNA was purified using an equal volume of SPRI beads and resuspended in EB buffer (10 mM Tris-HCl, pH 8.0). The extracted total DNA (DNA ≥ 1 µg, concentration ≥ 20 ng/µL) was then quantified with the Quant-iT dsDNA HS kit (ThermoFisher Scientific) assay in a plate reader and diluted as appropriate.

The Illumina NovaSeq platform (2 × 250 bp) was used for DNA sequencing, and library preparation was carried out using the Nextera XT Library Prep Kit, following the manufacturer's protocol, but with the following modifications: input DNA was increased 2-fold, and PCR elongation time was increased to 45 s. DNA quantification and library preparation were carried out on a Hamilton Microlab STAR automated liquid handling system (Hamilton Bonaduz AG, Reno, Nevada, USA). Libraries were sequenced on an Illumina NovaSeq 6000 (Illumina, San Diego, CA, USA), using a 250 bp paired-end protocol. Genomic information analysis was performed following the workflow reported by Ortega-Urquieta, 2022 [26]. Trimmomatic version 0.30 was used to remove adapter sequences and eliminate low-quality bases. The SPAdes version 3.15.4 generated a de novo assembly, and the contigs were ordered regarding the genome of *Bacillus cabrialesii* subsp. *cabrialesii* TE3^{T} (GenBank accession number GCA_004124315.2), using Mauve Contig Mover version 2.4.0. Plasmid detection was performed with PlasmidFinder 2.1. The genome sequence of strain PE1 was analyzed for contamination using CheckM version 1.0.18 [27].

To affiliate the strain PE1 at a species level, its genome was compared to its more closely related strains (Table 1; 16S rRNA similarity ≥ 99.6%) by using the overall genome relatedness indices (OGRIs): average nucleotide identity (ANI) by the OrthoANI algorithm [28] and the Genome to Genome Distance Calculator (GGDC) version 3.0 by BLAST [29]. A whole-genome-based phylogenetic tree was constructed using Type (strain) Genome Server (TYGA) (https://tygs.dsmz.de/, accessed on 10 January 2024) [29].

Table 1. 16S rRNA gene and OGRIs-based taxonomic affiliation of strain PE1.

Taxon Name	Strain	GenBank Accession Number	Similarity (%)	OrthoANI	GGDC (Formula 2)
Bacillus cabrialesii subsp. *cabrialesii*	TE3 ^{T}	MK462260	100	100	100
Bacillus inaquosorum	KCTC 13429 ^{T}	AMXN01000021	100	93.95	54.5
Bacillus tequilensis	KCTC 13622 ^{T}	AYTO01000043	99.86	93.58	52.7
Bacillus stercoris	JCM 30051 ^{T}	MN536904	99.86	92.19	46.4
Bacillus spizizenii	NRRL B-23049 ^{T}	CP002905	99.86	93.76	53.4
Bacillus subtilis	NCIB 3610 ^{T}	ABQL01000001	99.8	92.42	47.8
Bacillus halotolerans	ATCC 25096 ^{T}	LPVF01000003	99.73	87.46	33.4
Bacillus mojavensis	RO-H-1 ^{T}	JH600280	99.66	87.57	33.2

^{T} Type strain.

2.3. Genome Annotation and Genome Mining

The genome annotation for strain PE1 was performed using the Rapid Annotation Using Subsystem Technology (RAST) server version 2.0 and the RASTtk pipeline based on the PathoSystems Resource Integration Center (PATRIC). A second annotation was conducted using Proksee, which incorporates the Rapid Prokaryotic Genome Annotation (Prokka), and this process generated the circular chromosome map of strain PE1, including coding sequences (CDSs), tRNAs, rRNAs, guanine–cytosine (GC), and skew content. To identify biosynthetic gene clusters associated with biocontrol, the genome of strain PE1 was submitted to the Antibiotics & Secondary Metabolite Analysis Shell (AntiSMASH) 7.1 web server (https://antismash.secondarymetabolites.org, accessed on 18 February 2024) [30], under the 'relaxed' parameter.

2.4. Evaluation of the Antagonistic Activity of Strain PE1 against Fusarium languescens CE2 through Extracellular Metabolites

Fusarium languescens CE2 was obtained from a national microbial culture collection, named COLMENA (Coleccion de Microorganismos Edaficos y Endofitos Nativos, [31]). This is a phytopathogenic strain previously isolated from the Yaqui Valley, Mexico [9]. In this study, the strain CE2 was used as a model for *Fusarium*. This assay was carried out following the methods described by Montoya-Martinez et al. (2023) [9] and Baard et al. (2023) [32], with modifications. Thus, a liquid bacterial culture was prepared by inoculating a 1×10^4 CFU of strain PE1 into 20 mL of NB. The culture was incubated at 30 °C, with constant shaking at 120 rpm, for 72 h. Afterward, the bacterial culture was centrifuged at 5000 rpm for 10 min, and the resulting supernatant was collected and filtered through a hydrophilic syringe filter (0.22 µm). The effectiveness of the cell-free supernatant (CF) against *F. languescens* CE2 was tested using two methods: a well-diffusion method and casting agar plates with 50% CF. To perform the well-diffusion method, a 0.6 cm diameter agar plug containing growing mycelia from *F. languescens* CE2 was placed at the center of a fresh Potato Dextrose Agar (PDA) plate. Then, three paper discs, each with a diameter of 0.6 cm, were placed equidistantly on the plate, and 25 µL of cell-free supernatant was added over each disc. The plates were sealed and incubated at 30 °C for three days. The antagonistic effect of the cell-free supernatant on *F. languescens* CE2 mycelial growth was measured in terms of area (mm^2) using ImageJ 1.54g [33]. For the second method, agar plates with 50% cell-free supernatant were used; thus, Potato Dextrose Agar (PDA) was prepared at double concentration and sterilized, and then the cell-free supernatant was added to a 50% concentration v/v. Then, a 0.6 cm diameter agar plug containing growing mycelia from *F. languescens* CE2 was placed at the center of each plate. The inoculated plates were then incubated at 30 °C for six days, and the area (mm^2) with mycelial growth was measured. For the control treatment, the cell-free supernatant was replaced with sterile distilled water.

The experiment was conducted with three replicates. Statistical analyses were performed using R Studio Version 4.3.0 [34]. Data were analyzed using a one-way ANOVA ($p \leq 0.05$). The presented values represent the mean between replicates, and the bars represent the standard deviation (SD).

3. Results

3.1. Genomic Analysis

The bacterial DNA was subjected to sequencing, yielding a total of 1,017,168 paired-end reads (2×250 bp). Subsequent assembly of the obtained reads resulted in the draft genome of strain PE1, comprising 27 contigs (≥500 bp). The assembled genome encompassed a total length of 4,071,293 bp, with a G + C content of 44.13%, an N50 value of 357, 305 bp, and 5 L50. Importantly, plasmids were not detected within the genome of strain PE1, indicating its genomic stability and absence of extrachromosomal genetic elements.

Based on the 16S rRNA gene, strain PE1 was taxonomically affiliated with the genus *Bacillus*, showing 100% similarity to *Bacillus cabrialesii* subsp. *cabrialesii* TE3^T, 100% to *B. inaquosorum* KCTC 13429 ^{T}, and 99.86% to *B. tequilensis* KCTC 13622 ^{T} (Table 1). Based on the OGRIs analysis, this strain was strongly affiliated with *Bacillus cabrialesii* subsp. *cabrialesii* due to these values being higher than those delimiting the species affiliation (ANI ≥ 95–96% and GGDC ≥ 70%) (Table 1). Finally, this taxonomic affiliation was confirmed by the construction of a whole-genome-based phylogenomic tree (Figure 1), showcasing the close evolutionary relationship of strain PE1. Thus, this strain belongs to *Bacillus cabrialesii* subsp. *cabrialesii*.

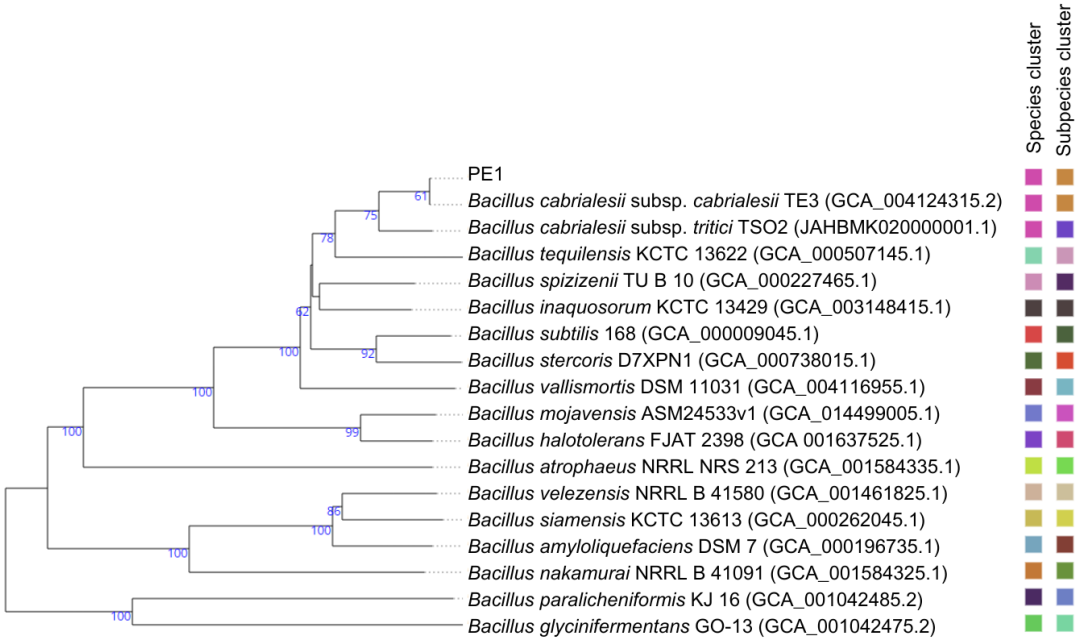

Figure 1. Phylogenetic relationship between strain PE1 and closely related species based on genome sequences constructed by TYGS. Tree inferred with FastME 2.1.6.1 [35] from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of the GBDP distance formula d5. The numbers above the branches are GBDP pseudo-bootstrap support values > 60% from 100 replications, with an average branch support of 73.0%. The tree was rooted at the midpoint [36].

3.2. Genome Annotation

According to the RAST prediction, the genome of *B. cabrialesii* subsp. *cabrialesii* PE1 contains 94 RNA sequences and 4261 protein-coding DNA sequences (CDSs) distributed across 331 subsystems, as shown in Figure 2. Notable subsystems in the genome of strain PE1 include coding sequences related to biocontrol, such as virulence, disease, and defense (36 CDSs). These are further subdivided into resistance to antibiotics and toxic compounds, invasion and intracellular resistance, and bacteriocins and ribosomally synthesized antibacterial peptides. Additionally, the iron acquisition and metabolism (30 CDSs) subsystem includes genes related to siderophores, which function as iron chelators, decreasing the accessibility of iron to phytopathogenic microorganisms. The carbohydrates subsystem (272 CDSs) contains coding sequences related to the production of acetoin and butanediol. These volatile components are capable of inducing systemic resistance in plants [37]. The cell wall and capsule (86 CDSs) subsystem contains coding sequences for exopolysaccharide biosynthesis (EpsC and EpsD), which are genes related to colonization [22]. Additionally, subsystems related to bacterial resilience for the development of agricultural bioproducts were identified, including the stress response (43 CDSs), which covers osmotic and oxidative stress. Furthermore, complementing these results, the circular chromosome map based on Prokka predicted a total of 5395 CDSs, 130 tRNAs, and 1 tmRNA (Figure 3).

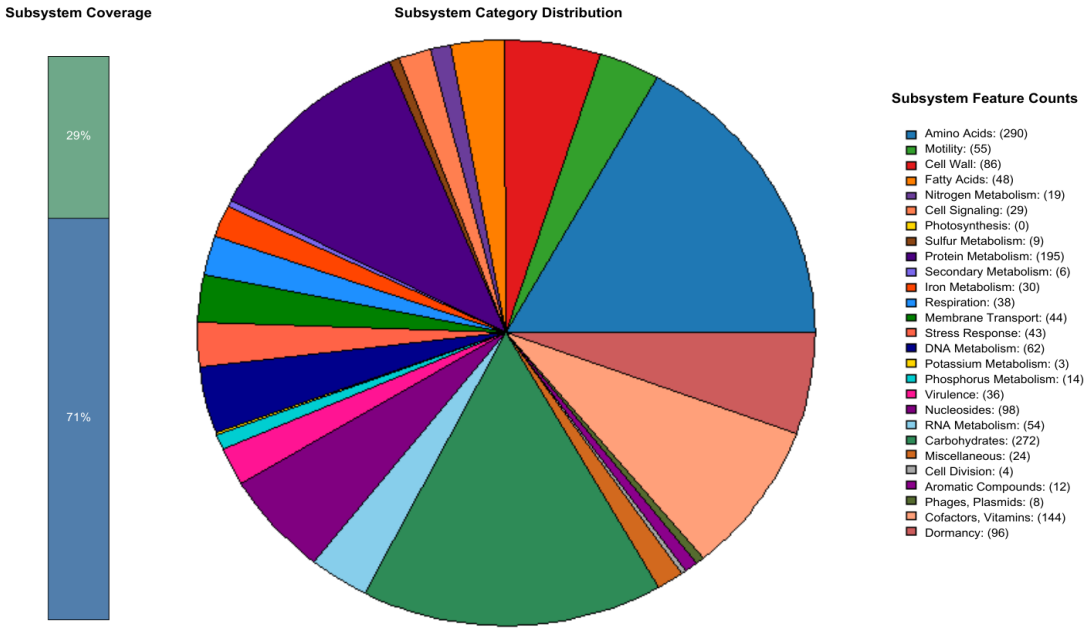

Figure 2. Pie chart of the subsystem category distribution of CDSs from *Bacillus cabrialesii* subsp. *cabrialesii* PE1 constructed by RAST server version 2.0 and R studio version 4.3.0. CDSs, 4261; and subsystems, 331.

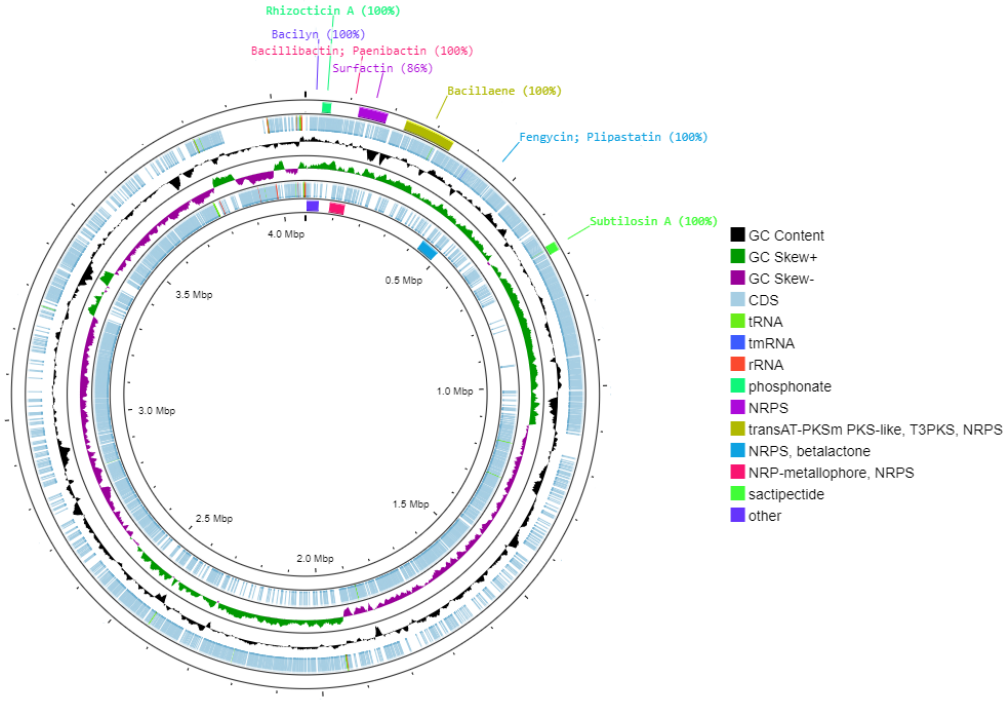

Figure 3. Circular chromosome map of *Bacillus cabrialesii* subsp. *cabrialesii* PE1, including the distribution of CDS, tRNAs, rRNAs, and GC content skew generated through genome annotation from PROKKA. Additionally, the identified biosynthetic gene clusters associated with biocontrol by AntiSMASH [30] are also shown.

3.3. Genome Mining

The AntiSMASH 7.1 web server was used for genome mining on *Bacillus cabrialesii* subsp. *cabrialesii* PE1, revealing seven distinct genomic regions. Notable biosynthetic gene clusters were detected in regions 5, 6, 14, 20, and 21 (Table 2 and Figure 3).

Table 2. Biosynthetic gene clusters found in *B. cabrialesii* subsp. *cabrialesii* PE1 obtained from genome mining by the antiSMASH web server.

Region	From	To	BGCs Type	Most Similar Known Cluster	Similarity (%)
5.1	37,565	57,940	Phosphonate	Rhizocticin A	100
6.1	121,022	186,413	NRPS	Surfactin	86
14.1	230,573	345,237	TransAT-PKSm PKS-like, T3PKS, NRPS	Bacillaene	100
14.2	424,703	487,830	NRPS, betalactone	Fengycin/plipastatin	100
20.1	82,439	134,222	NRP-metallophore, NRPS	Bacillibactin; paenibactin	100
20.2	662,012	682,579	Sactipectide	Subtilosin A	100
21.1	3451	44,869	Other	Bacilyn	100

3.4. Antagonistic Activity of Bacillus cabrialesii subsp. cabrialesii PE1 against Fusarium Languescens CE2, through Extracellular Metabolites

Extracellular metabolites produced by *B. cabrialesii* subsp. *cabrialesii* PE1 significantly inhibited the growth of *F. languescens* CE2, as demonstrated by the reduction of 41.6% in the mycelial growth area, from 969.60 ± 55.72 mm^2 (control treatment) to 566.20 ± 29.54 mm^2 (Figure 4a). On the other hand, 50% of the cell-free supernatant (CF) obtained from *B. cabrialesii* subsp. *cabrialesii* PE1 also showed a significant inhibitory effect on the mycelial growth of *F. languescens* CE2, from 1835.18 ± 125.09 mm^2 (control treatment) to 83.31 ± 6.32 mm^2 95.4% (Figure 4b).

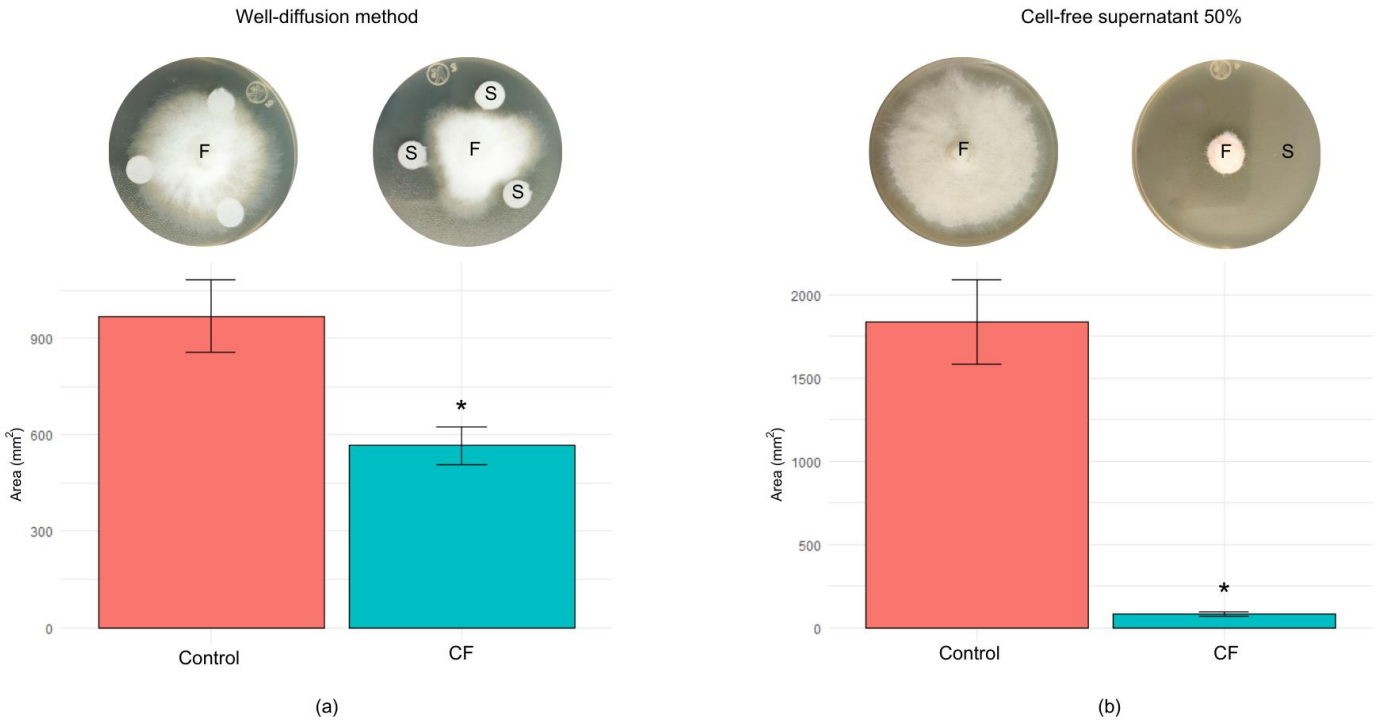

Figure 4. Antagonistic activity of extracellular metabolites from *B. cabrialesii* subsp. *cabrialesii* PE1 against *F. languescens* CE2 in PDA: (**a**) well-diffusion method, 3 days of incubation; and (**b**) cell-free supernatant 50% *v*/*v*, 6 days of incubation. The antagonistic effect of the cell-free supernatant on *F. languescens* CE2 mycelial growth was measured in terms of area (mm^2). * Statistically significant difference ($p \leq 0.05$). F: *F. languescens* CE2. S: cell-free supernatant from *B. cabrialesii* subsp. *cabrialesii* PE1.

4. Discussion

Sustainable plant disease management faces the challenge of meeting the global demand for safe and diversified food. Thus, bioprospecting is a key tool to reach this goal, which involves isolating microorganisms from the habitat where they will be used and

directly screening their potential against phytopathogens, under specific edaphoclimatic conditions [38,39]. Strain PE1 was isolated and genomically characterized under this objective. This work establishes the basis for studying and evaluating *Bacillus cabrialesii* subsp. *cabrialesii* PE1 as a biological control agent against *Fusarium languescens* in potato production in the Yaqui Valley, Mexico.

Biological control offers several opportunities for improved disease control, particularly where conventional approaches are limited or compromised; this is particularly relevant for *Fusarium*-related diseases, where chemical resistance and the potential harm to human and environmental health from fungicide use are major concerns [5,40]. In this sense, there are four main routes of action underlying biological control of plant diseases: (i) exploitation competition for resources, (ii) interference competition for space via antibiosis or toxic secondary metabolites, (iii) hyperparasitism, and (iv) induced resistance [39]. *Bacillus cabrialesii* subsp. *cabrialesii* PE1 demonstrated biocontrol potential against *F. languescens* CE2 by producing secondary metabolites, including potentially subtilosin A, bacillibactin, bacillaene, fengycin, surfactin, bacilyn, and rhizocticin A, as revealed by genome mining (Figures 2 and 3; Table 2). The genus *Bacillus* is well-characterized and exhibits multiple beneficial properties in plant nutrition and antimicrobial activity against phytopathogens. Among diverse species of this genus, *B. velezensis*, *B. subtilis*, *B. amyloliquefaciens*, and *B. cereus* have been reported to be active against *Fusarium* [41,42]. Additionally, *Bacillus cabrialesii* subsp. *Tritici* TSO2^{T} and *Bacillus cabrialesii* subsp. *cabrialesii* TE3^{T} have been reported as biocontrol agents [9,43]. The biocontrol capacity of these biological control strains is largely attributed to their ability to produce extracellular enzymes (cellulase, glucanases, proteases, chitinases, beta-xylosidase, chitin deacetylases, catalase, peroxide dismutase, and peroxidase); and antimicrobial secondary metabolites such as organic compounds (1,2-benzenedicarboxylic acid, 6-undecylamine, 2-methyloctacosane, 9-octadecenoic acid and 1-tetradecanamine, and N,N-dimethyl), lipopeptides (mycosubtilin, fengycin B, iturin A, surfactin A, iturin, bacillomycin, bacilysin, and mersacidin) and siderophores [41,44].

In vitro assays of *B. cabrialesii* subsp. *cabrialesii* PE1 demonstrated a reduction in the growth of *F. languescens* CE2 by 41.6% and 95.4%, respectively, depending on the concentration strategy using CF (Figure 4). This validates the production and diversity of secondary metabolites produced by *B. cabrialesii* subsp. *cabrialesii* PE1 and highlights the importance of exploring the action modes to quantify their roles in the biocontrol efficacy [45]. Similar metabolites found in the genome information have been associated with *Fusarium* biocontrol. For instance, *Bacillus subtilis* SG6 has been reported to act against *F. graminearum* by producing fengycins and surfactins [42]. Additionally, under field conditions, *Bacillus velezensis* LM2303 inhibited *F. graminearum*, where the observed inhibition was attributed to the presence of three antifungal metabolites (fengycin B, iturin A, and surfactin A) and eight antibacterial metabolites, including bacillaene [44], which were also identified in the strain PE1 genome. Another report showed strong antifungal activity by disrupting the cell walls, membranes, and cytoskeleton of *Fusarium oxysporum* f. sp. *cucumerinum* hyphae due to plipastatin [46], which was found in region 14.2 of the strain PE1 genome. Thus, the production of lipopeptides such as fengycin/plipastatin and surfactin, as well as polyketides such as bacillaene by strain PE1, may be involved in the demonstrated biocontrol activity in the in vitro assay. However, further deeper studies, such as metabolomics, are necessary to complement these findings.

Additionally, the iron acquisition and metabolism subsystem in the genome of *B. cabrialesii* subsp. *cabrialesii* PE1 includes 15 CDSs related to siderophores, which are iron chelators that decrease the accessibility of iron to phytopathogenic microorganisms. Siderophores are classified into three functional groups based on their structure: hydroxamate, catecholate, and carboxylate [47]. *Bacillus* sp. produces catecholate as its main siderophore, including bacillibactin [48], which has also been identified through genome mining in strain PE1. Siderophores play a crucial role in the biological control of Fusarium wilt of pepper [49], and they could be applicable in the case of biocontrol of *Fusarium* affecting potato.

The genome annotation also suggests that strain PE1 may possess additional biocontrol mechanisms. For example, the strain may produce acetoin and butanediol, which are volatile compounds that can induce systemic resistance in plants [37]. Furthermore, the strain may be associated with exopolysaccharide biosynthesis, which enhances colonization and biocontrol [22]. In this context, strain PE1 may have multiple biocontrol mechanisms that could be expressed based on the environment. Identifying the responsible mechanisms is a significant challenge due to the involvement of biotic and abiotic factors that can affect the performance of biocontrol agents and their mechanisms of action [50]. In many cases, complex interactions between plants, biocontrol agents, and pathogens involve various mechanisms [51,52]. Characterizing the genome of selected strains is a crucial step in developing biological control agents (BCAs), as it provides valuable information about their potential as biopesticides [22,52]. This process allows further analysis of strains based on their key attributes. Identifying and characterizing the genotypic traits of strains is essential in determining their potential as biocontrol agents.

5. Conclusions

This research showcases the biocontrol capabilities of *Bacillus cabrialesii* subsp. *cabrialesii* PE1 against the causal agent that affects potato cultivation, *Fusarium languescens* CE2. Genome mining identified seven biosynthetic gene clusters associated with biocontrol within this strain, providing insights into potential genomic determinants of its biocontrol ability. The in vitro assessments suggest that lipopeptides, such as fengycin and surfactin, as well as polyketides like bacillaene, may contribute to the observed biocontrol activity. However, further investigations are required, particularly in the field of metabolomics, to improve and refine these findings. Additionally, exploring the diverse functional activities and genes of strain PE1 is crucial to effectively use it as a biocontrol agent in the field.

Author Contributions: Conceptualization, S.d.l.S.-V. and B.V.-A.; methodology, formal analysis, all authors, and data curation, B.V.-A. and A.C.M.-M.; writing—original draft preparation, all authors; writing—review and editing, all authors; supervision, project administration, and funding acquisition, S.d.l.S.-V. All authors have read and agreed to the published version of the manuscript.

Funding: We acknowledge funding from GetGenome (a newly formed charitable organization that provides equitable access to genomics technology for early career researchers all over the world) and the Instituto Tecnológico de Sonora (PROFAPI_2024_0001). In addition, A.C.M.-M. was funded by Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT) for a postdoctoral fellowship (application number: 2306476).

Data Availability Statement: The complete genome sequence was deposited in DDBJ/ENA/GenBank and is openly available in NCBI under accession number JBAMMW000000000, under BioProject number PRJNA1080047, and BioSample number SAMN40099177.

Acknowledgments: The authors thank all members of the LBRM-COLMENA for their support in the development of this research project, mainly Abraham Ruíz Castrejón.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Calıskan, M.E.; Bakhsh, A.; Jabran, K. (Eds.) *Potato Production Worldwide*; Academic Press: Cambridge, MA, USA, 2022.
2. Waheed, A.; Chuang, L.; Murad, M.; Mushtaq, A.; Khalid, A.K.; Hamed, A.; Ghramh Zhongwei, W.; Daoyuan, Z. Sustainable Potato Growth under Straw Mulching Practices. *Sustainability* **2023**, *15*, 10442. [CrossRef]
3. Khedher, S.B.; Mejdoub-Trabelsi, B.; Tounsi, S. Biological potential of *Bacillus subtilis* V26 for the control of Fusarium wilt and tuber dry rot on potato caused by *Fusarium* species and the promotion of plant growth. *Biol. Control.* **2021**, *152*, 104444. [CrossRef]
4. García-Ávila, C.J.; Valenzuela-Tirado, G.A.; Florencio Anastasio, J.G.; Ruiz-Galván, I.; Moreno-Velázquez, M.; Hernández-Macías, B.; López-Buenfil, J.A.; Bravo-Pérez, D.; Pineda-Ríos, J.M.; Quezada-Salinas, A.; et al. Organisms associated with damage to post-harvest potato tubers. *Rev. Mex. Fitopatol.* **2018**, *36*, 308–320.
5. Lahlali, R.; Ezrari, S.; Radouane, N.; Kenfaoui, J.; Esmaeel, Q.; El Hamss, H.; Belabess, Z.; Barka, E.A. Biological Control of Plant Pathogens: A Global Perspective. *Microorganisms* **2022**, *10*, 596. [CrossRef] [PubMed]

6. Tiwari, R.K.; Kumar, R.; Sharma, S.; Sagar, V.; Aggarwal, R.; Naga, K.C.; Lal, M.C.; Chourasia, K.N.; Kumar, D.; Kumar, M. Potato dry rot disease: Current status, pathogenomics and management. *3 Biotech* **2020**, *10*, 503. [CrossRef] [PubMed]
7. Ferrigo, D.; Raiola, A.; Causin, R. Fusarium toxins in cereals: Occurrence, legislation, factors promoting the appearance and their management. *Molecules* **2016**, *21*, 627. [CrossRef] [PubMed]
8. Pfordt, A.; Ramos Romero, L.; Schiwek, S.; Karlovsky, P.; von Tiedemann, A. Impact of environmental conditions and agronomic practices on the prevalence of Fusarium species associated with ear-and stalk rot in maize. *Pathogens* **2020**, *9*, 236. [CrossRef]
9. Montoya-Martínez, A.C.; Figueroa-Brambila, K.M.; Escalante-Beltrán, A.; López-Montoya, N.D.; Valenzuela-Ruíz, V.; Parra-Cota, F.I.; Estrada Alvarado, M.I.; de los Santos-Villalobos, S. Biological Control Mechanisms of Bacillus cabrialesii subsp. tritici TSO2T against Fusarium languescens, the Causal Agent of Wilt in Jalapeño Peppers. *Horticulturae* **2023**, *9*, 964. [CrossRef]
10. Cota-Barreras, C.I.; García-Estrada, R.S.; León-Félix, J.; Valenzuela-Herrera, V.; Mora-Romero, G.A.; Leyva-Madrigal, K.Y.; Tovar-Pedraza, J.M. Phylogeny, distribution, and pathogenicity of fusarioid fungi associated with chickpea wilt in Sinaloa and Sonora, Mexico. Available online: https://doi.org/10.21203/rs.3.rs-2960826/v1 (accessed on 7 July 2023).
11. de Chaves, M.A.; Reginatto, P.; da Costa, B.S.; de Paschoal, R.I.; Teixeira, M.L.; Fuentefria, A.M. Fungicide resistance in Fusarium graminearum species complex. *Curr. Microbiol.* **2022**, *79*, 62. [CrossRef]
12. Mao, Y.; Zhang, Z.; Shen, J.; Yin, X.; Wang, T.; Zheng, X.; Duan, Y. The intrinsic resistance of Fusarium solani to the Fusarium-specific fungicide phenamacril is attributed to the natural variation of both T218S and K376M in myosin5. *Pestic. Biochem. Physiol.* **2023**, *196*, 105595. [CrossRef]
13. Zhang, N.; Xu, Y.; Zhang, Q.; Zhao, L.; Zhu, Y.; Wu, Y.; Li, Z.; Yang, W. Detection of fungicide resistance to fludioxonil and tebuconazole in *Fusarium pseudograminearum*, the causal agent of Fusarium crown rot in wheat. *PeerJ* **2023**, *11*, e14705. [CrossRef] [PubMed]
14. Mir, S.A.; Padhiary, A.; Ekka, N.J.; Baitharu, I.; Nayak, B. Environmental impacts of synthetic and biofungicides. In *Current Developments in Biotechnology and Bioengineering*; Elsevier: Amsterdam, The Netherlands, 2023; pp. 487–504. [CrossRef]
15. Sulaiman, M.A.; Bello, S.K. Biological control of soil-borne pathogens in arid lands: A review. *J. Plant Dis. Prot.* **2023**, *131*, 293–313. [CrossRef]
16. Ramlawi, S.; Chiu, J.O.; Cloutier, A.; Avis, T.J. Suppression of Fusarium dry rot of potato using beneficial bacterial treatments. *J. Plant Pathol.* **2021**, *103*, 269–281. [CrossRef]
17. Mnif, I.; Hammami, I.; Triki, M.A.; Azabou, M.C.; Ellouze-Chaabouni, S.; Ghribi, D. Antifungal efficiency of a lipopeptide biosurfactant derived from *Bacillus subtilis* SPB1 versus the phytopathogenic fungus, *Fusarium solani*. *Environ. Sci. Pollut. Res. Int.* **2015**, *22*, 18137–18147. [CrossRef] [PubMed]
18. Etesami, H.; Jeong, B.R.; Glick, B.R. Biocontrol of plant diseases by *Bacillus* spp. *Physiol. Mol. Plant Pathol.* **2023**, *126*, 102048. [CrossRef]
19. Chen, K.; Tian, Z.; He, H.; Long, C.A.; Jiang, F. *Bacillus* species as potential biocontrol agents against citrus diseases. *Biol. Control.* **2020**, *151*, 104419. [CrossRef]
20. Miljaković, D.; Marinković, J.; Balešević-Tubić, S. The significance of *Bacillus* spp. in disease suppression and growth promotion of field and vegetable crops. *Microorganisms* **2020**, *8*, 1037. [CrossRef] [PubMed]
21. Shimbori, E.M.; Querino, R.B.; Costa, V.A.; Zucchi, R.A. Taxonomy and biological control: New challenges in an old relationship. *Neotrop. Entomol.* **2023**, *52*, 351–372. [CrossRef]
22. Bertê, R.; Teixeira, G.M.; de Oliveira, J.P.; Nicoletto, M.L.A.; da Silva, D.V.; de Godoy, G.G.; Sanches, D.S.; de Resende, J.T.V.; Pereira, U.d.P.; da Rocha, U.N.; et al. Genome mining reveals high biosynthetic potential of biocontrol agent *Bacillus velezensis* B. BV10. *Genes* **2022**, *13*, 1984. [CrossRef]
23. Paterson, J.; Jahanshah, G.; Li, Y.; Wang, Q.; Mehnaz, S.; Gross, H. The contribution of genome mining strategies to the understanding of active principles of PGPR strains. *FEMS Microbiol. Ecol.* **2017**, *93*, fiw249. [CrossRef]
24. Montoya-Martínez, A.C.; Valenzuela-Ruíz, V.; Ortega-Urquieta, M.E.; Parra-Cota, F.I.; de los Santos-Villalobos, S. Genomic mining for the identification of promising mechanisms of bioactivity in biological control agents. In *Biocontrol Agents for Improved Agriculture*; Academic Press: Cambridge, MA, USA, 2024; pp. 143–163.
25. Panth, M.; Hassler, S.C.; Baysal-Gurel, F. Methods for management of soilborne diseases in crop production. *Agriculture* **2020**, *10*, 16. [CrossRef]
26. Ortega-Urquieta, M.E.; Valenzuela-Ruíz, V.; Mitra, D.; Hyder, S.; Elsheery, N.I.; Kumar Das Mohapatra, D.; Parra-Cota, F.I.; de los Santos-Villalobos, S. Draft Genome Sequence of *Priestia* sp. Strain TSO9, a Plant Growth-Promoting Bacterium Associated with Wheat (*Triticum turgidum* subsp. *durum*) *in the Yaqui Valley, Mexico. Plants* **2022**, *11*, 2231. [CrossRef] [PubMed]
27. Parks, D.H.; Imelfort, M.; Skennerton, C.T.; Hugenholtz, P.; Tyson, G.W. CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res.* **2015**, *25*, 1043–1055. [CrossRef] [PubMed]
28. Yoon, S.H.; Ha, S.M.; Lim, J.; Kwon, S.; Chun, J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek* **2017**, *110*, 1281–1286. [CrossRef] [PubMed]
29. Meier-Kolthoff, J.P.; Auch, A.F.; Klenk, H.P.; Göker, M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinform.* **2013**, *14*, 60. [CrossRef] [PubMed]
30. Blin, K.; Shaw, S.; Augustijn, H.E.; Reitz, Z.L.; Biermann, F.; Alanjary, M.; Fetter, A.; Terlouw, B.R.; Metcalf, W.W.; Helfrich, E.J.N.; et al. antiSMASH 7.0: New and improved predictions for detection, regulation, chemical structures and visualisation. *Nucleic Acids Res.* **2023**, *51*, W46–W50. [CrossRef] [PubMed]

31. de los Santos-Villalobos, S.; Díaz-Rodríguez, A.M.; Ávila-Mascareño, M.F.; Martínez-Vidales, A.D.; Parra-Cota, F.I. COLMENA: A culture collection of native microorganisms for harnessing the agro-biotechnological potential in soils and contributing to food security. *Diversity* **2021**, *13*, 337. [CrossRef]
32. Baard, V.; Bakare, O.O.; Daniel, A.I.; Nkomo, M.; Gokul, A.; Keyster, M.; Klein, A. Biocontrol potential of *Bacillus subtilis* and *Bacillus tequilensis* against four *fusarium* species. *Pathogens* **2023**, *12*, 254. [CrossRef]
33. Rueden, C.T.; Schindelin, J.; Hiner, M.C.; DeZonia, B.E.; Walter, A.E.; Arena, E.T.; Eliceiri, K.W. ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinform.* **2017**, *18*, 529. [CrossRef]
34. R Core Team. R version 4.3.0 (2023–04–21 ucrt) "Already Tomorrow" Copyright (C) 2023 The R Foundation for Statistical Computing Platform: x86_64–w64–mingw32/x64 (64–bit). 2023. Available online: https://www.r-project.org/ (accessed on 25 February 2024).
35. Lefort, V.; Desper, R.; Gascuel, O. FastME 2.0: A comprehensive, accurate, and fast distance-based phylogeny inference program. *Mol. Biol. Evol.* **2015**, *32*, 2798–2800. [CrossRef]
36. Farris, J.S. Estimating phylogenetic trees from distance matrices. *Am. Nat.* **1972**, *106*, 645–667. [CrossRef]
37. Yi, H.S.; Ahn, Y.R.; Ryu, C.M. Impact of a bacterial volatile 2, 3-butanediol on Bacillus subtilis rhizosphere robustness. *Front. Microbiol.* **2016**, *7*, 180263. [CrossRef] [PubMed]
38. Ayilara, M.S.; Adeleke, B.S.; Babalola, O.O. Bioprospecting and challenges of plant microbiome research for sustainable agriculture, a review on soybean endophytic bacteria. *Microb. Ecol.* **2023**, *85*, 1113–1135. [CrossRef] [PubMed]
39. Collinge, D.B.; Jensen, D.F.; Rabiey, M.; Sarrocco, S.; Shaw, M.W.; Shaw, R.H. Biological control of plant diseases–What has been achieved and what is the direction? *Plant Pathol.* **2022**, *71*, 1024–1047. [CrossRef]
40. Rampersad, S.N. Pathogenomics and management of Fusarium diseases in plants. *Pathogens* **2020**, *9*, 340. [CrossRef] [PubMed]
41. Todorović, I.; Moënne-Loccoz, Y.; Raičević, V.; Jovičić-Petrović, J.; Muller, D. Microbial diversity in soils suppressive to Fusarium diseases. *Front. Plant Sci.* **2023**, *14*, 1228749. [CrossRef] [PubMed]
42. Zhao, Y.; Selvaraj, J.; Xing, F.; Zhou, L.; Wang, Y.; Song, H.; Tan, X.; Sun, L.; Sangare, L.; Elodie-Folly, Y.M.; et al. Antagonistic action of *Bacillus subtilis* strain SG6 on *Fusarium graminearum*. *PLoS ONE* **2014**, *9*, e92486. [CrossRef] [PubMed]
43. Ruiz, V.V.; Santoyo, G.; Godínez, L.J.G.; Chávez, L.A.C.; Cota, F.I.P.; Villalobos, S.d.L.S. Complete genome sequencing of *Bacillus cabrialesii* TE3T: A plant growth-promoting and biological control agent isolated from wheat (*Triticum turgidum* subsp. *durum) in the Yaqui Valley. Curr. Res. Microb. Sci.* **2023**, *4*, 100193.
44. Chen, L.; Heng, J.; Qin, S.; Bian, K. A comprehensive understanding of the biocontrol potential of *Bacillus velezensis* LM2303 against Fusarium head blight. *PLoS ONE* **2018**, *13*, e0198560. [CrossRef]
45. Villa-Rodriguez, E.; Moreno-Ulloa, A.; Castro-Longoria, E.; Parra-Cota, F.I.; de Los Santos-Villalobos, S. Integrated omics approaches for deciphering antifungal metabolites produced by a novel *Bacillus* species, *B. cabrialesii* TE3T, against the spot blotch disease of wheat (*Triticum turgidum* L. subsp. durum). *Microbiol. Res.* **2021**, *251*, 126826. [CrossRef]
46. Gong, A.D.; Li, H.P.; Yuan, Q.S.; Song, X.S.; Yao, W.; He, W.J.; Zhang, J.B.; Liao, Y.C. Antagonistic mechanism of iturin A and plipastatin A from *Bacillus amyloliquefaciens* S76-3 from wheat spikes against *Fusarium graminearum*. *PLoS ONE* **2015**, *10*, e0116871. [CrossRef] [PubMed]
47. Boulahouat, S.; Cherif-Silini, H.; Silini, A.; Bouket, A.C.; Luptakova, L.; Alenezi, F.N.; Belbahri, L. Biocontrol Efficiency of Rhizospheric *Bacillus* against the Plant Pathogen *Fusarium oxysporum*: A Promising Approach for Sustainable Agriculture. *Microbiol. Res.* **2023**, *14*, 892–908. [CrossRef]
48. Dimopoulou, A.; Theologidis, I.; Benaki, D.; Koukounia, M.; Zervakou, A.; Tzima, A.; Diallinas, G.; Hatzinikolaou, D.G.; Skandalis, N. Direct antibiotic activity of bacillibactin broadens the biocontrol range of *Bacillus amyloliquefaciens* MBI600. *Msphere* **2021**, *6*, e00376-21. [CrossRef]
49. Ghosh, S.K.; Bera, T.; Chakrabarty, A.M. Microbial siderophore—A boon to agricultural sciences. *Biol. Control.* **2020**, *144*, 104214. [CrossRef]
50. Palmieri, D.; Ianiri, G.; Del Grosso, C.; Barone, G.; De Curtis, F.; Castoria, R.; Lima, G. Advances and perspectives in the use of biocontrol agents against fungal plant diseases. *Horticulturae* **2022**, *8*, 577. [CrossRef]
51. Singh, S.; Kumar, V.; Dhanjal, D.S.; Singh, J. Biological control agents: Diversity, ecological significances, and biotechnological applications. In *Natural Bioactive Products in Sustainable Agriculture*; Springer: Singapore, 2020; pp. 31–44.
52. Bonaterra, A.; Badosa, E.; Daranas, N.; Francés, J.; Roselló, G.; Montesinos, E. Bacteria as biological control agents of plant diseases. *Microorganisms* **2022**, *10*, 1759. [CrossRef]