Perception of Biocontrol Potential of *Bacillus inaquosorum* KR2-7 against Tomato Fusarium Wilt through Merging Genome Mining with Chemical Analysis

Maedeh Kamali ¹, Dianjing Guo ²*, Shahram Naeimi ³ and Jafar Ahmadi ⁴

¹ College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong, China; mkamali@cityu.edu.hk
² State Key Laboratory of Agrobiotechnology and School of Life Sciences, Chinese University of Hong Kong, Hong Kong, China
³ Department of Biological Control Research, Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran 19858-13111, Iran; sh.naeimi@areeo.ac.ir
⁴ Department of Genetics and Plant Breeding, Imam Khomeini International University, Qazvin 34149-16818, Iran; j.ahmadi@eng.ikiu.ac.ir

*Correspondence: djguo@cuhk.edu.hk; Tel.: +852-3943-6298

Simple Summary: *Bacillus* is a bacterial genus that is widely used as a promising alternative to chemical pesticides due to its protective activity toward economically important plant pathogens. Fusarium wilt of tomato is a serious fungal disease limiting tomato production worldwide. Recently, the newly isolated *B. inaquosorum* strain KR2-7 considerably suppressed Fusarium wilt of tomato plants. The present study was performed to perceive potential direct and indirect biocontrol mechanisms implemented by KR2-7 against this disease through genome and chemical analysis. The potential direct biocontrol mechanisms of KR2-7 were determined through the identification of genes involved in the synthesis of antibiotically active compounds suppressing tomato Fusarium wilt. Furthermore, the indirect mechanisms of this bacterium were perceived through recognizing genes that contributed to the resource acquisition or modulation of plant hormone levels. This is the first study that aimed at the modes of actions of *B. inaquosorum* against Fusarium wilt of tomatoes and the results strongly indicate that strain KR2-7 could be a good candidate for microbial biopesticide formulations to be used for biological control of plant diseases and plant growth promotion.

Abstract: Tomato Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), is a destructive disease that threatens the agricultural production of tomatoes. In the present study, the biocontrol potential of strain KR2-7 against *Fol* was investigated through integrated genome mining and chemical analysis. Strain KR2-7 was identified as *B. inaquosorum* based on phylogenetic analysis. Through the genome mining of strain KR2-7, we identified nine antifungal and antibacterial compound biosynthetic gene clusters (BGCs) including fengycin, surfactin and Bacillomycin F, bacillaene, macrolactin, sporulation killing factor (skf), subtilosin A, bacilysin, and bacillibactin. The corresponding compounds were confirmed through MALDI-TOF-MS chemical analysis. The gene/gene clusters involved in plant colonization, plant growth promotion, and induced systemic resistance were also identified in the KR2-7 genome, and their related secondary metabolites were detected. In light of these results, the biocontrol potential of strain KR2-7 against tomato Fusarium wilt was identified. This study highlights the potential to use strain KR2-7 as a plant-growth promotion agent.

Keywords: Fusarium wilt; biocontrol; *B. inaquosorum* KR2-7; genome mining; gene clusters; MALDI-TOF-MS; secondary metabolites
1. Introduction

Tomato Fusarium wilt, caused by Fusarium oxysporum f. sp. lycopersici (Fol), is one of the most destructive diseases, causing a considerable loss in the production of both field and greenhouse tomatoes worldwide [1]. Since Fusarium wilt is a difficult disease to control [2–4], control strategies including physical and cultural methods, chemical fungicides treatment, and the cultivation of resistance tomato cultivars [5] achieved limited efficacy [6]. In addition, excessive usage of agrochemicals imposed serious negative impacts on the environment, causing the pollution of soil and groundwater reservoirs, an accumulation of chemical residues in the food chain, an emergence of pesticide-resistance pathogens, and health hazards [7]. As a result, biocontrol microbes have been suggested as a promising alternative to agrochemicals in plant disease control. Numerous biocontrol microbes, especially Bacillus strains, have been commercially developed as biopesticides and biofertilizers worldwide [7].

Biocontrol microbes protect the crops from an invasion of phytopathogens via (1) direct modes of action, e.g., the antibiosis and production of antimicrobial secondary metabolites [8,9]; and (2), the indirect modes of action, including induced systemic resistance (ISR) and the competition for nutrients and space [10,11]. The investigation of biocontrol microbes through conventional genetic and biochemical approaches could not unveil the full potential of these microbes due to the absence of appropriate natural triggers or stress signals under laboratory conditions [12]. With the development of high-throughput DNA sequencing technologies and genome mining, along with MS-based analytical methods (e.g., GC/LC-MS, LC-ESI-MS, and MALDI-TOF-MS), more potential biocontrol microbes can be revealed. For instance, the Bacillus amyloliquefaciens FZB42 genome contains nine giant gene clusters synthesizing secondary metabolites which are involved in the suppression of soil-born plant pathogens. Several gene/gene clusters are implicated in swarming motility, plant colonization, biofilm formation, and the synthesis of plant growth-promoting volatile compounds and hormones [13]. A wide range of extracellular proteins and phytase in the FZB42 secretome were detected through two-dimensional electrophoresis, MALDI-TOF-MS, and the proteomics approach, indicating this strain can grow on the plant’s surface and supply phosphorus for the plant under phosphorus starvation. Additionally, four members of the macrolactin family were identified in an FZB42 culture filtrate by combining mass spectrometric and ultraviolet-visible data which perfectly agree with the overall structure of the macrolactin gene cluster found in the FZB42 genome [13]. Recently, the genome analysis of plant-protecting bacterium B. velezensis 9D-6 demonstrated that this strain can synthesize 13 secondary metabolites, of which surfacin B and surfactin C were detected as antimicrobial compounds against Clavibacter michiganensis through LC-MS/MS [14]. Furthermore, the genome mining of B. inaquosorum strain HU Biol-II revealed that this bacterial genome contains eight bioactive metabolite clusters and the production of seven metabolites was confirmed through HPLC MS/MS [15].

In our previous study, the B. inaquosorum strain KR2-7 was isolated from the rhizosphere soil of the tomato (Solanum lycopersicum) and was introduced as a highly effective biocontrol agent against Fol with a biocontrol efficiency of 80% under greenhouse conditions [16]. To better understand the biocontrol mechanisms of strain KR2-7 against Fol, whole-genome sequencing was conducted to identify putative gene clusters for secondary metabolites biosynthesis and to characterize gene/gene clusters involved in plant colonization, plant growth promotion, and induced systemic resistance (ISR). Moreover, secondary metabolites and other compounds related to identified BGCs and gene/gene clusters were detected using MALDI-TOF-MS analysis to confirm the results of genome mining.

2. Materials and Methods

2.1. Strains and Culture Conditions

The fungal pathogen Fol strain Fo-To-S-V-1 used in this study was obtained from the culture collection from the Iranian Research Institute of Plant Protection. The fungus
was maintained on a potato dextrose agar (PDA, Merck, Germany) slant at 4 °C and was sub-cultured onto a fresh PDA plate at 27 °C for 7 days for further tests.

Strain KR2-7 was maintained on nutrient agar (NA, Merck, Germany; with a 0.3% beef extract, 0.5% peptone, and 1.5% agar) plate with a periodic transfer to a fresh medium. For long-term storage, it was kept at −80 °C in lysogeny broth (LB, Merck, Germany) with 20% glycerol (v/v).

2.2. Dual Culture Assay

In order to investigate the antagonism efficiency of strain KR2-7 against various tomato pathogens, five destructive fungal pathogens, including *Alternaria alternata* f. sp. *lycopersici*, *Athelia rolfsii*, *Botrytis cinerea*, *Rhizoctonia solani*, and *Verticillium albo-atrum*, were selected. The antifungal activity of strain KR2-7 against each pathogen was evaluated through a dual culture assay in three replications. In the dual culture assay, strain KR2-7 was simultaneously cultured 3cm apart from the 5-mm plug of a pathogen in a 9 cm PDA plate. The control plate was inoculated only with the pathogen. Plates were incubated at 27 °C. The fungal growth was checked daily by measuring the diameter of the colony for a period of three days. The percentage of fungal growth inhibition (PFGI) was calculated by the formula (1) developed by Skidmore and Dickinson [17], where \( R_1 \) is the maximum radius of the growing fungal colony in the control plate, and \( R_2 \) is the radius of the fungal colony that grew in the presence of strain KR2-7:

\[
PFGI = \left( \frac{R_1 - R_2}{R_1} \right) \times 100
\]

2.3. MALDI-TOF-MS Analysis of KR2-7 Secondary Metabolites

The secondary metabolite analysis was performed from the whole-cell surface extract of bacterium obtained during the dual culture of KR2-7 and *Fol*. The bacterial surface extract was prepared according to the methodology described by Vater et al. [18]. The Dual culture was done on potato dextrose agar (PDA) instead of Landy agar. Strain KR2-7 was streaked on one side of the plate and a 5 mm plug of *Fol* was placed on the opposite side simultaneously and incubated at 27 °C. After 24 h, two loops of bacterial cells from the interface of the bacterium-fungus in the inhibition zone were suspended in 500 µL of 70% acetonitrile with 0.1% trifluoroacetic acid for 2 min. The suspension was gently vortexed to produce a homogenized suspension. The bacterial cells were pelleted by centrifuging at 5000 rpm for 10 min. The cell-free supernatant was transferred to a new microcentrifuge tube and stored at 4 °C for further analysis. One microliter of supernatant liquid was spotted onto the target of the mass spectrometer with an equal volume of α-cyano-4-hydroxycinnamic acid (CHCA) matrix and was air-dried. The sample mass fingerprints were obtained using an ultralifeXtreme MALDI-TOF/TOF-MS (Bruker, Billerica, MA, USA) within a mass range of 100–3000 Da. The MALDI-TOF-MS analysis was performed at the school of life sciences, Chinese University of Hong Kong (CUHK), Hong Kong. The whole-cell surface extract of strain KR2-7 grown on a potato dextrose agar was used as a control.

2.4. Genome Sequencing, Assembly, and Annotation

The genomic DNA of strain KR2-7 was extracted using a commercial DNA extraction kit (Thermo Fisher Scientific, Waltham, MA, USA). The whole-genome sequencing was performed using the Illumina HiSeq 4000 and PacBio RSII platforms (BGI, Shenzhen, China). The quality control of raw sequences was performed by FastQC v0.11.9 and the de novo assembly was done using SPAdes v3.14.1. The genome was annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP), and Bacterial Annotation System (BASys) webserver. The proteome of KR2-7 was subjected to BLASTP against the Cluster of Orthologous Group (COGs) database at E-value < 1 × 10^{-5} to identify the Cluster of Orthologous Groups (COGs) [19].
2.5. Genome Phylogeny

In this study, 32 *Bacillus* strains belonging to various species were selected among those recorded in the NCBI GenBank database. For all the selected strains, the nucleotide and the corresponding amino acid sequences were retrieved from the GenBank database. Whole-genome alignments were performed using REALPHY (http://realphy.unibas.ch (accessed on 22 December 2021); [20]) and the phylogenetic tree was constructed using the MEGA v. 7 [21] by maximum likelihood method [22], with evolutionary distances computed using the general time-reversible model [23]. Branch validity was evaluated by the bootstrap test with 1000 replications. The average nucleotide identity (ANI) values of selected *Bacillus* strains were calculated using the server EzBioCloud (http://www.ezbiocloud.net/tools/ani (accessed on 21 August 2021); [24]). According to the algorithm developed by Goris et al. [25], 95~96% cut-off value was used for the species boundary [26]. The web-based DSMZ service (http://ggdc.dsmz.de (accessed on 7 January 2022); [27]) with 70% species and sub-species cut-off was used to estimate the in silico genome-to-genome distance values for the selected strains.

2.6. Pathway Analysis

The annotated genome was analyzed using KEGG (Kyoto Encyclopedia of Genes and Genomes) to determine the existing pathways, which were then manually validated through matching the assigned gene functions to the corresponding KEGG pathway.

2.7. Genome-Wide Identification of Secondary Metabolite Biosynthesis Gene Clusters

The antibiotics and secondary metabolite analysis shell (antiSMASH) is a comprehensive resource that allows the automatic genome-wide identification and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genomes [28,29]. Thereby, the KR2-7 genome was submitted to the antiSMASH web server (https://antismash.secondarymetabolites.org) (accessed on 22 December 2021) to detect the putative BGCs for secondary metabolites. Each identified BGC in the KR2-7 genome was aligned against corresponding BGC in *B. subtilis* strain 168 and *B. amyloliquefacience* stain FZB42 using Geneious Prime v.2021.2.2. to find out the BGCs similarity between KR2-7, 168 and, FZB42.

3. Results

3.1. General Genomic Features of Strain KR2-7

The assembled genome of *B. inaquosorum* KR2-7 contained 4 contigs, with an N50 of 2,144,057 bp and 700X sequence coverage. The KR2-7 genome was obtained with a length of 4,248,657 bp, the G+C content of 43.1%, and 4265 predicted genes consisting of 4017 protein-coding genes, 50 rRNA genes, and 83 tRNA genes. Interestingly, strain KR2-7 possesses the larger number of genes contributing to amino acid transport and metabolism (322 genes), carbohydrate transport and metabolism (278 genes), inorganic ion transport and metabolism (200 genes), and secondary metabolites biosynthesis, transport and catabolism (76) compared to the reputable biocontrol agent *B. velezensis* strain FZB42 using Geneious Prime v.2021.2.2. to find out the BGCs similarity between KR2-7, 168 and, FZB42. The genome sequence of *B. inaquosorum* KR2-7 was deposited in NCBI GenBank under the accession number QZDE0000000.2.

3.2. Genome Phylogeny

The genomes of 31 *Bacillus* strains were selected for aligning with the KR2-7 genome and phylogenomic analysis. The selected strains and their corresponding genome sequence accession numbers were presented in Table 1. Selected *Bacillus* strains were accurately distributed on branches of the maximum likelihood phylogenomic tree (Figure 1).
Table 1. Average nucleotide identity (ANI) and Genome-to-Genome Distance Calculation (GGDC) values between each selected Bacillus strain and strain KR2-7.

| Species                        | Strain   | Accession Number | ANI (%) | GGDC  |
|--------------------------------|----------|------------------|---------|-------|
| *Bacillus altitudinis*         | P-10     | NZ_CP024204.1    | 71.39   | 0.2368|
|                                | B15      | NZ_CP014783.1    | 77.31   | 0.2087|
|                                | CC178    | NC_022653.1      | 77.35   | 0.2082|
|                                | FZB42    | NC_009725.1      | 77.65   | 0.2081|
|                                | L-H15    | NZ_CP010556.1    | 77.38   | 0.2104|
|                                | L-S60    | NZ_CP011278.1    | 77.37   | 0.2101|
|                                | S499     | NZ_CP014700.1    | 77.36   | 0.2097|
|                                | Y2       | NC_017912.1      | 77.43   | 0.2086|
| *Bacillus amyoliquefaciens*    | GQJK17   | NZ_CP022653.1    | 80.4    | 0.1895|
|                                | UCMB-5137| NZ_CP011802.1    | 80.3    | 0.1925|
| *Bacillus atrophaeus*          | GLB197   | NZ_CP018574.1    | 71.45   | 0.2368|
|                                | KLBMP 4941| NZ_CP016790.1   | 68.97   | 0.1585|
| *Bacillus cellulasensis*       | YC4-R4   | NZ_CP026736.1    | 68.87   | 0.1619|
| *Bacillus flexus*              | G25-68   | NZ_CP017080.1    | 68.82   | 0.1484|
| *Bacillus megaterium*          | Gnyt1    | NZ_CP020743.1    | 68.46   | 0.1317|
| *Bacillus muralis*             | 2691     | NZ_CP015506.1    | 69.26   | 0.1506|
| *Bacillus mycoides*            | 2691     | NZ_CP015506.1    | 69.26   | 0.1506|
| *Bacillus oceanisdiminimus*    | MDJK30   | NZ_CP020352.1    | 73.09   | 0.2242|
| *Bacillus paralicheniformis*   | SAFR-032 | NC_009848.4      | 71.3    | 0.2341|
| *Bacillus pumilus*             | TUAT1    | NZ_AP014928.1    | 71.2    | 0.2366|
| *Bacillus sp.*                 | B25 (2016b)| CP016285.1      | 68.41   | 0.177 |
|                                | WP8      | NZ_CP010075.1    | 71.11   | 0.2347|
| *Bacillus subtilis*            | B8n5     | NC_014976.1      | 93.06   | 0.0706|
|                                | HJ5      | NZ_CP007173.1    | 93.1    | 0.0703|
|                                | XF-1     | NC_020244.1      | 93.01   | 0.0707|
| *Bacillus subtilis subsp. inaquosorum* | KCTC 13429 | NZ_CP029465.1 | 99.26 | 0.0075|
| *Bacillus subtilis subsp. inaquosorum* | DE111 | NZ_CP013984.1 | 98.8 | 0.01222|
| *Bacillus subtilis subsp. spizizenii* | W23 | NC_014479.1 | 94.18 | 0.0585|
| *Bacillus subtilis subsp. subtilis* | 168 | NC_009725.1 | 93.03 | 0.0707|
| *Bacillus thuringiensis subsp. kurstaki* | HD-1 | NZ_CP004870.1 | 68.83 | 0.1575|
| *Bacillus vallismortis*        | NBIF-001 | NZ_CP020893.1 | 77.57 | 0.2081|
| *Bacillus velezensis*          | SQR9     | NZ_CP006890.1    | 77.38   | 0.2095|
| *Bacillus weihenstephanensis*  | KBAB4    | NC_010184.1      | 68.46   | 0.1386|
Figure 1. Maximum Likelihood phylogenomic tree of strain KR2-7 and selected Bacillus strains based on REALPY. Numbers at nodes represent the percentages of occurrence of nodes in 1000 bootstrap trials. The Listeria monocytogenes strain HCC23 (CP001175.1) was served as an outgroup. Moreover, closely related Bacillus species such as B. amyloliquefaciens and B. velezensis were distributed on the same branch (Figure 1). The genome-based phylogeny approaches well recognized B. methylotrophicus, B. amyloliquefaciens subsp. plantarum, and B. oryzicola as the heterotypic synonyms of B. velezensis [30]. Recently, three subspecies of Bacillus subtilis, including B. subtilis subsp. inaquosorum, B. subtilis subsp. Spizizenii, and B. subtilis subsp. stercoris were promoted to species status through comparative genomics. Each subspecies encompasses unique bioactive secondary metabolite genes which cause the unique phenotypes [31]. According to REALPHY results, strain KR2-7 was identified as B. inaquosorum, owing to being placed within the B. subtilis branch close to B. subtilis subsp. inaquosorum strain KCTC 13429 and strain DE111 in the phylogenomic tree (Figure 1). Notably, the results of ANI and GGDC analysis were consistent with REALPHY results as the KR2-7 genome displayed the highest ANI values (99.26%) and the lowest GGDC values (0.0075) with respect to the genome of strain KCTC 13429 (Table 1). Interestingly, the phylogeny analysis of several B. amyloliquefaciens strains based on core-genome was consistent with the ANI and GGDC values [32]. Altogether, the aforesaid genome-based phylogeny approaches identified strain KR2-7 as Bacillus inaquosorum.
3.3. Secondary Metabolites Biosynthetic Gene Clusters in the KR2-7 Genome

Genome mining of the strain KR2-7 revealed that more than 700 kb (i.e., nearly 17% of the genome) is devoted to 13 putative BGCs. Of the 13 found BGCs, nine were identified to contain one polyketide synthase (PKS) for macrolactin; five non-ribosomal peptide synthetases (NRPSs) for bacillibactin, bacillomycin F, bacilysin, fengycin, and surfactin; one PKS-NRPS hybrid synthetases (PKS-NRPS) for bacillaene; one thiopeptide synthase for subtilosin A, and one head-to-tail cyclised peptide for the sporulation killing factor. The nine annotated BGCs encode secondary metabolites which contribute to plant growth promotion through the fungal/bacterial pathogen suppression, ISR, nutrient uptake, and plant colonization (Table 2) [33–36]. The distribution of identified BGCs within the KR2-7 genome underlies its vigorous potential in plant disease biocontrol application [16]. The coding genes of secondary metabolites in KR2-7 were different from those in B. velezensis FZB42, while these genes showed more similarity with those in B. subtilis 168 (Table 2).

Interestingly, the BGC of bacillomycin F in KR2-7 was absent in B. subtilis 168 and B. velezensis FZB42 (Table 2) as this gene cluster conserved in B. inaquosorum [31]. Moreover, the KR2-7 genome contains four unannotated BGCs (data not shown) which showed less similarity to compounds listed in the MIBiG database.

Table 2. The comparison of secondary metabolites biosynthetic gene clusters in B. inaquosorum strains KR2-7, B. subtilis 168 and B. velezensis strain FZB42.

| Metabolite                  | Synthetase Type | Gene Cluster | Function               | Gene Similarity with Strain |
|-----------------------------|-----------------|--------------|------------------------|----------------------------|
| Bacillaene                  | PKS-NRPS        | pksABCDE, acpK, pksFGHIJLMNRS | Antibacterial           | 89.63% 75.25%              |
| Bacillibactin               | NRPS            | dhiABCDEFG   | Nutrient uptake        | 92.25% 73.07%              |
| Bacilysin                   | NRPS            | bacABCDEF, gafA | Antibacterial           | 93.50% 80.67%              |
| Fengycin                    | NRPS            | ppsABCDE     | Antifungal             | 92.01% 72.05%              |
| Macrolactin                 | PKS             | pksABCDE, acpK, pksFGHIJLMNRS | Antibacterial           | - 74.12%                   |
| Bacillomycin F              | NRPS            | ituABCDEFG   | Antifungal, ISR        | - -                        |
| Sporulation killing factor  | Head-to-tail cyclised peptide | skfABCDEFG | Antibacterial           | 96.08% -                     |
| Subtilosin A                | Thiopeptide     | sboA, albABCDEF | Antibacterial           | 91.86% -                     |
| Surfactin                   | NRPS            | srfAAP, AB, AC, AD | Antifungal, Antibacterial, Colonization, ISR | 92.20% 74.65%              |

3.4. Antifungal Secondary Metabolites Production in Strain KR2-7

The KR2-7 genome mining showed that this strain harbors three BGCs with antifungal function, including fengycin, surfacing, and bacillomycin F (a variant of iturin) belonging to Bacillus cyclic-lipopeptides (CLPs). Bacillus CLPs represented the powerful fungitoxicity properties by interfering with cell membrane integrity, permeabilizing the cell membrane, and perturbing membrane osmotic balance due to the formation of ion-conducting pores [37].

Strain KR2-7 not only suppressed the Fusarium wilt of tomato caused by Fusarium oxysporum f. sp. lycopersici [16] but also showed a broad-spectrum antifungal activity towards various phytopathogenic fungi including Alternaria alternata f. sp. lycopersici, Athelia rolfsii, Botrytis cinerea, Rhizoctonia solani, and Verticillium albo-atrum (Figure 2).
Fengycin (plipastatin), the powerful fungitoxic compound—especially against filamentous fungi [37]—is synthesized by NRPS and encoded by a 39302 bp gene cluster with five genes including ppsA-E in KR2-7, which showed a 92% and 72.05% similarity to the fengycin gene cluster of B. subtilis 168 and B. amyloliquefaciens FZB42, respectively (Table 2). The first three genes (ppsABC) each encode two amino acid modules. The fourth gene (ppsD) encodes three amino acid modules, and the last gene (ppsE) encodes one amino acid module (Figure 3). Ions of m/z values 1471.8, 1485.7, 1487.9, 1499.9, 1501.9, 1513.8, 1515.9, 1527.8, 1529.9 and, 1543.8 were observed in a whole-cell surface extract of KR2-7 grown on the control plate (thereafter, control cell extract) only four aforesaid peaks (m/z 1501.9, 1515.9, 1529.9 and, 1543.8) were detected (Table 3, Figure S2). The result indicated that the KR2-7 strain secreted various fengycin homologues to inhibit the growth of Fol.

Figure 2. Antifungal activity of strain KR2-7 towards various phytopathogenic fungi. (A1–E1): a 5-mm agar plug of each phytopathogenic fungi including Alternaria alternata, Athelia rofssii, Botrytis cinerea, Rhizoctonia solani, and Verticillium albo-atrum was cultured on the center of the PDA plate for 6 days at 28 °C, respectively. (A2–E2): strain KR2-7 was simultaneously cultured 3 cm apart from the plug of (A2): Alternaria alternata, (B2): Athelia rofssii, (C2): Botrytis cinerea, (D2): Rhizoctonia solani, (E2): Verticillium albo-atrum.

Figure 3. The biosynthetic gene cluster of fengycin in strain KR2-7.
More strikingly, a 37074 bp gene cluster encoding bacillomycin F was also identified immediately downstream of the fengycin gene cluster of KR2-7 (Figure 4). The bacillomycin F is one of seven main variants within the iturin family [40] encoded by a gene cluster consisting of four genes designated ituD, ituA, ituB and, ituC. The gene cluster code a cyclic heptapeptide in which the first three amino acids are shared among iturin family members, whereas the remaining four amino acids are conserved in B. inaquosorum [31]. Furthermore, iturins are characterized by a heptapeptide of α-amino acids attached to a β-amino fatty acid chain with a length of 14 to 17 carbons [37]. They possess potent antifungal activity against a wide variety of fungi and yeast, but bounded antibacterial and no antiviral actions [41–43]. Furthermore, these molecules also showed strong haemolytic activity, which limits their clinical use [44]. The antifungal mechanism of iturins launches by their interaction with the target cell membrane and osmotic perturbation of the membrane, owing to the formation of ion-conducting pores. Subsequently, the change in the permeability of a membrane is conducive to the release of biomolecules, such as proteins, nucleotides, and lipids from cells, which ultimately causes cell death [44,45]. In the dual culture cell extract of KR2-7, six mass peaks assigned to C16, C18 and C19 forms of iturin were observed while they were absent in the control cell extract of KR2-7 (Table 4, Figure S3). This result indicated that strain KR2-7 produced different variants of iturins to limit the growth of Fol hyphae.

Table 3. Assignments of all fengycin mass peaks obtained by MALDI-TOF mass spectrometry of whole cells of strain KR2-7 grown on control and dual culture plates.

| Mass Peak (m/z) | Assignment | Reference |
|----------------|------------|-----------|
| On PDA control |            |           |
| 1501.9         | Ala-6-C16 fengycin [M + H, Na, K]⁺ | [38]      |
| 1515.9         | Ala-6-C17 fengycin [M + H, Na, K]⁺ | [38]      |
| 1529.9         | Val-6-C16 fengycin [M + H, Na, K]⁺ | [38]      |
| 1543.8         | Val-6-C17 fengycin [M + H, Na, K]⁺ | [38]      |
| On PDA dual culture |          |           |
| 1471.9         | Ala-6-C15 fengycin [M + H, Na, K]⁺ | [38]      |
| 1485.7         | C16-Fengycin A [M + Na] | [39]      |
| 1487.9         | Ala-6-C15 fengycin [M + H, Na, K]⁺ | [38]      |
| 1499.9         | Ala-6-C17 fengycin [M + H, Na, K]⁺ | [38]      |
| 1501.9         | Ala-6-C16 fengycin [M + H, Na, K]⁺ | [38]      |
| 1513.8         | C18-fengycin A [M + Na] | [39]      |
| 1515.9         | Ala-6-C17 fengycin [M + H, Na, K]⁺ | [38]      |
| 1527.8         | Val-6-C17 fengycin [M + H, Na, K]⁺ | [38]      |
| 1529.9         | Val-6-C16 fengycin [M + H, Na, K]⁺ | [38]      |
| 1543.8         | Val-6-C17 fengycin [M + H, Na, K]⁺ | [38]      |
| 1501.9         | Ala-6-C16 fengycin [M + H, Na, K]⁺ | [38]      |
| 1515.9         | Ala-6-C17 fengycin [M + H, Na, K]⁺ | [38]      |
| 1527.8         | Val-6-C16 fengycin [M + H, Na, K]⁺ | [38]      |
| 1529.9         | Val-6-C17 fengycin [M + H, Na, K]⁺ | [38]      |
| 1543.8         | Ala-6-C15 fengycin [M + H, Na, K]⁺ | [38]      |

![Figure 3. The biosynthetic gene cluster of fengycin in strain KR2-7.](image)

![Figure 4. The biosynthetic gene cluster of bacillomycin F in strain KR2-7.](image)
Table 4. Assignments of iturin mass peaks obtained by MALDI-TOF mass spectrometry of whole cells of strain KR2-7 grown on control and dual culture plates.

| Mass Peak (m/z) | Assignment | Reference |
|----------------|------------|-----------|
| 1106.6         | C17-iturin [M + Na]^+ | [18]       |
| 1122.6         | C17-iturin [M + K]^+ | [18]       |
| 1134.6         | C19-iturin [M + Na]^+ | [18]       |
| 1136.6         | C18-iturin [M + K]^+ | [18]       |
| 1092.6         | C16-iturin [M + Na]^+ | [18]       |
| 1098.6         | C16-iturin [M + H]^+ | [18]       |
| 1106.6         | C17-iturin [M + Na]^+ | [18]       |
| 1112.6         | C19-iturin [M + H]^+ | [18]       |
| 1120.6         | C18-iturin [M + Na]^+ | [18]       |
| 1122.6         | C17-iturin [M + K]^+ | [18]       |
| 1134.6         | C19-iturin [M + Na]^+ | [18]       |
| 1136.6         | C18-iturin [M + K]^+ | [18]       |
| 1150.6         | C19-iturin [M + K]^+ | [18]       |

Similar to fengycin, surfactin was synthesized by NRPS and encoded by a srf gene cluster that spans 26073 bp in the KR2-7 genome. The gene cluster harbors four genes (srfAA-AD) and showed a 92.20% and 74.65% similarity to those of B. subtilis 168 and B. amyloliquefaciens FZB42, respectively. The product of the srf gene cluster is a linear array of seven modules, six of which are encoded by srfAA and srfAB genes and the last module is encoded by a srfAC gene (Figure 5). The fourth gene (srfAD) encodes thioesterase/acyltransferase (Te/At-domain) which triggers surfactin biosynthesis [37]. Hence, the srf gene encodes an essential enzyme (phosphopantetheinyl transferase) for the non-ribosomal synthesis of lipopeptides and the synthesis of polyketides. The regulatory gene yczE encoding an integral membrane protein was detected within the KR2-7 genome (Figure 5). Surfactin enables bacteria cells to interact with plant cells as a bacterial elicitor for stimulating ISR [37], especially through the activation of jasmonate- and salicylic acid-dependent signaling pathways [46]. Several studies indicated the ISR-elicitor role of surfactin against phytopathogens in various host plants, e.g., tomato [47], wheat [48], citrus fruit [49], lettuce [50], and grapevine [51]. Comparing the MALDI-TOF mass spectra of KR2-7 grown on a PDA control and dual culture revealed that surfactin contributed to the suppression of Fol as eight mass peaks assigned to C13, C14 and C15 surfactin homologs were detected in dual culture cell extracts, while only four of which were observed in the control cell extract (Table 5, Figure S3). Furthermore, the bacterium produced more surfactin to suppress Fol. Additionally, C14 and C15 surfactin tend to stimulate stronger ISR rather than those with shorter chain lengths [47]. Moreover, suppression of taxonomically diverse fungal pathogens including Fusarium oxysporum, F. moniliforme, F. solani, F. verticillioides, Magnaporthe grisea, Saccharicola bicolor, Cochliobolus haraeniensis, and Alternaria alternata by the surfactin family demonstrated that surfactins are strong fungitoxic compounds [52–55].
Table 5. Assignments of surfactin mass peaks obtained by MALDI-TOF mass spectrometry of whole cells of strain KR2-7 grown on control and dual culture plates.

| Mass Peak (m/z) | Assignment                  | Reference |
|----------------|------------------------------|-----------|
| 1044.6         | C14-surfactin [M + Na, K]^+   | [18]      |
| 1046.6         | C13-surfactin [M + K]^+       | [18]      |
| 1058.6         | C15-surfactin [M + Na]^+      | [18]      |
| 1060.5         | C14-surfactin [M + Na, K]^+   | [18]      |
| 1074.6         | C15-surfactin [M + Na, K]^+   | [18]      |

On PDA control

| Mass Peak (m/z) | Assignment                  | Reference |
|----------------|------------------------------|-----------|
| 1030.6         | C13-surfactin [M + Na]^+      | [18]      |
| 1032.7         | C13-surfactin [M + K]^+       | [18]      |
| 1044.6         | C14-surfactin [M + Na, K]^+   | [18]      |
| 1046.6         | C13-surfactin [M + K]^+       | [18]      |
| 1058.6         | C15-surfactin [M + Na]^+      | [18]      |
| 1060.5         | C14-surfactin [M + Na, K]^+   | [18]      |
| 1074.6         | C15-surfactin [M + Na, K]^+   | [18]      |

On PDA dual culture

3.5. Antibacterial Secondary Metabolites Production in Strain KR2-7

The KR2-7 genome contained six BGCs coding for antibacterial compounds including bacillaene and macrolactin, sporulation killing factor (skf), subtilosin A, bacilysin, and surfactin. Several studies on surfactin and its isoforms proved that these metabolites played a major role in combating bacterial plant diseases, such as fruit bloch caused by Acidovorax citrulli in melon [56], tomato wilt caused byRalstonia solanacearum[57], and root infection by Pseudomonas syringae in Arabidopsis [58]. Moreover, surfactin produced by B. subtilis R14 exhibited pronounced antagonistic efficacy against several multidrug-resistance bacterial strains of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Enterococcus faecalis [59].

Bacillaene is a polyketide known as a selective bacteriostatic agent that inhibits prokaryotic, not eukaryotic growth by disrupting protein synthesis [60]. Its antimicrobial efficacy against various bacteria (Myxococcus xanthus and Staphylococcus aureus) and fungi (Fusarium spp) have been reported [60–62]. In the KR2-7 genome, bacillaene was synthesized by a PKS/NRPS hybrid pathway and encoded by a giant pks gene cluster (76,355 Kbp) containing 16 genes (pksA-S and acpK) showing an 89.63% and 75.25% similarity to those of B. subtilis 168 and B. velezensis FZB42, respectively (Table 2, Figure 6B). Another polyketide, macrolactin, can be encoded by a 54,225 kbp gene cluster in strain KR2-7 and showed a 74.12% similarity to the mln cluster of B. velezensis FZB42 (Table 2, Figure 6A). Macrolactins are a large class of macrolide antibiotics that inhibited the growth of several bacteria, including Ralstonia solanacearum, Staphylococcus aureus, and Burkholderia epacian [63,64]. In the dual culture cell extract of KR2-7, one ion corresponding to 7-o-succinyl macrolactin A ([M + Na]^+ = 525.4), and another ion corresponding to bacillaene A ([M + H]^+ = 583.5) were detected (Figure 6C,D), while they were not observed in the control cell extract of KR2-7.
Bacilysin (also known as tetaine) is a dipeptide suppressing a wide variety of destructive phytopathogenic bacteria, e.g., Erwinia amylovora, Xanthomonas oryzae pv. oryzae, X. oryzae pv. Oryzicola, and Clavibacter michiganense subsp. sepedonicum [65–67]. This bactericidal property is due to the inhibition of glucosamine-6-phosphate synthase by the anticapsin moiety of bacilysin. Its inhibition repression the biosynthesis of peptidoglycans, the essential constituents of the bacterial cell wall [68,69]. In the KR2-7 genome, bacilysin can be encoded by a 7128 bp bac gene cluster consisting of seven genes (bacA-E, ywfAG), and display high gene similarity to those of B. subtilis 168 (Table 2, Figure 7). This metabolite and its derivatives were detected neither in the KR2-7 control cell extract nor dual culture cell extract, likely due to culture conditions or the assay method.

Furthermore, the KR2-7 genome encompassed two distinct gene clusters encoding bacteriocins, including subtilosin A and sporulation killing factors (SKFs). Subtilosin A is a macrocyclic anionic antimicrobial peptide originally obtained from wild-type strain B. subtilis 168 [70] but is also produced by B. amyloliquefaciens and B. atrophaeus [71,72]. This bacteriocin displayed a bactericidal effect on a broad spectrum of bacteria, including Gram-positive and Gram-negative bacteria and both aerobes and anaerobes [73], possibly through an interaction with membrane-associated receptors, or binding to the outer cell membrane, and is conducive to membrane permeabilization [73–75]. Subtilosin A is ribosomally synthesized by an alb gene cluster containing eight genes (albA-G, sboA) spanning 6.8 kbp in the KR2-7 genome (Figure 8). The sboA gene encodes presubtilosin, and albA-G genes encode proteins whose functions are presubtilosin processing and subtilosin export [76].
The mass peaks corresponding to subtilosin A and its homologs appeared neither in the KR2-7 control cell extract nor the dual culture cell extract. These peaks are detectable by altering the culture condition and/or evaluating the procedure.

Figure 8. The biosynthetic gene cluster of subtilosin A antibacterial metabolite in strain KR2-7.

The KR2-7 genome also harbored a 5976 bp skf gene cluster encompassing skfABCEFGH, and involves the production and release of killing factors during sporulation (Figure 9). During the early stages of sporulation, sporulating cells of *B. subtilis* exude extracellular killing factors to kill the nonsporulating sister cells whose immunity to these toxins was not developed. As a result, the nutrient from the dead cells are released and then used by the sporulating cells to resume their growth. This phenomenon is termed “cannibalism” and causes a delay in sporulation [77,78]. The SKF bacteriocin produced by the sporulating cells can destroy other soil-inhabiting bacteria. Similarly, the expression of *skf* genes in *B. subtilis* inhibits the growth of *X. orzae* pv. *oryzae*, the causative agent of rice bacterial blight [79].

Figure 9. The biosynthetic gene cluster of sporulation killing factor antibacterial metabolite in strain KR2-7.

3.6. Plant Colonization by Strain KR2-7

The most crucial step for a PGPR (Plant Growth Promoting Rhizobacteria) agent to survive, enhance plant growth, and suppress plant disease is the efficient colonization of plant tissues. The plant colonization process comprises two steps. In the first step, PGPR agents reach the surface of plant tissue either by passive movement in water flow or by flagellar movement. The second step is to establish the plant-bacterium interaction reliant on bacterial biofilm formation [36,80].

The KR2-7 genome harbored the gene clusters for flagellar assembly (*flg* cluster, *flh* cluster and, *fli* cluster) and bacterial chemotaxis (*che* cluster) together with other genes known to be necessary for swarming motility, including *lag*, two stator elements (*motAB*), as well as regulatory genes *surAB*, *surAB*, *surB* and, *surC* (Table 6). In the step of efficient colonization, the PGPR agent forms bacterial biofilm and not only strengthens the plant-bacterium interaction but protects the plant root system as a bio-barrier against pathogen attacks [80]. The main component of bacterial biofilm is the extracellular polymeric substances (EPS) with a chemical composition including proteins, neutral polysaccharides, charged polymers, and amphiphilic molecules [80]. The *eps* cluster (*epsC-O*) encoding exopolysaccharide of biofilm and its regulatory genes *sinR* and *arbA* (repressors) and, *sinI* (antirepressor), the *yqxm-sipW-tasA* gene cluster encoding amyloid fiber (TasA protein of biofilm) and *pgcA* encoding phosphoglucomutase were found in the KR2-7 genome (Table 6). Moreover, the involvement of surfactin in cell adhesion and biofilm formation due to its 3D topology and amphiphilic nature has been illustrated [81,82]. Baise et al. [58] declared that deleting surfactin gene expression in *B. subtilis* strain 6051 led to disability to form robust biofilm on *Arabidopsis* root surface, and reduced the suppression of disease caused by *Pseudomonas syringae*. Besides, the deficiency in surfactin production in *B. subtilis*, strain UMAF6614 resulted in a biofilm formation defect on melon phyllloplane and partially reduced the suppression of bacterial soft root rot, bacterial leaf spot, and cucurbit powdery-mildew by the biocontrol stain [83].
Table 6. Genes and gene clusters involved in plant-bacterium interaction in the genome of KR2-7.

| Bioactivity          | Gene/Gene Cluster | From     | To       | Product                                                                 | Remark                                                                 |
|----------------------|-------------------|----------|----------|-------------------------------------------------------------------------|-------------------------------------------------------------------------|
| Root colonization    | yfiQ              | 1181545  | 1180457  | Putative membrane-bound acyltransferase YfiQ                            | Involved in surface adhesion [13,84]                                    |
|                      | sacB              | 2852367  | 2850946  | Levan sucrase                                                           | Levan contributed to the aggregation of wheat root-adhering soil [85]   |
|                      | swrB              | 319026   | 318514   | Swarming motility protein swrB                                         | Essential for swarming motility [86]                                    |
|                      | swrC              | 1348113  | 1344916  | Swarming motility protein SwrC                                         | Self-resistance to surfactin [86]                                      |
|                      | sfp               | 1712320  | 1712994  | 4'-phosphopantetheinyl transferase sfp                                  | Necessary for lipopeptide and polyketide synthesis which is essential for surface motility and biofilm formation [13,86] |
|                      | swrAA             | 2770348  | 2770776  | Swarming motility protein swrAA                                         | Essential for swarming motility [87]                                    |
|                      | swrAB             | 2770855  | 2772051  | Swarming motility protein swrAB                                         | Essential for swarming motility [87]                                    |
|                      | sfp               | 3829945  | 3830526  | Elongation factor P                                                     | Essential for swarming motility [88]                                    |
| Swarming motility    | flhA              | 328577   | 326544   | Flagellar biosynthesis protein flhA                                     | Flagellar assembly                                                      |
|                      | flhB              | 329692   | 328610   | Flagellar biosynthetic protein flhB                                     | Flagellar assembly                                                      |
|                      | flhR              | 330489   | 329692   | Flagellar biosynthetic protein flhR                                     | Flagellar assembly                                                      |
|                      | flhQ              | 330757   | 330479   | Flagellar biosynthetic protein flhQ                                     | Flagellar assembly                                                      |
|                      | flhP              | 331428   | 330763   | Flagellar biosynthetic protein flhP                                     | Flagellar assembly                                                      |
|                      | flhY              | 333625   | 332483   | Flagellar motor switch phosphatase FlhY                                 | Flagellar assembly                                                      |
|                      | flhM              | 334613   | 333606   | Flagellar motor switch protein FlhM                                     | Flagellar assembly                                                      |
|                      | flhL              | 335060   | 334638   | Flagellar protein FlhL                                                  | Flagellar assembly                                                      |
|                      | flgG              | 336163   | 335312   | Flagellar basal-body rod protein flgG                                   | Flagellar assembly                                                      |
|                      | flhK              | 338009   | 336546   | Probable flagellar hook-length control protein                          | Flagellar assembly                                                      |
|                      | flhJ              | 339085   | 338642   | Flagellar FlhJ protein                                                  | Flagellar assembly                                                      |
|                      | flhI              | 340404   | 339088   | Flagellum-specific ATP synthase                                         | Flagellar assembly                                                      |
|                      | flhH              | 341153   | 340401   | Probable flagellar assembly protein                                     | Flagellar assembly                                                      |
|                      | flhF              | 342162   | 341146   | Flagellar motor switch protein FlhG                                     | Flagellar assembly                                                      |
|                      | flhG              | 343785   | 342175   | Flagellar M-ring protein                                                | Flagellar assembly                                                      |
|                      | flhE              | 344151   | 343831   | Flagellar hook basal body complex protein FlhE                          | Flagellar assembly                                                      |
|                      | flgC              | 344618   | 344163   | Flagellar basal-body rod protein flgC                                   | Flagellar assembly                                                      |
|                      | flgB              | 345010   | 344615   | Flagellar basal-body rod protein FlgB                                   | Flagellar assembly                                                      |
|                      | motA              | 654985   | 655887   | Motilite protein A                                                      | Motilite protein A                                                      |
|                      | motB              | 655835   | 656650   | Motilite protein B                                                      | Motilite protein B                                                      |
| Swarming motility    | flhO              | 2629891  | 2630742  | Flagellar hook basal body complex protein flhO                           | Flagellar assembly                                                      |
|                      | flhP              | 2630949  | 2631584  | Flagellar hook basal body complex protein flhP                           | Flagellar assembly                                                      |
|                      | flhM              | 2751851  | 2752117  | Negative regulator of flagellin synthesis                               | Flagellar assembly                                                      |
|                      | flgK              | 2752634  | 2754151  | Flagellar hook-associated protein 1                                     | Flagellar assembly                                                      |
|                      | flgL              | 2754161  | 2755057  | Flagellar hook-associated protein 3                                     | Flagellar assembly                                                      |
|                      | lag               | 2756494  | 2757405  | Flagellin                                                               | Flagellar assembly                                                      |
|                      | flhD              | 2757987  | 2759483  | Flagellar hook-associated protein 2                                     | Flagellar assembly                                                      |
|                      | flhS              | 2759505  | 2759906  | Flagellar protein FlhS                                                 | Flagellar assembly                                                      |
|                      | flhT              | 2759903  | 2760244  | Flagellar protein FlhT                                                 | Flagellar assembly                                                      |
|                      | cheD              | 320333   | 319833   | Chemoreceptor glutamine deamidase CheD                                  | Bacterial chemotaxis                                                    |
|                      | cheC              | 320959   | 320330   | CheY-P phosphatase CheC                                                | Bacterial chemotaxis                                                    |
|                      | cheW              | 321457   | 320978   | Chemotaxis protein CheW                                                | Bacterial chemotaxis                                                    |
|                      | cheA              | 323488   | 321470   | Chemotaxis protein CheA                                                | Bacterial chemotaxis                                                    |
|                      | cheB              | 324555   | 323485   | Chemotaxis response regulator protein-glutamate methyltransferase      | Bacterial chemotaxis                                                    |
|                      | cheY              | 332463   | 332095   | Chemotaxis protein CheY                                                | Bacterial chemotaxis                                                    |
|                      | cheV              | 616969   | 616058   | Chemotaxis protein CheV                                                | Bacterial chemotaxis                                                    |
|                      | cheR              | 3987238  | 3988153  | Chemotaxis protein methyltransferase                                   | Bacterial chemotaxis                                                    |
| Bioactivity | Gene/Gene Cluster | From     | To       | Product                                                                 | Remark                                                                 |
|-------------|-------------------|----------|----------|-------------------------------------------------------------------------|------------------------------------------------------------------------|
| Biofilm formation |                  |          |          |                                                                         |                                                                        |
| pgsA        | 273607            | 273026   |          | CDP-diacylglycerol-glycerol-3-phosphate-3-Phosphatidyl transferase       | Member of pgsB-pgsC-pgsA-pgsE gene cluster encoding PGA which is        |
|             |                   |          |          |                                                                         | contributed to robustness and complex morphology of the colony          |
|             |                   |          |          |                                                                         | biofilms [89]                                                           |
| pgsA        | 1083817           | 1082072  |          | Phosphoglucomutase                                                     | Phosphoglucomutase plays an important role in biofilm formation [90]   |
| yhdK        | 1903365           | 1902379  |          | Sensor histidine kinase yhdK                                           | Transcriptional regulation of biofilm formation [91,92]                |
| sigW        | 1928876           | 1928313  |          | RNA polymerase sigma factor sigW                                      | Transcriptional regulation of biofilm formation [91,92]                |
| sigH        | 2011463           | 2010807  |          | RNA polymerase sigma-H factor                                          | Involves in the initial stage of biofilm formation [93]               |
| abrB        | 2082858           | 2083148  |          | Transition state regulatory protein AbrB                              | Transcriptional regulation of biofilm formation [94]                  |
| epsC-O      | 2731363           | 2874940  |          | Gene cluster for capsular poly-saccharide biosynthesis                | Encoding exopolysaccharide which is essential for biofilm formation [91]|
|             | lytS              | 3426013  | 3427803  | Sensor protein lytS                                                    | Transcriptional regulation of biofilm formation [91,92]                |
| yqxM        | 3813390           | 3814151  |          | Protein yqxM                                                          | Belongs to yqxM-sipW-tasA gene cluster that is necessary for biofilm  |
|             |                   |          |          |                                                                         | formation [95]                                                         |
| tasA        | 3814771           | 3815556  |          | Spore coat-associated protein N                                       | Required for development of complex colony architecture [94]          |
| sinR        | 3816019           | 3815651  |          | HTH-type transcriptional regulator sinR                                | Transcriptional regulation of biofilm formation [91,92]                |
| sinI        | 3816289           | 3816020  |          | Protein sinI                                                          | Transcriptional regulation of biofilm formation [91,92]                |
| spo0A       | 3849786           | 3850625  |          | Stage 0 sporulation protein A                                         | Involved in the initial stage of biofilm formation [93]               |
| resE        | 3951273           | 3953042  |          | Sensor histidine kinase resE                                          | Transcriptional regulation of biofilm formation [91,92]                |
| ymcA        | 261856            | 261425   |          | Protein ymcA                                                          |                                                                         |
| ylbF        | 467892            | 467434   |          | Regulatory protein ylbF                                               |                                                                         |
| yqkK        | 3725618           | 3726187  |          | Protein yqkK                                                          |                                                                         |
| sipW        | 3814123           | 3814707  |          | Signal peptide I W                                                    |                                                                         |
| moaD        | 594413            | 594180   |          | Molybdopterin synthase sulfur carrier subunit                          |                                                                         |
| moaE        | 594879            | 594406   |          | Molybdopterin synthase catalytic subunit                               |                                                                         |
| moaC        | 1469542           | 1469000  |          | Molybdenum cofactor biosynthesis protein C                             | Nitrogen assimilation                                                  |
| moaA        | 2592667           | 2593692  |          | Molybdenum cofactor biosynthesis protein A                             |                                                                         |
| moaB        | 3369200           | 3369751  |          | Molybdenum cofactor biosynthesis protein B                             |                                                                         |
| nasA-F      | 1758586           | 1768809  |          | Gene cluster for Nitrate transport and reduction                       |                                                                         |
| narK        | 2532129           | 2533373  |          | Nitrite extrusion protein                                              | Nitrogen assimilation                                                  |
| fnr         | 2533450           | 2534190  |          | Anaerobic regulatory protein                                           |                                                                         |
| arfM        | 2534994           | 2535563  |          | Probable transcription regulator arfM                                  |                                                                         |
| narG-J      | 2535783           | 2542171  |          | Gene cluster for Nitrate reductase                                     |                                                                         |
| nrgB        | 2619035           | 2618670  |          | Nitrogen regulatory PII-like protein                                    |                                                                         |
| nrgA        | 2620246           | 2619032  |          | Ammonium transporter nrgA                                              |                                                                         |
| corA        | 694316            | 692961   | 380769   | Magnesium transporter corA                                             | Magnesium assimilation                                                |
| mntH        | 1626982           | 1628297  |          | Manganese transport protein mntH                                       | Manganese assimilation                                                |
| mntA-D      | 3237914           | 3241819  |          | Gene cluster for Manganese binding/transport protein                   |                                                                         |
| mntR        | 3824735           | 3825202  |          | Transcriptional regulator mntR                                        |                                                                         |
Table 6. Cont.

| Bioactivity | Gene/Gene Cluster | From       | To         | Product                                             | Remark                          |
|-------------|------------------|------------|------------|-----------------------------------------------------|---------------------------------|
| Ktr system potassium uptake protein C | ktrC       | 573312     | 572647     | Potassium assimilation                               |                                 |
| Putative gamma-glutamylcyclotransferase ykqA | ykqA       | 574212     | 573469     |                                                     |                                 |
| Ktr system potassium uptake protein D | ktrD       | 674275     | 672926     |                                                     |                                 |
| Putative potassium channel protein yugO | yugO       | 3174241    | 3173258    |                                                     |                                 |
| Ktr system potassium uptake protein B | ktrB       | 3203187    | 3201850    |                                                     |                                 |
| Ktr system potassium uptake protein A | ktrA       | 3205862    | 3203194    |                                                     |                                 |
| Ferrichrome ABC transporter                | yeIQ       | 1683673    | 1682711    | Iron assimilation                                    |                                 |
| Putative iron binding lipoprotein yvcC    | yvcC       | 2985220    | 2986197    |                                                     |                                 |
| Putative iron (III) ABC transport ATPase component | yusV       | 3009672    | 3010583    |                                                     |                                 |
| Gene cluster encoding Bacillibactin       | dhaABC    | 3101063    | 3112861    |                                                     |                                 |
| Gene cluster for teichuronic acid biosynthesis | tuaA-H    | 2733487    | 2742546    |                                                     | Bivalent cations assimilation   |
| Glycosyl hydrolase yvdK                   | yvdK       | 2839830    | 2842166    |                                                     |                                 |
| HTH-type transcriptional regulator alsR   | alsR       | 2672398    | 2671511    | These genes encode enzymes of the biosynthetic pathway from pyruvate to 3-hydroxy-2-butanone |                                 |
| Acetolactate synthase                     | alsS       | 2672549    | 2674270    |                                                     |                                 |
| Alpha-acetolactate decarboxylase          | alsD       | 2674320    | 2675099    |                                                     |                                 |
| (R, R)-butanediol dehydrogenase           | bdiA       | 1448068    | 1449108    | This gene encodes enzyme to catalyse 3-hydroxy-2-butanone to 2,3-butanediol |                                 |
| Carbon-nitrogen hydrolase                 | yhcX       | 1092936    | 1091395    | These genes are involved in indole acetic acid biosynthesis |                                 |
| N-acetyltransferase                       | ysnE       | 3489604    | 3489101    |                                                     |                                 |
| Putative aldehyde dehydrogenase dhaS      | dhaS       | 4136609    | 4135263    |                                                     |                                 |
| 3-phytase                                  | phy        | 4084788    | 4085936    | Phytohormones biosynthesis gene                      |                                 |
| Trehalose cluster transcriptional repressor | treR       | 1237083    | 1236367    | These genes are involved in trehalose biosynthesis  |                                 |
| Trehalose-6-phosphate hydrolase PTS system trehalose-specific EIIBC component | treA       | 1238792    | 1237104    |                                                     |                                 |
| Trehalose cluster transcriptional repressor | treP       | 1240275    | 1238863    |                                                     |                                 |
| Acetolactate synthase small subunit        | ilvH       | 3492606    | 3493124    | These genes are parts of leucine, valine, and isoleucine biosynthesis pathway |                                 |
| Acetolactate synthase large subunit        | ilvB       | 3490858    | 3492609    |                                                     |                                 |
| Ketal-acid reductoisomerase                | ilvC       | 3493148    | 3494176    |                                                     |                                 |
| Arginine decarboxylase                     | speA       | 501879     | 503375     | These genes may transform amino acids to plant growth-promoting substances [80] |                                 |
| Spermidine synthase                       | speE       | 2512298    | 2513128    |                                                     |                                 |
| Agmatinase                                 | speB       | 2513189    | 2514061    |                                                     |                                 |

3.7. Genes Involved in Bacterium-Plant Interactions

Quite apart from the antagonistic mechanisms of bacterial biocontrol strains, these bacterial microorganisms are also involved in plant growth augmentation through making nutrients available for host plants, production of plant growth-promotion hormones, and the induction of systemic resistance within the plant by specific metabolite secretion [96,97]. Similar to other biocontrol microorganisms, the KR2-7 genome contains the genes/gene clusters related to plant growth promotion (Table 6).

The KR2-7 genome contained moaA-E genes encoding molybdenum cofactor and may be a relic of a nitrogen-fixing gene cluster or a cofactor for nitrogen assimilation [80]. Moreover, the genes for nitrate reduction (narG-J), nitrate transport (narK), probable transcription regulator genes (arfM), regulatory protein (fnr), an ammonium acid transporter (nrgA), and its regulator gene (nrgB), along with the nas gene cluster (nasA-F), were also identified in the KR2-7 genome. The nas gene cluster is involved in nitrite transport and reduction (Table 6).

In addition to nitrogen assimilation, the KR2-7 genome encompassed potassium transporting genes, including ktr system potassium uptake proteins (ktrA-D), a putative potassium channel protein (yugO), and putative gamma-glutamyl cyclotransferase (ykqA) [80,98]. Furthermore, the presence of genes for transportation of magnesium (mgfE, corA), fer-
rochrome (vdK), manganese (mntH), and a gene cluster for manganese binding/transport (mntA-D), along with the transcription regulator protein (mntR), were identified in the KR2-7 genome. These genes uptake the nutrients or detoxify the heavy metal ions for both the bacteria and host plants [80]. An 11.7 kb dlb gene cluster (dlbABDEF) encoding siderophore bacillibactin was identified in the KR2-7 genome.

Furthermore, ions of m/z values 883.4 and 905.2 were detected in the KR2-7 dual culture cell extract and were identified as bacillibactin [M + H]+ and bacillibactin [M + Na]+ (Figure 10) by comparison with previously reported data [99,100]. Notably, the molecular ion peaks corresponding to bacillibactin were not observed in the control cell extract of KR2-7. Siderophores are low-molecular-weight molecules with a high affinity for ferric iron that solubilize iron from minerals and organic compounds under iron limitation conditions [101].

Volatile organic compounds (VOCs) produced by PGPR agents play a significant role in promoting plant growth through the regulation of synthesis or metabolism of phytohormones [107], the induction of systemic disease resistance [108,109], and the control of plant pathogens [110]. A 2,3-butanediol and 3-hydroxy-2-butanone (acetoin) are the best-known growth-promoting VOCs that produced B. subtilis and B. amyloliquefaciens. The genome of the KR2-7 harbored als gene cluster (alsR, alsS, alsD), along with the bdhA gene is together required for the biosynthesis pathway of 2,3-butanediol from pyruvate. In this pathway, alsS encodes the acetolactate synthase enzyme, which catalyzes the condensation of two pyruvate molecules into acetolactate. Then, acetolactate decarboxylase, encoded by alsD, converts decarboxylate acetolactate into acetoin. The alsR regulates two aforesaid steps. Finally, the bdhA encoded (R, R)-butanediol dehydrogenase enzyme catalyzes 3-hydroxy-2-butanoate (acetoin) to 2,3-butanediol [111]. In addition, the KR2-7 genome contained ilvH, ilvB, ilvC genes and a leu gene cluster (leuABCD) which are required for the biosynthesis pathway of three branched-chain amino acids (BCAA), including leucine, isoleucine, and valine. Acetolactate is a central metabolite between 2,3-butanediol and BCAA biosynthesis and can involve in both anabolism and catabolism by acetolactate decarboxylase. It was reported that acetolactate decarboxylase is an enzyme with a dual role that can direct acetolactate flux to catabolism in favour of valine and leucine biosynthesis or can catalyze the second step of the 2, 3-butanediol anabolism pathway [112]. Bacillus spp. can enhance plant growth through the synthesis of plant growth-promoting hormones, such as auxin, indole-3-acetic acid (IAA), and gibberellic acid. The genome of KR2-7 may encompass

Figure 10. MALDI-TOF MS analysis of bacillibactin produced by strain KR2-7 grown on a PDA dual culture. (A) m/z 883.4: bacillibactin [M + H]+; (B) m/z 905.2: bacillibactin [M + Na]+.
genes/gene clusters responsible for the biosynthesis of indole acetic acid, phytase, and trehalose (Table 6). Moreover, a large variety of PGPRs produce polyamines, such as putrescine, spermine, spermidine, and cadaverine, and are known to be involved in promoting plant growth and improving abiotic stress tolerance in plants [113]. The genes coding for arginine decarboxylase (SpeA), agmatinase (SpeB), and spermidine synthase (SpeE), which directly polyamines biosynthesis, were also found in the KR2-7 genome (Table 6).

4. Discussion

Previously, the Bacillus subtilis species complex was composed of four close subspecies, i.e., subspecies subtilis, spizizenii, inaquosorum, and stercoris, which were differentiated through a phylogenetic analysis of multiple protein-coding genes and genome-based comparative analysis [114,115]. B. subtilis subsp. Inaquosorum was deemed as a distinctive taxon encompassing strains KCTC 13429 and NRRL B-14697 [116]. Recent phylogenomic studies clearly distinguished subspecies spizizenii from subspecies spizizenii, as the estimated ANI among them was smaller than the defined ANI for species delineation (95% ANI) [117]. In addition to a low ANI value (<95%), the BGC of subtilin exclusively presents in the genomes of subspecies spizizenii, but was not characterized in subspecies inaquosorum genome [115]. Accordingly, B. inaquosorum KR2-7 was clearly differentiated from B. subtilis subsp. spizizenii W23 because of the low ANI value among them (94.18%) and the lack of subtilin gene cluster in the genome content of strain KR2-7. In addition, it was reported that B. inaquosorum is the only species to produce bacillomycin F. It was approved by detecting a unique MALDI-TOF-MS biomarker at m/z 1120 in the MALDI-TOF-MS spectra of B. inaquosorum that cannot be produced by other species [114]. Since this unique biomarker (m/z value 1120.6) was observed in the MALDI-TOF-MS spectra of strain KR2-7, it can be concluded that this strain is a B. inaquosorum. Recently, the ability of B. inaquosorum strain HU Biol-II in producing bacillomycin F was confirmed through HPLC MS/MS [15]. Most recently, subspecies spizizenii, inaquosorum, and stercoris were promoted to species status through a comparative genome study [118]. This study determined that each subspecies had unique secondary metabolite genes encoding unique phenotypes, thereby each subspecies can be promoted to species. According to the aforesaid results, strain KR2-7 was identified as a B. inaquosorum.

The genome-driven data highlighted the plant-beneficial functions of strain KR2-7. This strain can efficiently colonize the plant root surface, relying on its swarming motility and biofilm formation abilities. Efficient root colonization of biocontrol bacteria is necessary for suppressing phytopathogens, and biofilm formation is an essential prerequisite for persistent root colonization [119,120]. The biofilm-deficient mutant of B. pumilus HR10 produced weakened biofilms with reduced contents of extracellular polysaccharides and proteins, and thereby could not efficiently control pine seedling damping-off disease [121]. Hence, the suppression of tomato Fusarium wilt by strain KR2-7 [16] may contribute to efficient tomato root colonization of this strain.

In addition to efficient root colonization, strain KR2-7 is able to directly suppress soil-dwelling phytopathogens through producing eight antimicrobial secondary metabolites, e.g., fengycin, surfactin, bacillomycin F, macrolactin, bacilaene, bacilysin, subtilosin A, and sporulation killing factor. The combination of obtained data via MALDI-TOF-MS with our previous observations [16] confirmed that strain KR2-7 produced at least four bioactive metabolites (including fengycin, surfactin, macrolactin, and bacilaene) to directly protect the tomato plant from the invasion and penetration of Fol. The cyclic lipodecapeptide fengycin exhibits strong fungitoxic properties by inhibiting phospholipase A2 and aromatase functions [122], disruption of biological membrane integrity [123], deformation and permeabilization of hyphae [124,125], and induction of ISR [126]. In this context, the strong antifungal activity of B. inaquosorum strain HU Biol-II against a diverse group of fungi highly pertained to the fengycin produced by this strain. Interestingly, 97.47% of the ppsA/E gene cluster in KR2-7 was similar to the fengycin gene cluster in strain HU Biol-II [15]. Fengycin produced by B. subtilis SQR9 and B. amyloliquefaciens NJN-6 significantly inhibited
the growth of *F. oxysporum* [127,128]. Moreover, fengycin BS155 isolated from *B. subtilis* BS155 destroyed *Magnaporthe grisea* through damaging the plasma membrane and cell wall, disruption of mitochondrial membrane potential (MMP), chromatin condensation, and the induction of reactive oxygen species (ROS) [129]. In addition to fengycin, the contribution of other secondary metabolites in the biocontrol of various pathogens has been reported. The supernatant of *B. subtilis* GLB191, consisting of surfactin and fengycin, highly controlled grapevine downy mildew caused by *Plasmopara viticola* by means of direct antagonistic activity and the stimulation of plant defence [51]. Furthermore, the strong antifungal effect of *B. velezensis* strains Y6 and F7 against *Ralstonia solanacearum* and *F. oxysporum* was attributed to the production of fengycin, iturin, and surfactin, among which iturin played a key role in the suppression of *F. oxysporum* [130]. The biocontrol mechanism of *B. amyloliquefaciens* DH-4 against *Penicillium digitatum*, the causal agent of citrus green mold, was secreting a cocktail of antimicrobial compounds consisting of macrolactin, bacillaene, iturins, fengycin, and surfactin [100].

Additionally, strain KR2-7 can exert hormones, such as IAA, phytase, and trehalose for root uptake and rebalance hormones in the host plant to boost growth and stress response. Phytate (inositol hexa- and penta-phosphates) is the predominant form of soil organic phosphorus, which is unavailable for plant uptake due to the rapid immobilization of phosphorus and the lack of adequate phytase levels in plants [131]. Phytase is a phosphatase enzyme responsible for the transformation of organic phytate to inorganic phosphate, which is acquirable for plant roots. Similarly, phytase-producing Bacillus strains can effectively enhance plant growth through the liberation of reactive phosphorus from phytate and make this element available for plant uptake. In the presence of phytate, the comparison of the culture filtrate of *B. amyloliquefaciens* strain FZB45 with those of a phytase-deficient mutant provided evidence that the phytase activity of strain FZB45 enhanced the growth of corn seedling [132]. The bacterization of Brassica juncea with Bacillus sp. PB-13 considerably boost phosphorus content and growth parameters in 30-day-old seedlings [133]. More recently, the soil inoculation of Bacillus strain SD01N-014 resulted in the enhancement of soil phosphorus content and the promotion of maize seedling growth [134]. Accordingly, extracellular phytase activity of strain KR2-7 mediated with phy gene can be expectable. In addition, the presence of genes involved in the biosynthesis of IAA and trehalose in the KR2-7 genome (Table 6) is an indication of this strain’s potential in the mitigation of salt toxic stress on plants. Inoculation of tomato plants subjected to salt stress with OxtreS (trehalose over-expressing strain) mutant of *Pseudomonas* sp. UW4 considerably boosted the dry weight, root and shoot length, and chlorophyll content of the tomato plant [135]. Moreover, canola seedlings treated with over-expressed IAA transformant of UW4 represented longer primary root with an increased number of root hairs than seedlings treated with wild-type UW4 [136]. The growth promotion of root hairs by IAA improves the assimilation of water and nutrients from the soil, which in turn raises plant biomass [136]. Similarly, Japanese cypress seedlings inoculated with *B. velezensis* CE 100 showed significant increases in growth parameters and biomass due to the production of indole-3-acetic acid (IAA) by CE 100 strain [137].

5. Conclusions

According to genome-driven data, along with chemical analysis results, strain KR2-7 most likely exploits four possible modes of action to control tomato Fusarium wilt, as shown in Figure 11:

1. Inhibition of the pathogen growth through the diffusion of antifungal and antibacterial secondary metabolites and biofilm formation;
2. Stimulation of ISR in tomato via the production of surfactin and volatile organic compounds;
3. Promotion of plant health and growth by producing plant growth promotion hormones and polyamines, supplying iron for tomato, depriving the pathogen of iron,
and relieving heavy metal stress in the soil as a result of siderophore bacillibactin activity;

(4) Efficient colonization of plant roots.

The described modes of action were highly based on the identified gene clusters encoding secondary metabolites and characterized gene/gene clusters involved in plant colonization, plant growth promotion, and ISR. Furthermore, future studies using integrated omics approaches and the mutagenesis of strain KR2-7 are required to approve the aforesaid possible modes of action of strain KR2-7 and exact functions of the putative genes and gene clusters in the suppression of fungal pathogen Fol.

Figure 11. Schematic presentation of putative biocontrol mechanism of strain KR2-7 against Fol. (A) An untreated tomato plant in which Fol (yellow 16-point star) penetrated root tissue, colonized and blocked the vascular system to prevent water and nutrients from being transferred to plant organs. It caused yellowing began with bottom leaves, followed by wilting, browning, and defoliation. Growth is typically stunted, and little or no fruit develops. (B) Strain KR2-7 (blue rod) reaches to tomato root and colonizes on the root surface through its motility potential and biofilm formation. As a result of root colonization, strain KR2-7 diffuses a wide variety of antifungal and antibacterial secondary metabolites to establish a protective zone (green dash line semicircular) in the tomato rhizosphere. Strain KR2-7 directly limits the invasion of Fol fungal pathogen through diffused antifungal secondary metabolites and also control the bacterial pathogens of tomato by means of produced antibacterial secondary metabolites. Meanwhile, volatile organic compounds and surfactin stimulate tomato systemic resistance to provide ISR-mediated protection (yellow dash line arrow) against phytopathogens. Moreover, tomato growth is enhanced assisted by growth-promoting hormones, polyamines, and siderophore bacillibactin.
Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/biology11010137/s1, Figure S1. Circular map of the *B. inaquosorum* KR2-7 genome. Outermost circle (1st); all genes are color-coded according to their functions (see top right); 2nd circle: GC content (black); 3rd circle: GC skew+ (green); 4th circle: GC skew− (violet); 5th circle: scale (bps). GC views were prepared using CGView Server V1.0 (http://wishart.biology.ualberta.ca/cgview/), Table S1: The comparison of COG functional categories between *B. subtilis* KR2-7 and *B. velezensis* FZB42, Figure S2: The fengycin mass peaks detected by MALDI-TOF mass spectrometry in (A) control cell extract of KR2-7 and (B) dual culture cell extract of KR2-7, Figure S3: The iturin, mycosubtilin, and surfactin mass peaks detected by MALDI-TOF mass spectrometry in (A) control cell extract of KR2-7 and (B) dual culture cell extract of KR2-7.

Author Contributions: M.K.; Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing—original draft and supplementary materials, Visualization. D.G.; Supervision, review & editing, Funding acquisition. S.N.; Supervision, Methodology, review & editing. J.A.; Supervision, review & editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Guangdong Science and Technology Department (project ID: 2020A0505090009), and partially supported by a fund from State Key Laboratory of Agrobiotechnology (project ID: 8300052).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The genome sequence of *B. inaquosorum* KR2-7 was deposited in NCBI GenBank under the accession number QZDE00000002.

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

References
1. Agrios, G.N. *Plant Pathology*, 5th ed.; Elsevier Academic Press: London, UK, 2005; pp. 522–534.
2. Borrero, C.; Ordovas, J.; Trillas, M.I.; Aviles, M. Tomato *Fusarium* wilt suppressiveness. The relationship between the organic plant growth media and microbial communities as characterised by Biolog. *Soil. Biol. Biochem.* 2006, 38, 1631–1637. [CrossRef]
3. Elmer, W.H. Effects of acibenzolar-S-methyl on the suppression of *Fusarium* wilt of cyclamen. *Crop Prot.* 2006, 25, 671–676. [CrossRef]
4. L’Haridon, F.; Aimé, S.; Duplessis, S.; Alabouvette, C.; Steinberg, C.; Olivain, C. Isolation of differentially expressed genes during interactions between tomato cells and a protective or a non-protective strain of *Fusarium oxysporum*. *Physiol. Mol. Plant Pathol.* 2011, 76, 9–19. [CrossRef]
5. Ajilogba, C.F.; Babalola, O.O. Integrated Management Strategies for Tomato Fusarium Wilt. *Biocentrol. Sci.* 2013, 18, 117–127. [CrossRef] [PubMed]
6. Arie, T. *Fusarium* diseases of cultivated plants, control, diagnosis, and molecular and genetic studies. *J. Pestic. Sci.* 2019, 44, 275–281. [CrossRef] [PubMed]
7. Cawoy, H.; Bettiol, W.; Fickers, P.; Ongena, M. Bacillus-based biological control of plant diseases. In *Pesticides in the Modern World-Pesticides Use and Management*, 1st ed.; Stoytcheva, M., Ed.; IntechOpen Press: London, UK, 2011; pp. 273–302. Available online: https://www.intechopen.com/chapters/21989 (accessed on 21 May 2021).
8. Raaijmakers, J.M.; Mazzola, M. Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. *Annu. Rev. Phytopathol.* 2012, 50, 403–424. [CrossRef] [PubMed]
9. Kohl, J.; Kolnaar, R.; Ravensberg, W.J. Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. *Front. Plant Sci.* 2019, 10, 845. Available online: https://www.frontiersin.org/article/10.3389/fpls.2019.00845 (accessed on 21 May 2021). [CrossRef]
10. Conrath, U.; Beckers, G.J.M.; Langenbach, C.J.G.; Jaskiewicz, M.R. Priming for enhanced defense. *Annu. Rev. Phytopathol.* 2015, 53, 97–119. [CrossRef] [PubMed]
11. Spadaro, D.; Droby, S. Development of biocontrol products for postharvest diseases of fruit: The importance of elucidating the mechanisms of action of yeast antagonists. *Trends Food Sci. Technol.* 2016, 47, 39–49. [CrossRef]
12. 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] [PubMed]
13. Chen, X.; Kountouts, A.; Schöl, R.; Eisenreich, A.; Schneider, K.; Heinemeyer, I.; Morgenstern, B.; Voss, B.; Hess, W.R.; Reva, O.; et al. Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. *Nat. Biotechnol.* 2007, 25, 1007–1014. [CrossRef]

[CrossRef]
14. Grady, E.N.; MacDonald, J.; Ho, M.T.; Weselowski, B.; McDowell, T.; Solomon, O.; Renaud, J.; Yuan, Z.C. Characterization and complete genome analysis of the surfactin-producing, plant-protecting bacterium Bacillus velezensis 9D-6. BMC Microbiol. 2019, 19, 5. [CrossRef]

15. Knight, C.A.; Bowman, M.J.; Frederick, L.; Day, A.; Lee, C.; Dunlap, C.A. The first report of antifungal lipopeptide production by a Bacillus subtilis subsp. inaquosorum strain. Microbiol. Res. 2018, 216, 40–46. [CrossRef] [PubMed]

16. Kamali, M.; Ahmadi, J.; Naeimi, S.; Guo, D. Characterization of Bacillus isolates from the rhizosphere of tomato suppressing Fusarium wilt disease. Acta Phytopathol. Entomol. Hung. 2019, 54, 53–68. [CrossRef]

17. Skidmore, A.M.; Dickinson, C.H. Colony interactions and hyphal interference between Septoria Nodorum and phytophyl fungus. Trans. Brit. Mycol. Soc. 1976, 66, 57–64. [CrossRef]

18. Vater, J.; Gao, X.; Hitzeroth, G.; Wilde, C.; Franke, P. “Whole cell”-matrix assisted laser desorption ionization-time of flight mass spectrometry, an emerging technique for efficient screening of biocombinatorial libraries of natural compounds- present state of research. Comb. Chem. High Throughput Screen. 2003, 6, 557–567. [CrossRef] [PubMed]

19. Tatusov, R.L.; Fedorova, N.D.; Jackson, J.D.; Jacobs, A.R.; Kiryutin, B.; Koonin, E.V.; Krylov, D.M.; Mazumder, R.; Mekhedov, S.L.; Nikolskaya, A.N.; et al. The COG database: An updated version includes eukaryotes. BMC Bioinform. 2003, 4, 41. [CrossRef] [PubMed]

20. Bertels, F.; Silander, O.K.; Pachkov, M.; Rainey, P.B.; van Nimwegen, E. Automated reconstruction of whole-genome phylogenies from short-sequence reads. Mol. Biol. Evol. 2014, 31, 1077–1088. [CrossRef]

21. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 2013, 30, 2725–2729. [CrossRef]

22. Felsenstein, J. Evolutionary trees from DNA sequences: A maximum likelihood approach. J. Mol. Evol. 1981, 17, 368–376. [CrossRef]

23. Tavare, S. Some probabilistic and statistical problems in the analysis of DNA sequences. Am. Math. Soc. 1986, 17, 57–86.

24. Yoon, S.H.; Ha, S.M.; Kwon, S.; Lim, J.; Kim, Y.; Seo, H.; Chun, J. Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. Int. J. Syst. Evol. Microbiol. 2017, 67, 1613–1617. [CrossRef] [PubMed]

25. Goris, J.; Konstantinidis, K.T.; Klappenbach, J.A.; Coenye, T.; Vandamme, P.; Tiedje, J.M. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int. J. Syst. Evol. Microbiol. 2007, 57, 81–91. [CrossRef] [PubMed]

26. Richter, M.; Rosselló-Mora, R. Shifting the genomic gold standard for the prokaryotic species definition. Proc. Natl. Acad. Sci. USA 2009, 106, 19126–19131. [CrossRef] [PubMed]

27. 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 Biol. 2013, 14, 60. [CrossRef]

28. Medema, M.H.; Blin, K.; Cimermancic, P.; de Jager, V.; Zakrzewski, P.; Fischbach, M.A.; Weber, T.; Takano, E.; Breitling, R. antiSMASH: Rapid identification, annotation, and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res. 2011, 39, W339–W346. [CrossRef] [PubMed]

29. Weber, T.; Blin, K.; Duddela, S.; Krug, D.; Kim, H.U.; Brucoleri, R.; Lee, S.Y.; Fischbach, M.A.; Müller, R.; Wohlleben, W.; et al. antiSMASH 3.0- a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res. 2015, 43, W237–W243. [CrossRef] [PubMed]

30. Dunlap, C.A.; Kim, S.J.; Kwon, S.W.; Rooney, A.P. Bacillus velezensis is not a later heterotypic synonym of Bacillus amyloliquefaciens; Bacillus methylotrophicus, Bacillus amyloliquefaciens subsp. plantarum and “Bacillus oryzicola” are later heterotypic synonyms of Bacillus velezensis based on phylogenomics. Int. J. Syst. Evol. Microbiol. 2016, 66, 1212–1217. [CrossRef]

31. Dunlap, C.A.; Bowman, M.J.; Rooney, A.P. Inner Lipopeptide Diversity in the Bacillus subtilis species group—important antifungals for plant disease biocontrol applications. Front. Microbiol. 2019, 10, 1794. [CrossRef] [PubMed]

32. Belbahri, L.; Chenari Bouket, A.; Reiki, I.; Alenezi, F.N.; Vallat, A.; Luptakova, L.; Petrovova, E.; Oszako, T.; Cherrad, S.; Vacher, S.; et al. Comparative genomics of Bacillus amyloliquefaciens strains reveals a core genome with traits for habitat adaptation and a secondary metabolites rich accessory genome. Front. Microbiol. 2017, 8, 1438. [CrossRef]

33. Stein, T. Bacillus subtilis antibiotics: Structures, syntheses and specific functions. Mol. Microbiol. 2005, 56, 845–857. [CrossRef] [PubMed]

34. Schneider, K.; Chen, X.; Vater, J.; Franke, P.; Nicholson, G.; Borris, R.; Süßmuth, R.D. Macrolactin is the polyketide biosynthesis product of the pks2 cluster of Bacillus amyloliquefaciens FZB42. J. Nat. Prod. 2007, 70, 1417–1423. [CrossRef] [PubMed]

35. Ongena, M.; Henry, G.; Thornart, P. The roles of cyclic lipopeptides in the biocontrol activity of Bacillus amyloliquefaciens strain LM2303 against Fusarium head blight. PLoS ONE 2018, 13, e0198560. [CrossRef]

36. Ongena, M.; Jacques, P. Bacillus lipopeptides: Versatile weapons for plant disease biocontrol. Trends Microbiol. 2008, 16, 115–125. [CrossRef] [PubMed]

37. Koumoutsi, A.; Chen, X.H.; Henne, A.; Liesegang, H.; Hitzeroth, G.; Franke, P.; Vater, J.; Borris, R. Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive cyclic lipopeptides in Bacillus amyloliquefaciens strain FZB42. J. Bacteriol. 2004, 186, 1084–1096. [CrossRef] [PubMed]

38. Dimkic, I.; Stankovic, S.; Nišavic, M.; Petkovic, M.; Ristivojevic, P.; Fira, D.; Beric, T. The profile and antimicrobial activity of Bacillus lipopeptide extracts of five potential biocontrol strains. Front. Microbiol. 2017, 8, 925. [CrossRef] [PubMed]
40. Mhammedi, A.; Peypoux, F.; Besson, F.; Michel, G. Bacillomycin f, a new antibiotic of iturin group: Isolation and characterization. J. Antimicrob. Chemother. 1982, 35, 306–311. [CrossRef] [PubMed]

41. Phae, C.G.; Shoda, M.; Kubota, H. Suppressive effect of Bacillus subtilis and its products on phytopathogenic microorganisms. J. Ferment. Bioeng. 1990, 69, 1–7. [CrossRef]

42. Moyne, A.L.; Shelby, R.; Cleveland, T.E.; Tuzun, S. Bacillomycin D: An iturin with antifungal activity against Aspergillus flavus. J. Appl. Microbiol. 2001, 90, 622–629. [CrossRef]

43. Yu, G.Y.; Sinclair, J.B.; Hartman, G.L.; Bertagnolli, B.L. Production of iturin A by Bacillus amyloliquefaciens suppressing Rhizoctonia solani. Soil Biol. Biochem. 2002, 34, 955–963. [CrossRef]

44. Aranda, F.J.; Teruel, J.A.; Ortiz, A. Further aspects on the haemolytic activity of the antibiotic lipopeptide iturin A. Biochim. Biophys. Acta 2005, 17, 51–56. [CrossRef] [PubMed]

45. Besson, F.; Michel, G. Action of mycosubtilin, an antifungal antibiotic of Bacillus subtilis. J. Antibiot. 1995, 48, 1037–1050. [CrossRef] [PubMed]

46. Garcia-Gutierrez, L.; Zerouli, M.; Romero, D.; Cubero, J.; Vicente, A.; Perez-Garcia, A. The antagonistic strain Bacillus subtilis UMAF6639 also confers protection to melon plants against cucurbit powdery mildew by activation of jasmonate-and salicylic acid-dependent defence responses. Microb. Biotechnol. 2013, 6, 264–274. [CrossRef] [PubMed]

47. Henry, G.; Deleu, M.; Jourdan, E.; Thonart, P.; Ongena, M. The bacterial lipopeptide surfactin targets the lipid fraction of the plant plasma membrane to trigger immune-related defence responses. Cell Microbiol. 2011, 13, 1824–1837. [CrossRef] [PubMed]

48. Khong, N.G.; Randoux, B.; Tayeh, C.; Coutte, F.; Bourdon, N.; Tisserant, B.; Laruelle, F.; Jacques, P.; Reignault, P. Induction of resistance in wheat against powdery mildew by bacterial cyclic lipopeptides. Commun. Agric. Appl. Biol. Sci. 2012, 77, 39–51. [PubMed]

49. Warwongthongwak, W.; Leelasuphakul, W.; McCollum, G. Cyclic LIPopeptides from Bacillus subtilis ABS-S14 elicit defense-related gene expression in citrus fruit. PLoS ONE 2019, 9, e109386. [CrossRef]

50. Chowdhury, S.P.; Hartmann, A.; Gao, X.; Borriss, R. Biocontrol mechanism by root-associated Bacillus amyloliquefaciens FZB42. J. Agric. Food Chem. 2012, 60, 2976–2981. [CrossRef] [PubMed]
65. Chen, X.H.; Scholz, R.; Borris, M.; Junge, H.; Mogel, G.; Kunz, S.; Borris, R. Difficidin and bacilysin produced by plant-associated *Bacillus amyloliquefaciens* are efficient in controlling fire blight disease. *J. Biotechnol.* 2009, 140, 38–44. [CrossRef] [PubMed]

66. Wu, L.; Wu, H.; Chen, L.; Yu, X.; Borris, R.; Gao, X. Difficidin and bacilysin from *Bacillus amyloliquefaciens* FZB42 have antibacterial activity against *Xanthomonas oryzae* rice. *Sci. Rep.* 2015, 5, 12975. [CrossRef] [PubMed]

67. Wu, L.; Wu, H.; Chen, L.; Lin, L.; Borris, R.; Gao, X. Bacilysin overproduction in *Bacillus amyloliquefaciens* FZB42 markerless derivative strains FZB42P and FZBSPA enhances antibacterial activity. *Appl. Microbiol. Biotechnol.* 2015, 99, 4255–4263. [CrossRef] [PubMed]

68. Steinborn, G.; Hajirezaei, M.R.; Hofemeister, J. bac genes for recombinant bacilysin and anticapsin production in *Bacillus* host strains. *Arch. Microbiol.* 2005, 183, 71–79.

69. Mahlstedt, S.A.; Walsh, C.T. Investigation of anticapsin biosynthesis reveals a four-enzyme pathway to tetrahydrotyrosine in *Bacillus subtilis*. *Biochemistry* 2010, 49, 912–923. [CrossRef]

70. Babasaki, K.; Takao, T.; Shimoniishi, Y.; Kurahashi, K. Subtilosin A, a new antibiotic peptide produced by *Bacillus subtilis* 168: Isolation, structural analysis, and biogenesis. *J. Biochem.* 1985, 98, 585–603. [CrossRef] [PubMed]

71. Stein, T.; Düsterhus, S.; Stroh, A.; Entian, K.D. Subtilosin production by two *Bacillus subtilis* subspecies and variance of the sbo-alb cluster. *Appl. Environ. Microbiol.* 2004, 70, 2349–2353. [CrossRef] [PubMed]

72. Sutyak, K.E.; Wirawan, R.E.; Aroucheva, A.A.; Chikindas, M.L. Isolation of the *Bacillus subtilis* antimicrobial peptide subtilosin from the dairy product-derived *Bacillus amyloliquefaciens*. *J. Appl. Microbiol.* 2008, 104, 1067–1074. [CrossRef] [PubMed]

73. Shelburne, C.E.; An, F.Y.; Dholpe, V.; Ramamoorthy, A.; Lopatin, D.E.; Lantz, M.S. The spectrum of antimicrobial activity of the 18 bacteriocin subtilosin A. *J. Antimicrob. Chemother.* 2007, 59, 297–300. [CrossRef] [PubMed]

74. Wiedemann, I.; Breukink, E.; Kraaij, C.V.; Kuipers, O.S.; Bierbaum, G.; de Kruijff, B.; Sahl, H.G. Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. *J. Bacteriol.* 2000, 182, 3266–3273. [CrossRef] [PubMed]

75. Thennarasu, S.; Lee, D.K.; Poon, A.; Kawulka, K.E.; Vederas, J.C.; Ramamoorthy, A. Membrane permeabilization, orientation, and antimicrobial mechanism of subtilosin A. *Chemotherapie.* 2019, 1–9. [CrossRef] [PubMed]

76. Zheng, G.; Hehn, R.; Zuber, P. Mutational analysis of sbo-alb locus of *Bacillus subtilis*: Identification of genes required for subtilosin production and immunity. *J. Bacteriol.* 2000, 182, 5356–5366. [CrossRef] [PubMed]

77. González-Pastor, J.E.; Hobbs, E.C.; Losick, R. Cannibalism by sporulating bacteria. *Science* 2003, 301, 510–513. [CrossRef] [PubMed]

78. González-Pastor, J.E. Cannibalism: A social behavior in sporulating *Bacillus subtilis*. *FEMS Microbiol. Rev.* 2011, 35, 415–424. [CrossRef] [PubMed]

79. Lin, D.; Qu, L.J.; Gu, H.; Chen, Z. A 3.1-kb genomic fragment of *Bacillus subtilis* encodes the protein inhibiting growth of *Xanthomonas oryzae* pv. *oryzae*. *Appl. Microbiol. Biotechnol.* 2001, 91, 1044–1050. [CrossRef]

80. Guo, S.; Li, X.; He, P.; Ho, H.; Wu, W.; He, Y. Whole-genome sequencing of *Bacillus subtilis* XF-1 reveals mechanisms for biological control and multiple beneficial properties in plants. *J. Ind. Microbiol. Biotechnol.* 2015, 42, 925–937. [CrossRef] [PubMed]

81. Peypoux, F.; Bonmatin, J.M.; Wallach, J. Recent trends in the biochemistry of surfactin. *Appl. Microbiol. Biotechnol.* 1999, 51, 553–563. [CrossRef] [PubMed]

82. Bonmatin, J.M.; Laprêvote, O.; Peypoux, F. Diversity among microbial cyclic lipopeptides: Iturins and surfactins. Activity-structure relationships to design new bioactive agents. *Com. Chem. High Throughput Screen.* 2003, 6, 541–556. [CrossRef] [PubMed]

83. Zeriouh, H.; de Vicente, A.; Pérez-García, A.; Romero, D. Surfactin triggers biofilm formation of *Bacillus subtilis* in melon phylloplane and contributes to the biocontrol activity. *Environ. Microbiol.* 2013, 16, 2196–2211. [CrossRef] [PubMed]

84. Wipat, A.; Harwood, C.R. The *Bacillus subtilis* genome sequence: The molecular blueprint of a soil bacterium. *FEMS Microbiol. Ecol.* 1999, 28, 1–9. [CrossRef] [PubMed]

85. Bezzate, S.; Aymerich, S.; Chambert, R.; Czarne, S.; Berge, O.; Heulin, T. Disruption of the *Paenibacillus polymyxa* levansucrase gene impairs its ability to aggregate soil in the wheat rhizosphere. *Environ. Microbiol.* 2000, 2, 333–342. [CrossRef] [PubMed]

86. Kearns, D.B.; Chu, F.; Rudner, R.; Losick, R. Genes governing swarming in *Bacillus subtilis* and evidence for a phase variation mechanism controlling surface motility. *Mol. Microbiol.* 2004, 52, 357–369. [CrossRef] [PubMed]

87. Calvio, C.; Celandroni, F.; Ghezardi, E.; Amati, G.; Salvetti, S.; Cecilliani, F.; Galizzi, A.; Senesi, S. Swarming differentiation and swimming motility in *Bacillus subtilis* are controlled by swrA, a newly identified dicistronic operon. *J. Bacteriol.* 2005, 187, 5356–5366. [CrossRef] [PubMed]

88. Tsuge, K.; Obata, Y.; Shoda, M. Gene yerP, involved in surfactin self resistance in *Bacillus subtilis*. *Antimicrob. Agents Chemother.* 2001, 45, 3566–3573. [CrossRef] [PubMed]

89. Yu, Y.; Yan, F.; Chen, Y.; Jin, C.; Guo, J.H.; Chai, Y. Poly-γ-glutamic acid contribute to biofilm formation and plant root colonization in selected environmental isolates of *Bacillus subtilis*. *Front. Microbiol.* 2016, 7, 1811. [CrossRef]

90. Lazarevic, V.; Soldo, B.; Medico, N.; Pooley, H.; Bron, S.; Karamata, D. *Bacillus subtilis* alpha-Phosphoglucomutase is required for normal cell morphology and biofilm formation. *Appl. Environ. Microbiol.* 2005, 71, 39–45. [CrossRef] [PubMed]

91. Branda, S.S.; González-Pastor, J.E.; Dervyn, E.; Ehrlich, S.D.; Losick, R.; Kolter, R. Genes involved in formation of structural multicellular communities by *Bacillus subtilis*. *J. Bacteriol.* 2004, 186, 3970–3979. [CrossRef] [PubMed]

92. Kearns, D.B.; Chu, F.; Branda, S.S.; Kolter, R. Losick, R. A master regulator for biofilm formation by *Bacillus subtilis*. *Mol. Microbiol.* 2005, 55, 739–749. [CrossRef] [PubMed]
93. Branda, S.S.; González-Pastor, J.E.; Ben-Yehuda, S.; Losick, R.; Kolter, R. Fruiting body formation by \textit{Bacillus subtilis}. \textit{Proc. Natl. Acad. Sci. USA} \textbf{2001}, 98, 11621–11626. [CrossRef]

94. Chu, F.; Kearns, D.B.; McLoon, A.; Chai, Y.; Kolter, R.; Losick, R. A novel regulatory protein governing biofilm formation in \textit{Bacillus subtilis}. \textit{Mol. Microbiol.} \textbf{2008}, 68, 117–127. [CrossRef] [PubMed]

95. Chu, F.; Kearns, D.B.; Branda, S.S.; Kolter, R.; Losick, R. Targets of the master regulator of biofilm formation in \textit{Bacillus subtilis}. \textit{Mol. Microbiol.} \textbf{2006}, 59, 1216–1228. [CrossRef]

96. Harman, G.E. Multifunctional fungal plant symbionts: New tools to enhance plant growth and productivity. \textit{New Phytol.} \textbf{2011}, 189, 647–649. [CrossRef]

97. Shafi, J.; Tian, H.; Ji, M. \textit{Bacillus} species as versatile weapons for plant pathogens: A review. \textit{Biotechnol. Biotechnol. Equip.} \textbf{2017}, 31, 446–459. [CrossRef]

98. Holtmann, G.; Bakker, E.P.; Uozumi, N.; Bremer, E. KtrAB and KtrCD: Two K+ uptake systems in \textit{Bacillus subtilis} and their role in adaptation to hypertoncity. \textit{J. Bacteriol.} \textbf{2003}, 185, 1289–1298. [CrossRef]

99. Miethke, M.; Klotz, O.; Linne, U.; May, J.J.; Beckering, C.L.; Marahiel, M.A. Ferri-bacillibactin uptake and hydrolysis in \textit{Bacillus subtilis}. \textit{Mol. Microbiol.} \textbf{2006}, 61, 1413–1427. [PubMed]

100. Chen, K.; Tian, Z.; Luo, Y.; Cheng, Y.; Long, C. Antagonistic Activity and the Mechanism of \textit{Bacillus amylovoricfraciens} DH-4 Against Citrus Green Mold. \textit{Phytopathology} \textbf{2018}, 108, 1253–1262. [CrossRef]

101. Rajkumar, M.; Ae, N.; Prasad, M.N.; Freitas, H. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. \textit{Trends Biotechnol.} \textbf{2010}, 28, 142–149. [CrossRef] [PubMed]

102. Radzki, W.; Gutierrez Manero, F.J.; Algar, E.; Lucas García, J.A.; Garcia-Villaraco, A.; Ramos Solano, B. Bacterial siderophores efficiently provide iron to iron-starved tomato plants in hydroponics culture. \textit{Antonie Leeuwenhoek} \textbf{2013}, 104, 321–330. [CrossRef] [PubMed]

103. Lurthy, T.; Cantat, C.; Jeudy, C.; Declerck, P.; Gallardo, K.; Barraud, C.; Leroy, F.; Ourry, A.; Lehmann, P.; Salou, C.; et al. Impact of bacterial siderophores on iron status and ionome in pea. \textit{Front. Plant Sci.} \textbf{2020}, 11, 730. [CrossRef]

104. Arguelles-Arias, A.; Orgena, M.; Halimi, B.; Lara, Y.; Brans, A.; Joris, B.; Fickers, P. \textit{Bacillus amylovoricfraciens} GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. \textit{Microb. Cell Fact.} \textbf{2009}, 8, 63. [CrossRef]

105. Yu, X.; Ai, C.; Xin, L.; Zhou, G. The siderophore-producing bacterium, \textit{Bacillus subtilis} CAS15, has a biocontrol effect on Fusarium wilt and promotes the growth of pepper. \textit{Eur. J. Soil Biol.} \textbf{2011}, 47, 138–145. [CrossRef]

106. Woo, S.M.; Kim, S.D. Structural identification of siderophore (AH18) from \textit{Bacillus subtilis} AH18, a biocontrol agent of phytophthora blight disease in red-pepper. \textit{Kor. J. Microbiol. Biotechnol.} \textbf{2008}, 36, 326–335. [CrossRef]

107. Tahir, H.A.; Gu, Q.; Wu, H.; Raza, W.; Hanif, A.; Wu, L.; Colman, M.V.; Gao, X. Plant Growth Promotion by Volatile Organic Compounds Produced by \textit{Bacillus subtilis} SYS2. \textit{Front. Microbiol.} \textbf{2017}, 8, 171. [CrossRef] [PubMed]

108. Lee, B.; Farag, M.A.; Park, H.B.; Kloepper, W.J.; Lee, S.H.; Ryu, C.M. Induced resistance by a long-chain bacterial volatile: Elicitation of plant systemic defense by a C13 volatile produced by \textit{Pseudomonas syringae} pv. \textit{tomato}. \textit{PLoS ONE} \textbf{2012}, 7, e48744. [CrossRef] [PubMed]

109. Park, Y.S.; Dutto, S.; Ann, M.; Raaijmakers, J.M.; Park, K. Promotion of plant growth by \textit{Pseudomonas fluorescens} strain SS101 via novel volatile organic compounds. \textit{Biochem. Biophys. Res. Commun.} \textbf{2015}, 461, 361–365. [CrossRef] [PubMed]

110. Tahir, H.A.; Gu, Q.; Wu, H.; Niu, Y.; Hsu, R.; Gao, X. \textit{Bacillus} volatiles adversely affect the physiology and ultra-structure of \textit{Ralstonia solanacearum} and induce systemic resistance in tobacco against bacterial wilt. \textit{Sci. Rep.} \textbf{2017}, 7, 40481. [CrossRef] [PubMed]

111. Zhang, X.; Zhang, R.; Bao, T.; Yang, T.; Xu, M.; Li, H.; Xu, Z.; Rao, Z. Moderate expression of the transcriptional regulator \textit{ALsR} and \textit{ALsR} promotes root colonization and biocontrol activity of \textit{Bacillus amylovoricfraciens} SQR9 by abrB gene disruption. \textit{Appl. Microbiol. Biotechnol.} \textbf{2012}, 97, 8823–8830. [CrossRef] [PubMed]
120. Chowdhury, S.P.; Dietel, K.; Rändler, M.; Schmid, M.; Junge, H.; Borris, R.; Hartmann, A.; Grosch, R. Effects of *Bacillus amyloliquefaciens* FZB42 on lettuce growth and health under pathogen pressure and its impact on the rhizosphere bacterial community. *PLoS ONE* **2013**, *8*, e68818. [CrossRef]

121. Zhu, M.L.; Wu, X.Q.; Wang, Y.H.; Dai, Y. Role of biofilm formation by *Bacillus pumilus* HR10 in biocontrol against pine seedling damping-off disease caused by *Rhizoctonia solani*. *Forests* **2020**, *11*, 652. [CrossRef]

122. Steller, S.; Vater, J. Purification of the fengycin synthetase multienzyme system from *Bacillus subtilis* b213. *J. Chromatogr. B Biomed. Sci. Appl.* **2000**, *737*, 267–275. [CrossRef]

123. Deleu, M.; Paquot, M.; Nylander, T. Fengycin interaction with lipid monolayers at the air-aqueous interface—implications for the effect of fengycin on bacterial membranes. *J. Colloid Interface Sci.* **2005**, *283*, 358–365. [CrossRef]

124. Wang, J.; Liu, J.; Chen, H.; Yao, J. Characterization of *Fusarium graminearum* inhibitory lipopeptide from *Bacillus amyloliquefaciens* FZB45. *J. Chromatogr. B Biomed. Sci. Appl.* **2000**, *737*, 267–275. [CrossRef]

125. Hanif, A.; Zhang, F.; Li, P.; Li, C.; Xu, Y.; Zubair, M.; Zhang, M.; Jia, D.; Zhao, X.; Liang, J.; et al. Fengycin produced by *Fusarium oxysporum* inhibits *Fusarium graminearum* growth and mycotoxins biosynthesis. *Toxins* **2019**, *11*, 295. [CrossRef] [PubMed]

126. Ongena, M.; Jourdan, E.; Adam, A.; Paquot, M.; Brans, A.; Joris, B.; Arpigny, J.L.; Thonart, P. Surfactin and fengycin lipopeptides from *Fusarium graminearum* interact with bacterial and mammalian cell membranes. *Appl. Biochem. Biotechnol.* **2007**, *135*, 54–61. [CrossRef] [PubMed]

127. Cao, Y.; Xu, Z.; Ling, N.; Yuan, Y.; Yang, X.; Chen, L.; Shen, B.; Shen, Q. Isolation and identification of lipopeptides produced by *Bacillus velezensis* isolates against *Ralstonia solanacearum* and *Fusarium graminearum*. *J. Chromatogr. B Biomed. Sci. Appl.* **2013**, *84*, 295–301. [CrossRef] [PubMed]

128. Yuan, J.; Raza, W.; Huang, Q.; Shen, Q. The ultrasound-assisted extraction and identification of antifungal substances from *B. amyloliquefaciens* strain NNN-6 suppressing *Fusarium oxysporum*. *J. Basic Microbiol.* **2012**, *52*, 721–730. [CrossRef] [PubMed]

129. Zhang, L.; Sun, C. Fengycins, cyclic lipopeptides from marine *Bacillus subtilis* strains, kill the plant-pathogenic fungus *Magnaporthe grisea* by inducing reactive oxygen species production and chromatin condensation. *Appl. Environ. Microbiol.* **2018**, *84*, e00445-18. [CrossRef] [PubMed]

130. Idriss, E.E.; Makarewicz, O.; Farouk, A.; Rosner, K.; Greiner, R.; Richter, T.; Borris, R. Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. *Microbiology* **2002**, *148*, 2097–2109. [CrossRef]

131. Kumar, V.; Singh, P.; Jorquera, M.A.; Sangwan, P.; Kumar, P.; Verma, A.K.; Agrawal, S. Isolation of phytase-producing bacteria from Himalayan soils and their effect on growth and phosphorus uptake of Indian mustard (*Brassica juncea*). *World J. Microbiol. Biotechnol.* **2013**, *29*, 1361–1369. [CrossRef]

132. Duca, D.R.; Rose, D.R.; Glick, B.R. Indole acetic acid overproduction transformants of the rhizobacterium *Pseudomonas* sp. UW4 synergistically protect tomato plants against salt stress. *Front. Microbiol.* **2019**, *10*, 1392. [CrossRef] [PubMed]

133. Kumar, V.; Singh, P.; Jorquera, M.A.; Sangwan, P.; Kumar, P.; Verma, A.K.; Agrawal, S. Isolation of phytase-producing bacteria from Himalayan soils and their effect on growth and phosphorus uptake of Indian mustard (*Brassica juncea*). *World J. Microbiol. Biotechnol.* **2013**, *29*, 1361–1369. [CrossRef]

134. Liu, L.; Li, A.; Chen, J.; Su, Y.; Li, Y.; Ma, S. Isolation of a phytase-producing bacterial strain from agricultural soil and its characterization and application as an effective eco-friendly phosphate solubilizing bioinoculant. *Commun. Soil Sci. Plant* **2018**, *49*, 984–994. [CrossRef]

135. Orozco-Mosqueda, M.C.; Duan, J.; DiBernardo, M.; Zetter, E.; Campos-Garcia, J.; Glick, B.R.; Santoyo, G. The production of ACC deaminase and trehalose by the plant growth promoting bacterium *Pseudomonas* sp. UW4 synergistically protect tomato plants against salt stress. *Front. Microbiol.* **2019**, *10*, 1392. [CrossRef] [PubMed]

136. Duca, D.R.; Rose, D.R.; Glick, B.R. Indole acetic acid overproduction transformants of the rhizobacterium *Pseudomonas* sp. UW4. *Antonie Leeuwenhoek* **2018**, *111*, 1645–1660. [CrossRef] [PubMed]

137. Moon, J.-H.; Won, S.-J.; Maung, C.E.H.; Choi, J.-H.; Choi, S.-I.; Ajuna, H.B.; Ahn, Y.S. *Bacillus velezensis* CE 100 Inhibits Root Rot Diseases (Phytophthora spp.) and Promotes Growth of Japanese Cypress (*Chamaecyparis obtusa* Endlicher) Seedlings. *Microorganisms* **2021**, *9*, 821. [CrossRef]