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Biological Traits of Vertically Seed-Transmitted *Bacillus mojavensis* in *Triticum aestivum* L.

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Abstract: Seed-borne endophytic bacteria can influence host responses to biotic and abiotic stress conditions. Their presence in seeds is related to their ability to colonize plant tissues and to pass from parent plants to offspring. In this study, we investigated the ability of *Bacillus mojavensis* PS17 to pass into the next generation of spring wheat plants via seeds and the effect of the transmission mode on the functional traits of seed-transmitted colonies of PS17. The rifampicin-resistant PS17 strain at 100 µg/mL was used to track PS17 effectively throughout the wheat growth cycle. The results demonstrated the successful colonization of *B. mojavensis* PS17 and its ability to pass into the next plant generation through seeds. During plant development, the PS17 cell population was almost higher in the rhizosphere than in the aboveground parts of plants, including seeds at the grain-filling stage. The seed-transmitted *B. mojavensis* PS17 colonies exhibited identical biological traits to those of the parental PS17 strain. *Bacillus mojavensis* PS17 retained its ability to suppress the growth of pathogens, such as *Fusarium oxysporum* and *Alternaria alternata*, and produce hydrolases, including protease, lipase, amylase, and cellulase. These results highlight the potential of vertical transmission through seeds as a mode of spreading bacterial biocontrol agents in future plants.

Keywords: antibiosis; antagonism; biocontrol agents; endophytes; plant-microbe interaction; rhizosphere; spring wheat; transmission of bacterial endophytes

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1. Introduction

Biotic stresses caused by phytopathogens constrain sustainable agricultural production and affect global food security [1–3]. Seeds, as reproductive organs necessary for species survival, harbor diverse microbial communities, including beneficial symbionts and pathogenic microorganisms that significantly affect the development of next-generation plants. Chemical and physical seed pretreatments enhance seed traits and promote plant growth [4,5]. However, exploring sustainable green technologies is crucial [6,7]. To date, studies have highlighted the potential of seed-borne endophytic microorganisms as an alternative approach for managing seed-borne pathogens and abiotic factors that affect seeds at emergence and during early growth stages [8–10]. Seed-borne endophytic microorganisms can improve a plant's physiological and morphological characteristics via both direct and indirect mechanisms, including the production of siderophores and various phytohormones, as well as by inducing systemic acquired resistance and induced systemic resistance [11–16]. In addition, the competition between endophytic microorganisms and

plant pathogens for the same ecological niche within plants makes them potential instruments for plant disease management and crop yield enhancement [17–19]. According to Frank et al. [20], seed endophytes can originate from the surrounding environment or be inherited from parent plants through seed transmission. The transmission mode by which beneficial microorganisms can be transmitted from parent plants to offspring through seeds provides a range of advantages. This transmission mode protects plants against biotic and abiotic stress during the early stages of plant development [20–23]. Successful vertical transmission promotes the preservation of beneficial microorganisms in seeds for many generations, creating a self-sustaining method of pest and disease control [24]. Moreover, vertically transmitted microorganisms may eventually become dominant in seed microbiota, further increasing their efficiency and stability in plant ecosystems [25,26]. Vertical transmission of endophytic bacteria can be influenced by the following factors: the host plant characteristics, including genotype, growth stage, and immune response; the interaction with seed microbial communities present within their tissues; and the biological traits of microorganisms. For example, resistance to high osmotic pressure is characteristic of microorganisms that develop in seeds during the ripening period when starch accumulates and the water content decreases [27–29]. *Bacillus mojavensis* PS17 is a biological agent isolated from the wheat (*Triticum aestivum* L.) variety ''Sadokat" (Tatdjik Republic). Its ability to suppress the growth of phytopathogenic fungi, such as *Fusarium graminearum*, *Fusarium oxysporum*, *Fusarium chlamydosporum*, *Ascochyta pisi*, *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Verticillium dahliae*, and *Epicoccum nigrum,* to promote plant development and produce hydrolytic enzymes, such as chitinase, β-glucanase, cellulase, lipase, and protease, was reported in our previous study [30]. Considering the potential benefits of vertical transmission of endophytic bacteria, this study examined the ability of *B. mojavensis* PS17, a facultative endophyte, to be vertically transmitted to the next generation of plants via the seeds. We also evaluated the biological traits of seed-transmitted PS17 colonies in both soil and plant tissue. To ensure efficient tracking and establish their effectiveness under field conditions, we employed a rifampicin-resistant mutant strain, which is a well-established marker in such studies [31].

2. Materials and Methods

2.1. Microbial Strains and Growth Conditions

The microbial strains used in this study are listed in Table 1. Bacterial strains were cultivated on King's B (KB) medium and incubated at 28 ± 1 °C, while fungal strains were grown on Sabouraud medium (Merck, Darmstadt, Germany) and incubated at 25 ± 1 °C. *Bacillus mojavensis* PS17 was deposited in the All-Russia Collection of Industrial Microorganisms (NRC "Kurchatov Institute", Moscow, Russia) under number VKPM B-13415. The genomic sequence of *B. mojavensis* PS17 was deposited in GenBank under the accession number CP066516.1. The spring wheat variety "Ulianovsky 105" (Tatarstan, Kazan, Russia) was provided by the Centre of Agroecological Research, Kazan State Agrarian University (Kazan, Russia).

Table 1. The microbial strains used in this study.

Microbial Strains	Source	Reference
B. mojavensis PS17	From wheat seed	[30]
B. mojavensis PS17 (Rif^{100})	Rifampicin-resistant PS17 at 100 μg/mL	In this study
Bacillus amyloliquefaciens	From rhizosphere winter wheat	*
Bacillus halotolerans		*
Bacillus sp.		*
F. oxysporium	From onion (*Allium cepa* L.)	*
A. alternata	From spring wheat seed	*

* Microbial collection from the Centre of Agroecological Research, Kazan State Agrarian University.

2.2. Rifampicin-Resistant *B. mojavensis* PS17 Preparation

Rifampicin-resistant *Bacillus mojavensis* PS17 [PS17 (Rif^{100})] was obtained using a stepwise gradient method [31]. For this purpose, *B. mojavensis* PS17 wild-type was plated onto KB agar containing 0.1 µg/mL rifampicin and incubated overnight at 30 ± 1 °C. Rifampicin-resistant colonies were then selected and re-streaked onto KB agar containing a higher concentration (0.5 µg/mL) of rifampicin. This process of selection and re-streaking at increasing rifampicin concentrations (with 0.5 µg/mL increments) was repeated until a mutant was stably resistant to 100 µg/mL rifampicin.

2.3. Verification of Rifampicin Resistance Specificity

To ensure that the rifampicin resistance marker at the concentration of 100 µg/mL was specific to *B. mojavensis* PS17 (Rif^{100}) and that no other rifampicin resistance at this concentration was present in the soil or seed endophytic bacteria, a screening analysis was performed. The presence of pre-existing rifampicin-resistant bacterial strains was assessed at 100 µg/mL from garden soil and seed endophytes before use. For this purpose, 1 g of garden soil or wheat seed was serially diluted. After dilution, aliquots (100 µL) of 10^5 dilutions were plated onto KB agar amended with rifampicin (100 µg/mL) and nystatin (50 µg/mL). All plates were incubated at 30 ± 1 °C.

2.4. Bacterial Cell Suspension Preparation

The bacterial suspension was prepared from the bacterial culture *of B. mojavensis* PS17 (Rif^{100}) grown for 24 h in lysogeny broth (LB) [g/L: tryptone, 10 g; yeast extract, 5 g; and NaCl, 10 g] at 30 ± 1 °C. Cells were pelleted by centrifuging for 5 min at 4000 rpm and 4 ± 1 °C. The resulting supernatant was removed, and the precipitate was washed twice with sterile phosphate-buffered saline (PBS) [140 mM NaCl, 5 mM KH_2PO_4, 1 mM $NaHCO_3$, and pH 7.4]. After washing, the pellet was resuspended in PBS buffer to a final optical density (OD) of 0.1 at 595 nm, corresponding to a bacterial concentration of approximately 10^6 CFU/mL.

2.4.1. Vertical Transmission of *B. mojavensis* PS17

The vertical transmission of *B. mojavensis* PS17 was evaluated in a climate-controlled chamber (HPP 750 Memmert, Memmert, Germany) at 25 °C with 75% humidity and a 16 h light/8 h dark cycle. For this purpose, surface-sterilized wheat seeds were used, as studied by Simon et al. [32], alongside garden soil. Seeds were pretreated with a cell suspension of *B. mojavensis* PS17 using the semi-dry method of 1 L of cell bacterial culture suspension per 100 kg of seeds. Pretreated seeds (10 seeds per pot) were immediately sown in pots containing garden soil amended with sterile plant nutrient solution (PNS) [1.25 mM $Ca(NO_3)_2$; 1.25 mM KNO_3; 0.50 mM $MgSO_4$; 0.25 mM KH_4PO_4; 0.75 mg/L KI; 3.00 mg/L H_3BO_3; 10.0 mg/L $MnSO_4.H_2O$; 2.0 mg/L $ZnSO_4 \cdot 5H_2O$; 0.25 mg/L $Na_2MoO_4 \cdot 2H_2O$; 0.025 mg/L $CuSO_4 \cdot 5H_2O$; 0.025 mg/L $CoCI_2 \cdot 6H_2O$; and pH 5.8] to 60% of its water-holding capacity. Pots were watered twice daily with sterile tap water to maintain the soil moisture. Plants were cultivated up to the grain-filling stage. Samples were collected from different plant parts (leaves, stems, roots, and grains) at each growth stage (seedling and tillering, stem elongation, and milk development) to assess the endophytic colonization of PS17. Plants without bacterial treatment (pretreated only with water) were maintained as a control group to ensure the absence of any bacterial strain that could acquire spontaneous rifampicin resistance at 100 µg/mL in the soil and growing plants during the plant cultivation process. For statistical analysis, the experiment was repeated twice, and four pots per group were maintained. A significant difference between pretreatments was evaluated using one-way ANOVA and post hoc Tukey's test at $p < 0.05$.

2.4.2. Screening for *B. mojavensis* PS17 in Wheat

We screened for the presence of *B. mojavensis* PS17 in different wheat tissues, including soil, rhizosphere, roots, stems, leaves, and grains. Plant tissues were surface sterilized as

described by Simon et al. [32]. After sterilization, the tissues were homogenized in PBS buffer using a mortar and pestle, then 100 µL of obtained solutions were plated onto LB agar amended with rifampicin at a 100 µg/mL concentration and nystatin at 50 µg/mL to inhibit the growth of non-rifampicin-resistant bacteria and fungi. The plates were incubated at 30 ± 1 °C. Colonies growing on selective medium were presumed to be PS17 colonies. Subsequently, single-cell colonies were randomly selected and compared with the original PS17 strain using BOX-PCR and 16S rRNA, *recA*, *rpoB*, and *gyrA* gene sequence analyses.

2.5. DNA Fingerprinting Analysis

The total chromosomal DNA from bacterial strains was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. BOX-PCR was performed in a 25 µL volume, which included 2.5 µL of 10× PCR buffer, 0.4 µL of a 10 µM mixture of dNTPs, 0.5 µL of 10 µM primer BOXA1R (5′-CTACGGCAAGGCGACGCTGACG-3′), 5.0 µL of DNA template (50 ng), 1.0 µL of Taq DNA polymerase (1U), and nuclease-free water. Polymerase chain reaction (PCR) was performed using a thermocycler (Bio-Rad, Hercules, CA, USA) under initial denaturation at 95 °C for 2 min, followed by 30 cycles at 94 °C for 30 s. The primer annealing and elongation cycles were set to 58 °C for 30 s and 72 °C for 8 min, respectively. The final cycle was followed by a cycle at 72 °C for 10 min. The resulting PCR product was subjected to electrophoresis in 1% agarose gel.

2.6. Molecular Identification of Selected Bacterial Isolates

The identity of the selected bacterial strains was established by comparing the target genes presented in Table 2 with those of their parental *B. mojavensis* PS17 deposited in the National Center for Biotechnology Information (NCBI) GenBank.

Table 2. Primers used to identify isolated bacterial strains.

Target Genes	Oligonucleotide	Annealing Temperature	Reference
*rec*A	5′-GATCGTCAAGCAGCCTTAGAT-3′ 5′-TTACCGACCATAACGCCGAC-3′	55 °C	[33]
16S rRNA	5′-AGAGTTTGATCMTGGCTCAG-3′ 5′-AAGGAGGTGATCCAGCCGCA-3′	58 °C	[34]
*rpo*B	5′-ATCGAAACGCCTGAAGGTCCAAACAT-3′ 5′-ACACCCTTGTACCGTGACGACC-3′	58 °C	[35]
*gyr*A	5′-CAGTCAGGAAATGCGTACGTCCTT -3′ 5′-CAAGGTAATGCTCCAGGCATTGCT -3′	58 °C	[36]

PCR amplification of the target genes was performed using a QuantStudio 5 thermocycler (Applied Biosystems, Forester City, CA, USA). The PCR master mix contained 2.5 µL of 10× PCR buffer, 0.4 µL of a mixture of dNTPs (10 µM), 1.25 µL of each primer (10 µM), 2.5 µL of DNA sample (100 ng), 1.0 µL Taq DNA polymerase (1U), and nuclease-free water. The conditions included an initial denaturation at 95 °C for 3 min, followed by 36 cycles of denaturation at 95 °C for 15 s. The annealing temperature was set for 30 s, as described in Table 2, and the extension cycle was set at 72 °C for 40 s. The final extension cycle was performed at 72 °C for 10 min. After PCR amplification, the products were fragmented by electrophoresis in 1% agarose gel. Subsequently, DNA fragments were purified from the agarose gel using a cleanup kit (Evrogen, Moscow, Russia) according to the manufacturer's recommendations. Amplified fragments were determined by Evrogen (Moscow, Russia) using both forward and reverse primers. The resulting chromatograms were evaluated using the Clone Manager 9 software package (Sci Ed Software, Cologne, Germany) and blasted on NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 20 May 2024).

2.7. The Antagonistic Activity of the Selected Bacterial Isolates Against Phytopathogens

The ability of isolated bacterial colonies transmitted through seeds to inhibit the growth of the phytopathogenic fungi *F. oxysporium* and *A. alternata* was assayed using a dual culture method. For this purpose, a plug of phytopathogenic fungi was inoculated into the center of agar plates and allowed to grow for two days. Subsequently, 2 µL of the bacterial cell suspensions from the selected strains were co-inoculated at a distance around the periphery of the fungal inoculum. Plates were incubated at 28 ± 1 °C for up to ten days. The antagonistic activity against the fungi was determined by the formation of inhibition zones, which are clear areas around the bacterial colonies in which fungal growth is absent.

2.8. Hydrolytic Enzyme Production by Selected Bacterial Isolates

The ability of selected isolates to produce protease, cellulase, and amylase was determined by inoculating 2 µL of overnight bacterial culture onto basal medium (g/L: K_2HPO_4—5.8; KH_2PO_4—3; NH_4SO_4—1.0; $MgSO_4 \cdot 7H_2O$—0.2) amended with 1% skim milk powder (HiMedia, Mumbai India), carboxymethylcellulose sodium salt (HiMedia, India), and starch soluble AR (HiMedia, India), respectively. Lipase activity was assessed on lipase agar medium (peptone—10 g; NaCl—5 g; $CaCl_2 \cdot 2H_2O$—0.1 g; agar—18 g, and 10 mL (*v*/*v*) Tween-80). Subsequently, all plates were incubated at 30 ± 1 °C for up to four days. Cellulase and amylase were detected by flooding plates with 0.1% Congo red and 0.5% iodine solutions for 5 min, respectively. The plates were then destained with 0.1 N NaCl solution. The presence of a clearance zone (protease, amylase, and cellulase) or crystallite bubbles around the growing colonies indicated enzymatic activity.

3. Results

3.1. The Ability of B. mojavensis PS17 to Pass into Next-Generation Plants

The ability of PS17 to pass into next-generation plants through seeds was evaluated under laboratory conditions. The obtained results are presented in Figure 1. The presence of *B. mojavensis* PS17 was assessed in various wheat plant parts during tillering, stem elongation, heading, and grain-filling stages. The colony-forming units of PS17 after seed pretreatment were $6.37 \pm 0.15 \times 10^4$ cfu/g of plant tissue (Figure 1A). The presence of *B. mojavensis* PS17 remained relatively high in the rhizosphere during plant development. The colony-forming units of *B. mojavensis* PS17 in the rhizosphere were assayed as $3.87 \pm 0.14 \times 10^7$, $4.58 \pm 0.17 \times 10^6$, and $3.14 \pm 0.36 \times 10^5$ CFU/g of soil at the seedling and tillering (Figure 1B), stem elongation (Figure 1C), and grain-filling stages (Figure 1D), respectively. However, a decrease in PS17 colonies in the aboveground parts of the plants was observed only at the grain-filling stage, mostly in seeds. In contrast, the density of PS17 cells in the aboveground parts throughout the entire period of plant development did not exceed 10^7 CFU/g. While PS17 was detected in the aboveground plant parts, the population density was significantly lower than 10^4 CFU/g, particularly in seeds (Figure 1). In addition, the absence of bacterial growth in the control group confirmed the use of rifampicin as a selective marker under the tested conditions.

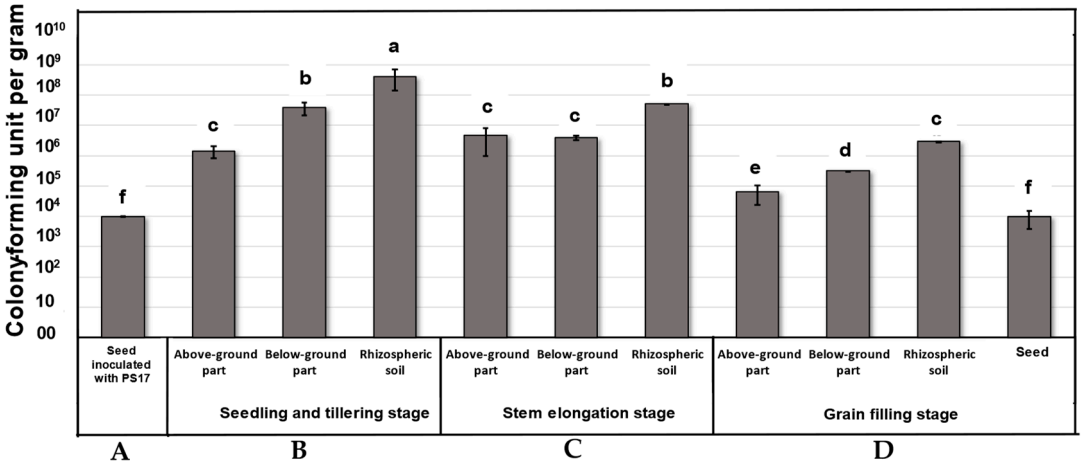

Figure 1. Colony-forming units of *B. mojavensis* PS17 screened in different parts of wheat tissues from seed inoculation (**A**), seedling and tillering (**B**), stem elongation (**C**), and grain-filling (**D**) growth stages. Statistical differences at p-value < 0.05 between groups are indicated by different lowercase letters.

3.2. Biological Traits of B. mojavensis PS17 Seed-Transmitted Colonies

Bacillus mojavensis PS17(Rif^{100}) was observed in all plant parts throughout the seedling, head differentiation, and grain-filling stages of spring wheat. All isolated bacterial colonies had identical morphologies, which were uniformly round in shape with a convex profile, similar to the original *B. mojavensis* PS17(Rif^{100}). Seed-transmitted colonies isolated from seeds (Figure 2C) were opaque, white, and homogeneous, exhibiting a slightly folded and scalloped edge with a hint of transparency compared to those isolated from roots (Figure 2A) and leaves (Figure 2B).

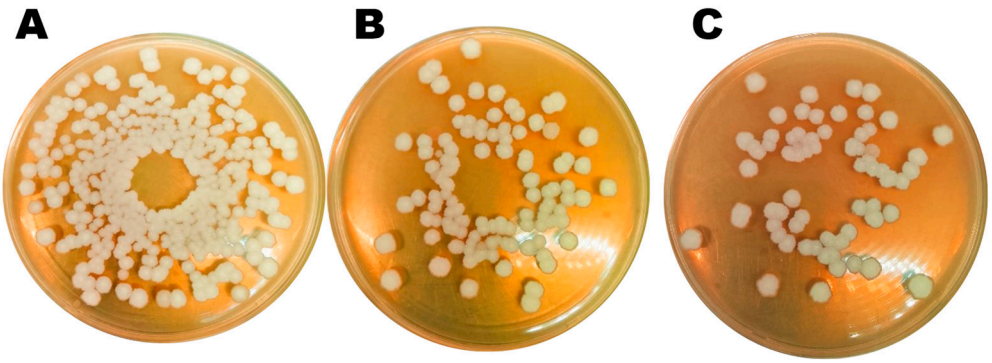

Figure 2. Screening *B. mojavensis* PS17 as an endophyte in wheat at the grain-filling stage. Bacterial colonies isolated from surface-sterilized roots (**A**), leaves (**B**), and seeds (**C**) of spring wheat. Bacterial strains were grown on LB agar medium amended with Rifampicin (100 µg/mL) for 48 h at 30 ± 1 °C.

3.2.1. The Antagonistic Activity of the Isolated Bacteria Against Phytopathogens

The abilities of seed-transmitted colonies of PS17(Rif^{100}) to inhibit the growth of phytopathogens compared with the parental strain are presented in Figure 3. Vertical transmission from seed to seed in spring wheat did not affect the ability of PS17 to produce antimicrobial compounds that inhibit the growth of the phytopathogenic fungi *F. oxysporium* (Figure 3A) and *A. alternata* (Figure 3B) used in this study.

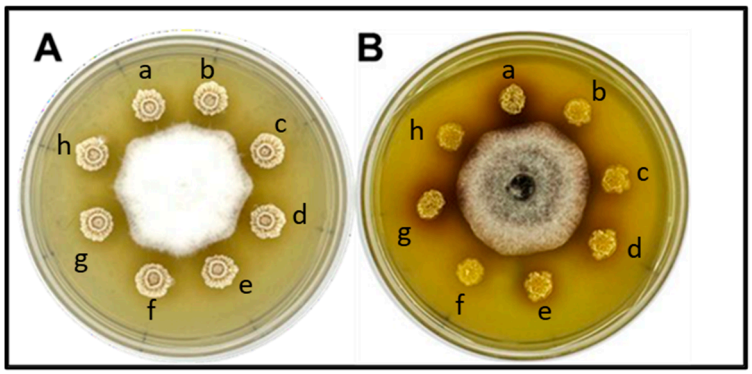

Figure 3. The antimicrobial activities of seed-transmitted colonies of PS17 against *F. oxysporium* (**A**) and *Alternaria alternata* (**B**) after seven days of incubation at 28 ± 1 °C. **a**–**b**—parental strain PS17; **c**–**h**—vertical transmitted bacterial colonies isolated from next-generation seeds.

3.2.2. Hydrolytic Enzyme Production by Selected Bacterial Strains

We evaluated hydrolytic enzyme production in selected PS17 mutant strains. All tested strains formed clear zones around them on media containing protease (Figure 4A), cellulase (Figure 4B), and amylase (Figure 4D) substrates, indicating their ability to produce these enzymes. In addition, among the isolated bacterial colonies transmitted through seeds, some did not exhibit lipase activity, which was observed by the absence of a transparent zone on the lipase medium (Figure 4C).

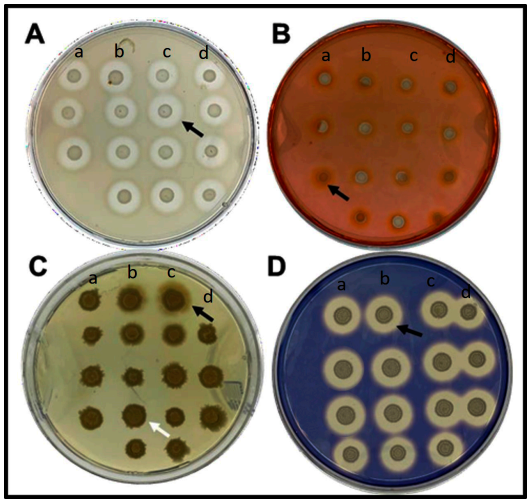

Figure 4. The abilities of seed-transmitted *B. mojavensis* PS17 colonies to produce the hydrolytic enzymes protease (**A**), cellulase (**B**), lipase (**C**), and amylase (**D**). The black and white arrows indicate the formation of crystals and halo zones surrounding the bacterial colonies, respectively. Parental strain PS17 (Rif^{100}) (**a**) and bacterial colonies isolated from next-generation seeds (**b**–**d**).

3.3. DNA Fingerprinting Analysis and Molecular Identification of Seed-Isolated Bacterial Strains

To confirm the taxonomy of PS17 isolates recovered from seeds and the rhizosphere at the grain-filling stage, 50 colonies were randomly selected. These selected colonies were compared with the original *B. mojavensis* PS17 strain using BOX-PCR fingerprinting. As shown in Figure 5, all selected isolates from seedling (Figure 5(AI)), tillering, and head differentiation (Figure 5(AII)) generated identical banding DNA patterns to those of the original PS17(Rif^{100}). Notably, all isolates generated the same number of box fragments, ranging from 500 to 1500 base pairs (bp).

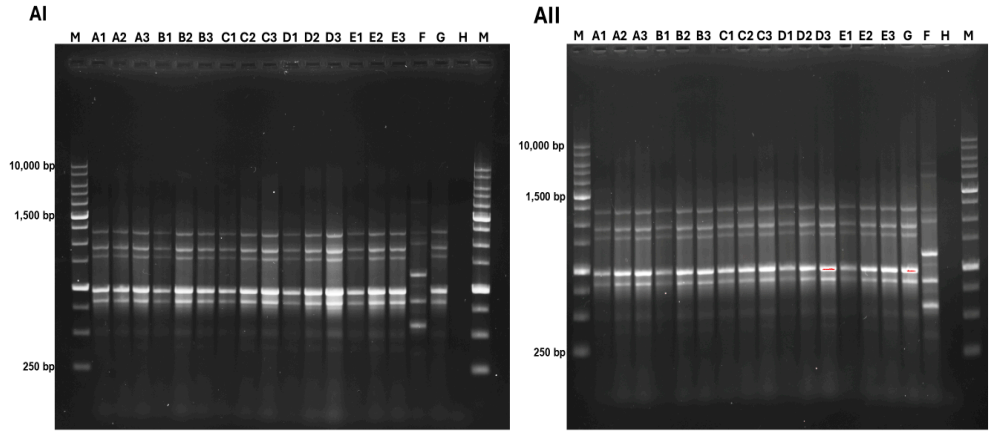

Figure 5. 1% agarose gel electrophoresis of the BOX-PCR products of bacterial strains isolated from spring wheat grown during seedling (**AI**) and tillering and head differentiation (**AII**). Line **M**—DNA ladder 1 kb; line **A1**–**A3**—bacterial strains isolated from rhizosphere; line **B1**–**B3**—bacterial strain isolated from surface-sterilized roots; line **C1**–**C3**—bacterial strain isolated from stems; line **D1**–**D3**—bacterial strain isolated from leaves; line **E1**–**E3**—parental *B. mojavensis* strain PS17(Rif^{100}); line **F**—*B. amyloliquefaciens*; line **G**—*B. halotolerans*; and line **H**—negative control.

An identical BOX-PCR profile was obtained at the grain-filling stage (Figure 6). All randomly selected bacterial colonies isolated from soil, the rhizosphere, and surface-sterilized roots, stems, leaves, and seeds, which grew on LB medium amended with Rifampicin, showed a fingerprint profile that was indistinguishable from that of their parent *B. mojavensis* PS17. Moreover, the generated profile boxes differed from those generated by related bacteria *B. amyloliquefaciens*, *B. halotolerans*, and *Bacillus* sp. used as control bacterial strains.

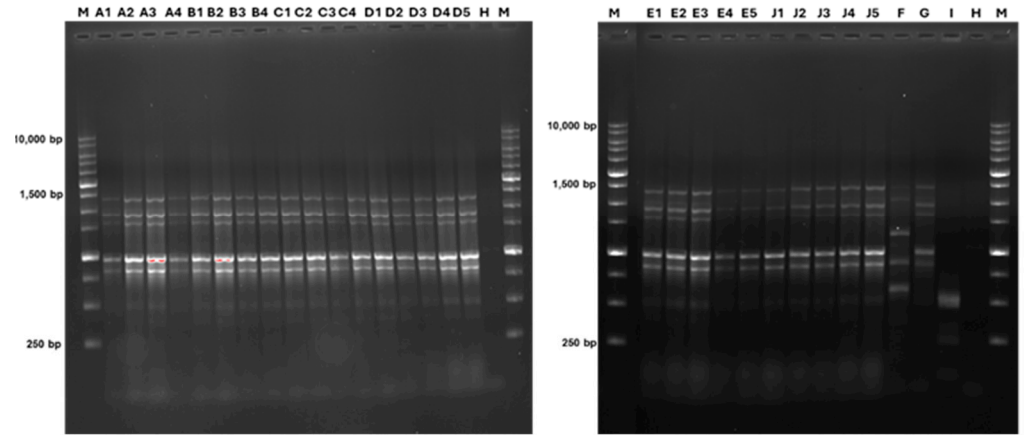

Figure 6. 1% agarose gel electrophoresis of the BOX-PCR products of bacterial strains isolated from spring wheat grown at the grain-filling stage. Line **M**—DNA ladder 1 kb; line **A1**–**A4**—bacterial strains isolated from the rhizosphere; line **B1**–**B4**—bacterial strain isolated from surface-sterilized roots; line **C1**–**C4**—bacterial strain isolated from surface-sterilized stems; line **D1**–**D5**—bacterial strain isolated from surface-sterilized leaves; Line **E1**–**E5**—bacterial strain isolated from surface-sterilized seeds; line **J1–J5**—parental *B. mojavensis* PS17(Rif^{100}); line **F**—*B. amyloliquefaciens;* line **G**—*B. halotolerans*; line **I**—*Bacillus* sp; and line **H**—negative control.

Additionally, specific genes (*rpo*B, *gyr*A, 16S rRNA, and *Rec*A) were sequenced and compared to the reference parental sequences available in the NCBI database to confirm the identity of the isolated bacterial strains. The 16S rRNA gene sequences showed a high-scoring segment pair, demonstrating 100% identity with *B. mojavensis* PS17 (accession number MW350040.1). Likewise, the coding sequences (CDS) for the *rpo*B, *gyr*A, and *Rec*A

genes were also identical, showing 100% similarity with the corresponding sequences of *B. mojavensis* PS17 (accession numbers PQ044378, PQ044379, and PQ044380, respectively). Based on the high sequence similarity to the reference strain, we concluded that all isolates belonged to *B. mojavensis*.

4. Discussion

Preserving the beneficial endophytic bacteria transmitted through seeds is a promising strategy for introducing microbiological agents into seed microbiota because successful colonization often leads to mutually beneficial interactions [37–39]. The efficacy of plant colonization by endophytic bacteria requires an initial adherence to seed surfaces and tolerance to natural defense compounds secreted by plants [37,40,41]. This suggests that *B. mojavensis* PS17 is closely associated with spring wheat. Moreover, a high PS17 cell density was recovered from various plant parts (roots, stems, and leaves) during the early seedling stage on LB amended with rifampicin (100 μg/mL). These findings indicated the ability of *B. mojavensis* PS17 to act as an endophyte. Successful plant colonization may be facilitated by the release of root exudates from spring wheat, which are known to play crucial roles in attracting and establishing beneficial microbial communities [42–45]. *Bacillus mojavensis* PS17 was detected in all analyzed plant tissues at different plant growth phases (germination, tillering, and head formation), as well as in seeds at the grain-filling stage (milk development), despite a slight decrease in the PS17 cell population at the grain-filling stage. The decreased PS17 population in seeds may be due to the seed coat forming a protective layer over the endosperm and embryo, which provides a safe environment that may affect the colonization ability of microorganisms [26,46,47]. Endophytic behavior is affected by complex interactions such as nutrient availability, host plant species, microbial communities, and environmental conditions [48]. The persistence of seed-transmitted endophytes is crucial for elucidating their ecological roles and potential applications in agriculture, particularly in crop protection. Several studies have reported that under stressful conditions, microorganisms undergo phenotypic variation, resulting in the formation of colonies with different phenotypic characteristics [49–51]. Likewise, Troxler et al. [52] reported that the root endophyte *Pseudomonas fluorescens* strain CHAO isolated from maize exhibited distinct biocontrol abilities compared with strains isolated from the rhizosphere. Similarly, Barnett et al. [53] observed functional differences in antagonistic activities among various colonies of the endophytic bacteria *Pseudomonas corrugate* 2140 after long-term residence within the plant. In this study, *B. mojavensis* PS17 demonstrated the ability to inhibit the growth of phytopathogenic fungi and produce lytic enzymes such as amylase and protease after its long-term survival in seed wheat, indicating its phenotypic persistence. BOX-PCR fingerprinting is a well-established method that seems to be able to identify bacteria at the strain level [54]. In a study conducted by Tacão et al. [55], BOX-PCR was used to discriminate various *Aeromonas* species, with most of the analyzed strains displaying unique banding patterns. In our study, all isolated bacterial strains exhibited identical banding patterns (Figure 5), indicating their genetic relatedness to *B. mojavensis* PS17.

5. Conclusions

Beneficial seed-borne endophytic microorganisms play a key role in the early seedling growth of plants and constantly affect the host plant's interaction with abiotic and biotic conditions. In this study, *B. mojavensis* PS17 demonstrated the ability to colonize the non-native host plant spring wheat and undergo vertical transmission to subsequent generations through seeds. Overall, our findings highlight the potential for using seed-mediated vertical transmission as a method for spreading biocontrol agents in future plants, which may eventually become dominant. However, field studies are required to validate the efficiency of vertical transmission and its persistence under varying soil and environmental conditions because these factors may affect this mode of transmission.

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References

1. Yildirim, E.; Dursun, A.; Kumlay, M.A.; Güvenç, Í. The effects of different salt, biostimulants, and temperature levels on seed germination of some vegetable species. *Acta Agrobot.* **2002**, *55*, 75–80. [CrossRef]
2. Bulgari, R.; Franzoni, G.; Ferrante, A. Biostimulants Application in Horticultural Crops under Abiotic Stress Conditions. *Agronomy* **2019**, *9*, 306. [CrossRef]
3. Savvides, A.; Ali, S.; Tester, M.; Fotopoulos, V. Chemical Priming of Plants Against Multiple Abiotic Stresses: Mission Possible? *Trends Plant Sci.* **2016**, *21*, 329–340. [CrossRef]
4. Moumni, M.; Brodal, G.; Romanazzi, G. Recent innovative seed treatment methods in the management of seed-borne pathogens. *Food Sec.* **2023**, *15*, 1365–1382. [CrossRef]
5. Sharma, K.K.; Singh, U.S.; Sharma, P.; Kumar, A.; Sharma, L. Seed treatments for sustainable agriculture—A review. *J. Appl. Nat. Sci.* **2015**, *7*, 521–539. [CrossRef]
6. Ibrahim, E.A. Seed priming to alleviate salinity stress in germinating seeds. *J. Plant Physiol.* **2016**, *192*, 38–46. [CrossRef] [PubMed]
7. Paparella, S.; Araújo, S.S.; Rossi, G.; Wijayasinghe, M.; Carbonera, D.; Balestrazzi, A. Seed priming: State of the art and new perspectives. *Plant Cell Rep.* **2015**, *34*, 1281–1293. [CrossRef]
8. Sturz, A.V.; Christie, B.R.; Nowak, J. Bacterial endophytes: Potential role in developing sustainable systems of crop production. *Crit. Rev. Plant Sci.* **2000**, *19*, 1–30. [CrossRef]
9. Shen, F.T.; Yen, J.H.; Liao, C.S.; Chen, W.C.; Chao, Y.T. Screening of Rice Endophytic Biofertilizers with Fungicide Tolerance and Plant Growth-Promoting Characteristics. *Sustainability* **2019**, *11*, 1133. [CrossRef]
10. Kloepper, J.W.; Ryu, C.M. Bacterial Endophytes as Elicitors of Induced Systemic Resistance. In *Microbial Root Endophytes, Soil Biology*; Schulz, B.J.E., Boyle, C.J.C., Sieber, T.N., Eds.; Springer: Berlin/Heidelberg, Germany, 2006; pp. 33–52. [CrossRef]
11. Gouda, S.; Das, G.; Sen, S.K.; Shin, H.S.; Patra, J.K. Endophytes: A Treasure House of Bioactive Compounds of Medicinal Importance. *Front. Microbiol.* **2016**, *7*, 1538. [CrossRef]
12. Khare, E.; Mishra, J.; Arora, N.K. Multifaceted Interactions Between Endophytes and Plant: Developments and Prospects. *Front. Microbiol.* **2018**, *9*, 2732. [CrossRef]
13. White, J.F.; Kingsley, K.L.; Zhang, Q.; Verma, R.; Obi, N.; Dvinskikh, S.; Elmore, M.T.; Verma, S.K.; Gond, S.K.; Kowalski, K.P. Review: Endophytic microbes and their potential applications in crop management. *Pest Manag. Sci.* **2019**, *75*, 2558–2565. [CrossRef] [PubMed]
14. Goggin, D.E.; Emery, R.J.N.; Kurepin, L.V.; Powles, S.B. A potential role for endogenous microflora in dormancy release, cytokinin metabolism, and the response to fluridone in Lolium rigidum seeds. *Ann. Bot.* **2015**, *115*, 293–301. [CrossRef]
15. Puente, M.E.; Li, C.Y.; Bashan, Y. Endophytic bacteria in cacti seeds can improve the development of cactus seedlings. *Environ. Exp. Bot.* **2009**, *66*, 402–408. [CrossRef]
16. Rout, M.E.; Chrzanowski, T.H.; Westlie, T.K.; Deluca, T.H.; Callaway, R.M.; Holben, W.E. Bacterial endophytes enhance competition by invasive plants. *Am. J. Bot.* **2013**, *100*, 1726–1737. [CrossRef]
17. Verma, H.; Kumar, D.; Kumar, V.; Kumari, M.; Singh, S.K.; Sharma, V.K.; Droby, S.; Santoyo, G.; White, J.F.; Kumar, A. The Potential Application of Endophytes in Management of Stress from Drought and Salinity in Crop Plants. *Microorganisms* **2021**, *9*, 1729. [CrossRef]
18. Senthilkumar, M.; Anandham, R.; Madhaiyan, M.; Venkateswaran, V.; Sa, T. Endophytic Bacteria: Perspectives and Applications in Agricultural Crop Production. In *Bacteria in Agrobiology: Crop Ecosystems*; Maheshwari, D.K., Ed.; Springer: Berlin/Heidelberg, Germany, 2011; pp. 61–96. [CrossRef]
19. Wu, W.; Chen, W.; Liu, S.; Wu, J.; Zhu, Y.; Qin, L.; Zhu, B. Beneficial relationships between endophytic bacteria and medicinal plants. *Front. Plant Sci.* **2021**, *12*, 646146. [CrossRef]
20. Frank, A.C.; Saldierna Guzmán, J.P.; Shay, J.E. Transmission of Bacterial Endophytes. *Microorganisms* **2017**, *5*, 70. [CrossRef]
21. Hallmann, J.; Quadt-Hallmann, A.; Mahaffee, W.F.; Kloepper, J.W. Bacterial endophytes in agricultural crops. *Can. J. Microbiol.* **1997**, *43*, 895–914. [CrossRef]
22. Firáková, S.; Šturdíková, M.; Múčková, M. Bioactive secondary metabolites produced by microorganisms associated with plants. *Biologia* **2007**, *62*, 251–257. [CrossRef]

23. Truyens, S.; Weyens, N.; Cuypers, A.; Vangronsveld, J. Bacterial seed endophytes genera, vertical transmission and interaction with plants. *Environ. Microbiol. Rep.* **2015**, *7*, 40–50. [CrossRef]
24. Carroll, G. Fungal endophytes in stems and leaves: From latent pathogen to mutualistic symbiont. *Ecology* **1988**, *69*, 2–9. [CrossRef]
25. Nelson, E.B. The seed microbiome: Origins, interactions, and impacts. *Plant Soil* **2018**, *422*, 7–34. [CrossRef]
26. Abdelfattah, A.; Tack, A.J.; Lobato, C.; Wassermann, B.; Berg, G. From seed to seed: The role of microbial inheritance in the assembly of the plant microbiome. *Trends Microbiol.* **2023**, *31*, 346–355. [CrossRef]
27. Elbeltagy, A.; Nishioka, K.; Suzuki, H.; Sato, T.; Sato, Y.I.; Morisaki, H.; Minamisawa, K. Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. *Soil Sci. Plant Nutr.* **2000**, *46*, 617–629. [CrossRef]
28. Mano, H.; Tanaka, F.; Watanabe, A.; Kaga, H.; Okunishi, S.; Morisaki, H. Culturable surface and endophytic bacterial flora of the maturing seeds of rice plants (*Oryza sativa)* cultivated in a paddy field. *Microbes Environ.* **2006**, *21*, 86–100. [CrossRef]
29. Kandel, S.L.; Joubert, P.M.; Doty, S.L. Bacterial endophyte colonization and distribution within plants. *Microorganisms* **2017**, *5*, 77. [CrossRef]
30. Diabankana, R.G.C.; Afordoanyi, D.M.; Safin, R.I.; Nizamov, R.M.; Karimova, L.Z.; Validov, S.Z. Antifungal properties, abiotic stress resistance, and biocontrol ability of *Bacillus mojavensis* PS17. *Curr. Microbiol.* **2021**, *78*, 3124–3132. [CrossRef]
31. Glandorf, D.C.M.; Brand, I.; Bakker, P.A.H.M. Stability of rifampicin resistance as a marker for root colonization studies of *Pseudomonas putida* in field. *Plant Soil* **1992**, *147*, 135–142. [CrossRef]
32. Simons, M.; Van Der Bij, A.J.; Brand, I.; De Weger, L.A.; Wijffelman, C.A.; Lugtenberg, B.J. Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. *Mol. Plant-Microbe Interact. MPMI* **1996**, *9*, 600–607. [CrossRef]
33. Mohkam, M.; Nezafat, N.; Berenjian, A.; Mobasher, M.A.; Ghasemi, Y. Identification of *Bacillus* probiotics isolated from soil rhizosphere using 16S rRNA, *recA*, and *rpoB* gene sequencing and RAPD-PCR. *Probiotics Antimicrob. Proteins* **2016**, *8*, 8–18. [CrossRef]
34. Lane, D.J.; Pace, B.; Olsen, G.J.; Stahl, D.A.; Sogin, M.L.; Pace, N.R. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 6955–6959. [CrossRef] [PubMed]
35. Ki, J.S.; Zhang, W.; Qian, P.Y. Discovery of marine *Bacillus* species by 16S rRNA and *rpoB* comparisons and their usefulness for species identification. *J. Microbiol. Methods* **2009**, *77*, 48–57. [CrossRef] [PubMed]
36. Chun, J.; Bae, K.S. Phylogenetic analysis of *Bacillus subtilis* and related taxa based on partial *gyrA* gene sequences. *Antonie Van Leeuwenhoek* **2000**, *78*, 123–127. [CrossRef] [PubMed]
37. Kumar, A.; Droby, S.; Singh, V.K.; Singh, S.K.; White, J.F. Entry, colonization, and distribution of endophytic microorganisms in plants. In *Microbial Endophytes*; Kumar, A., Radhakrishnan, E.K., Eds.; Woodhead Publishing: Cambridge, UK, 2020; pp. 1–33. [CrossRef]
38. Prieto, P.; Schilirò, E.; Maldonado-González, M.M.; Valderrama, R.; Barroso-Albarracín, J.B.; Mercado-Blanco, J. Root Hairs Play a Key Role in the Endophytic Colonization of Olive Roots by *Pseudomonas* spp. with Biocontrol Activity. *Microb. Ecol.* **2011**, *62*, 435–445. [CrossRef]
39. Liu, H.; Carvalhais, L.C.; Crawford, M.; Singh, E.; Dennis, P.G.; Pieterse, C.M.J.; Schenk, P.M. Inner Plant Values: Diversity, Colonization and Benefits from Endophytic Bacteria. *Front. Microbiol.* **2017**, *8*, 2552. [CrossRef] [PubMed]
40. De Weert, S.; Vermeiren, H.; Mulders, I.H.M.; Kuiper, I.; Hendrickx, N.; Bloemberg, G.V.; Vanderleyden, J.; De Mot, R.; Lugtenberg, B.J.J. Flagella-Driven Chemotaxis Towards Exudate Components Is an Important Trait for Tomato Root Colonization by *Pseudomonas fluorescens*. *Mol. Plant Microbe Interact.* **2002**, *15*, 1173–1180. [CrossRef]
41. Bacilio-Jiménez, M.; Aguilar-Flores, S.; Ventura-Zapata, E.; Pérez-Campos, E.; Bouquelet, S.; Zenteno, E. Chemical characterization of root exudates from rice (*Oryza sativa*) and their effects on the chemotactic response of endophytic bacteria. *Plant Soil* **2003**, *249*, 271–277. [CrossRef]
42. Compant, S.; Clément, C.; Sessitsch, A. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem.* **2010**, *42*, 669–678. [CrossRef]
43. Rosenblueth, M.; Martínez-Romero, E. Bacterial Endophytes and Their Interactions with Hosts. *Mol. Plant Microbe Interact.* **2006**, *19*, 827–837. [CrossRef]
44. Walitang, D.; Kim, C.G.; Jeon, S.; Kang, Y.; Sa, T. Conservation and transmission of seed bacterial endophytes across generations following crossbreeding and repeated inbreeding of rice at different geographic locations. *MicrobiologyOpen* **2019**, *8*, e00662. [CrossRef] [PubMed]
45. Podile, A.R.; Vukanti, R.; Ankati, S.; Kalam, S.; Dutta, S.; Durgeshwar, P.; Vaikuntapu, P. Root Colonization and Quorum Sensing are the Driving forces of Plant Growth Promoting Rhizobacteria (pgpr) for Growth Promotion. *Proc. Indian Natl. Sci. Acad.* **2014**, *80*, 407. [CrossRef]
46. Samreen, T.; Naveed, M.; Nazir, M.Z.; Asghar, H.N.; Khan, M.I.; Zahir, Z.A.; Choudhary, M. Seed associated bacterial and fungal endophytes: Diversity, life cycle, transmission, and application potential. *Appl. Soil Ecol.* **2021**, *168*, 104191. [CrossRef]
47. Paravar, A.; Piri, R.; Balouchi, H.; Ma, Y. Microbial seed coating: An attractive tool for sustainable agriculture. *Biotechnol. Rep.* **2023**, *37*, e00781. [CrossRef]
48. Wulff, E.G.; Van Vuurde, J.W.L.; Hockenhull, J. The ability of the biological control agent *Bacillus subtilis*, strain BB, to colonise vegetable brassicas endophytically following seed inoculation. *Plant Soil* **2003**, *255*, 463–474. [CrossRef]

49. Shahzad, R.; Khan, A.L.; Bilal, S.; Asaf, S.; Lee, I.J. What Is There in Seeds? Vertically Transmitted Endophytic Resources for Sustainable Improvement in Plant Growth. *Front. Plant Sci.* **2018**, *9*, 24. [CrossRef]
50. Bochner, B.R. Global phenotypic characterization of bacteria. *FEMS Microbiol. Rev.* **2008**, *33*, 191–205. [CrossRef]
51. Ackermann, M. A functional perspective on phenotypic heterogeneity in microorganisms. *Nat. Rev. Microbiol.* **2015**, *13*, 497–508. [CrossRef]
52. Troxler, J.; Zala, M.; Natsch, A.; Moënne-Loccoz, Y.; Défago, G. Autecology of the biocontrol strain *Pseudomonas fluorescens* CHA0 in the rhizosphere and inside roots at later stages of plant development. *FEMS Microbiol. Ecol.* **2006**, *23*, 119–130. [CrossRef]
53. Barnett, S.; Singleton, I.; Ryder, M.; Ogoshi, A.; Kobayashi, K.; Homma, Y.; Akino, S. The effect of growth conditions on phenotype plasticity in Pseudomonas corrugata 2140, and the phenotypic characterisation of a range of new phenotype variants. In Proceedings of the Fourth International Workshop on the Plant Growth-Promoting Rhizobacteria, Sapporo, Japan, 5–10 October 1997; Ogoshi, A., Kobayashi, K., Homma, Y., Kodama, F., Kondo, N., Akino, S., Eds.; Hokkaido University: Sapporo, Japan, 1997; pp. 417–420.
54. Marques, A.S.A.; Marchaison, A.; Gardan, L.; Samson, R. BOX-PCR-Based Identification of Bacterial Species Belonging to *Pseudomonas* Syringae—*P. Viridiflava* Group. Available online: https://www.scielo.br/pdf/gmb/v31n1/19.pdf (accessed on 20 September 2024).
55. Tacão, M.; Alves, A.; Saavedra, M.J.; Correia, A. BOX-PCR Is an Adequate Tool for Typing *Aeromonas* spp. Available online: https://link.springer.com/article/10.1007/s10482-005-3450-9 (accessed on 20 September 2024).