horticulturae

MDPI

Article

Plant Growth-Promoting Activity and Whole-Genome Analysis of *Pseudomonas marginalis* RA62c-B2 Isolated from *Vitis amurensis*

Alexey A. Ananev, Nikolay N. Nityagovsky, Andrey R. Suprun, Konstantin V. Kiselev and Olga A. Aleynova *

Laboratory of Biotechnology, Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences, 690022 Vladivostok, Russia; ananev.all@yandex.ru (A.A.A.); niknit1996@gmail.com (N.N.N.); suprun@biosoil.ru (A.R.S.); kiselev@biosoil.ru (K.V.K.)
* Correspondence: aleynova@biosoil.ru; Tel.: +7-4232-310708; Fax: +7-4232-310193

Abstract

Bacteria of the genus *Pseudomonas* act as effective plant growth promoters, realizing their potential through a complex of physiological mechanisms—from phytohormone synthesis to nutrient mobilization and suppression of phytopathogens. These properties position *Pseudomonas* strains as promising candidates for the development of environmentally friendly biopreparations in sustainable agriculture. The study presents a comprehensive characterization of the *Pseudomonas marginalis* RA62c-B2 strain isolated from Amur grapevine (*Vitis amurensis*), combining genome-wide analysis with laboratory and plant-based experimental validation. The genome of *P. marginalis* RA62c-B2 comprises 6,620,267 bp and encodes 6545 protein-coding sequences. Genomic analysis revealed a suite of functional genes that support plant growth promotion, including those engaged in indole-3-acetic acid (IAA) and cytokinin biosynthesis, organic acid production, nitrogen metabolism, phosphorus and potassium mobilization, iron assimilation, sulfur metabolism, resistance to osmotic and oxidative stress, and bioremediation potential. In conjunction with genomic data, metabolite profiling by HPLC-MS provided functional validation of the strain's biosynthetic pathways: tryptophan metabolites (indole-3-acetaldehyde and indole-3-carboxaldehyde) were identified, along with two cytokinins (*trans*-zeatin and N6-isopentenyladenine), detected in the *P. marginalis* RA62c-B2 culture extract. Furthermore, a single inoculation of rice seeds with *P. marginalis* RA62c-B2 significantly enhanced the growth parameters of seedlings and 30-day-old plants. These phenotypic effects are consistent with the strain's genetic profile, reflecting its multifaceted capacity to promote plant growth through multiple complementary mechanisms.

Keywords: grapevine; endophytes; genomic analysis; microbial metabolism; *Oryza sativa*; cytokinins

Check for updates

Academic Editor: Qiang-Sheng Wu

Received: 8 September 2026
Revised: 22 October 2026
Accepted: 23 October 2026
Published: 27 October 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Global food security and environmental sustainability require innovative, eco-friendly solutions to improve crop yields [1,2]. Microbial biostimulants based on plant growth-promoting bacteria (PGPB) represent a promising strategy to enhance plant performance while minimizing the use of synthetic inputs [3,4]. PGPB possess a number of key genes that enable plant growth promotion [5]. These genes are typically grouped into several functional categories: 1. phytohormone biosynthesis—this includes genes involved in the biosynthesis of auxins

(*iaaM*, *iaaH* (IAM pathway), *ipdC*, *aldH*, *aldA* (IPyA pathway), *nthA*, *iamA*, *oxd* (IAN pathway), *tdc*, *dcc* (TAM pathway), *tso* (TSO pathway) [6,7], cytokinins (*ipt*, *tmr*, *tzs*, *ptz*, *fasD/fas4*, *fasA*, *fasF*, *miaA*, *miaB*, *miaE*, *LOG*, *NoIPT1*, etc. [8])), and gibberellins (*cps*, *ks*, *ggps*, *cyp112*, *cyp114*, *cyp117*, *fd*, *sdr*, *cyp115*, *idi*, etc. [9]) or modulation of plant ethylene levels (*acdS*, *acdR*, *acdB*, *efe*, *tlpQ*, *nifA1/nifA2*, *gntR*, etc. [10]); 2. enhancement of nutrient availability—genes responsible for nitrogen fixation (*nifABDEHKLSUV*, *fixGJKLT*, *ntrC*, *hup*, *hox*, etc. [11]), phosphate solubilization (*gcd*, *pqqA–F*, *phoD*, *phoA*, *ppx*, *phyC*, *phyA*, *pstSCAB*, *phnCDE*, *ppk* etc. [12,13]), and iron sequestration (*entA–F*, *iroBCDN*, *iucABCD*, *iutA*, *pvd (NRPS + ABC)*, *fepA*, *fpvA*, *TonB/ExbB/ExbD*, *hmuRSTUV*, *phuRSTUVW*, *isdABCH*, *isdDEF*, *fyuA*, *ftnA*, *bfr*, *sufABCDSE*, *fur*, *rirA*, etc. [14,15]); 3. reduction of plant stress—genes associated with the synthesis of osmoprotectants, accumulation of non-enzymatic antioxidants, and activation of the plant antioxidant system (*proA*, *proB*, *proC*, *proU*, *opuA*, *betT*, *otsA*, *otsB*, etc. [16–18]).

Among PGPB, *Pseudomonas* species are among the most studied and applied, owing to their diverse mechanisms of action and compatibility with various crops [19]. For example, a tomato-derived *Pseudomonas aeruginosa* isolate (strain FG106) exhibited dual functionality as both a plant growth promoter and a potent inhibitor of phytopathogens, demonstrating strong suppression of growth and pathogenicity both *in vitro* and *in vivo* [20]. The combined treatment with *Pseudomonas* strains (*P. aeruginosa*, *P. putida*, *P. cepacia*, and *P. fluorescens*) resulted in enhanced rice growth and increased resistance of rice plants to heavy metal exposure [21]. *Pseudomonas* consortia, including *P. protegens* B21-024 and *P. putida* B21-09 from agricultural soils, were used to promote the growth of leafy greens in field conditions [22].

However, the discovery of novel, locally adapted microbial strains remains critical for addressing region-specific agricultural challenges. In this context, the Asian native wild grapevine species *Vitis amurensis* Rupr. offers a particularly promising ecological niche for identifying stress-resilient plant growth-promoting bacteria. This species typically grows on hillsides or in ravines at high elevations, where it is exposed to harsh environmental conditions. Notably, *V. amurensis* exhibits exceptional tolerance to cold, surviving temperatures as low as −40 °C [23], and demonstrates strong resistance to several widespread grapevine infections, including anthracnose, white rot, and powdery mildew [24,25]. These intrinsic traits suggest that the endophytic microbiome of *V. amurensis* may harbor bacterial strains with unique combinations of stress resilience and growth-promoting capabilities [26].

Although a wide variety of *Pseudomonas* species have been documented, the extent of their plant growth-promoting potential and the underlying molecular mechanisms remain incompletely characterized. To address this knowledge gap, we investigated *Pseudomonas marginalis* RA62c-B2, an endophytic strain isolated from *V. amurensis*, as a candidate biostimulant for plants. We employed an integrated approach combining whole-genome sequencing, specialized culture media, targeted biochemical assays, and plant bioassays to comprehensively characterize its plant growth-promoting traits.

2. Materials and Methods

2.1. Isolation and Identification of the Bacterial Strain

Leaf material from an asymptomatic *V. amurensis* plant served as the source for isolating the *Pseudomonas* sp. strain RA62c-B2. To ensure minimal carryover of surface microbes, a sequential surface sterilization procedure was applied: 1 min immersion in 70% ethanol, 2 min treatment with 10% H_2O_2, and five subsequent washes with sterile distilled water [26]. Sterilized leaf tissue was mechanically disrupted in a sterile mortar and brought to a suspension in 200 µL of sterile water. From this suspension, a 70 µL sample was spread over the surface of R2A agar medium. Cultivation was carried out at 26 °C for 48 h. The resulting colonies were subcultured to obtain a pure strain. The purified *Pseudomonas* sp. RA62c-B2 was deposited in the laboratory collection at the Laboratory of Biotechnology,

Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences (Vladivostok, Russia).

Gram staining was performed using the DIAKHIM kit (ABRIS+ Scientific Production Association, St. Petersburg, Russia). The presence of a capsule was assessed using the Burri–Gins staining method with the Micro-GINS-NITsF kit (NITsF LLC, St. Petersburg, Russia).

The cultivation and maintenance conditions for the bacterial strain have been detailed in a prior publication [27]. Genomic DNA extraction was performed with an adapted CTAB protocol [26,28].

Whole-genome sequencing was carried out by "SYNTOL" (Moscow, Russia), which employed the MiSeq Illumina platform (Illumina, San Diego, CA, USA). Genome assembly was performed with SPAdes (version 4.2.0) [29] and assembly quality was evaluated using the BUSCO tool (https://busco.ezlab.org/) [30].

Annotation was performed using bakta v 1.12.1 [31] and the eggNOG-mapper v 3 [32]. To identify genes associated with plant growth-promoting traits, the PGPT-Pred PlaBase predictor was applied [33]. The search for secondary metabolite gene clusters was performed using antiSMASH v. 8.0.4. [34]. To validate the accuracy of the genome annotation, a set of representative genes (*phoU*, *katB*, *cysA*, *miaB*, and *opuA*) was selected for further analysis. Primers were designed using Primer-BLAST (version 2.5.0) [35]. Gene-specific sequences were amplified by PCR using a commercial kit (Evrogen, Moscow, Russia). The 25 µL reaction mixture comprised 1× Taq buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of each oligonucleotide primer, 1 µL of template DNA, and 1 unit of Taq DNA polymerase. Amplification was conducted on a Bis-N thermocycler (DNA-Technology, Moscow, Russia) with an initial 2 min denaturation at 95 °C, followed by 39 cycles of 95 °C (15 s), 58 °C (10 s), and 72 °C (45 s), and a final 1 min extension at 72 °C. The amplified PCR products varied in length from 420 to 634 base pairs (Table S1).

2.2. Taxonomic Classification Using Genomic Data

Phylogenomic analysis was conducted via the Kbase platform (https://www.kbase.us/). A genome set comprising 17 genomes (see Table S1) was generated using the MASH algorithm v2.3 [36]. Phylogenetic analysis was performed using FastME v2.1.6.1 [37]. Orthologous Average Nucleotide Identity (OrthoANI) values were computed using the Orthologous Average Nucleotide Identity Tool (OAT) (https://www.ezbiocloud.net/tools/orthoani, accessed on 15 October 2026) [38], while digital DNA–DNA hybridization (dDDH) estimates were obtained with GGDC 3.0 [39].

2.3. Comparative Genomics

For comparative genomic assessment, the genome of *P. marginalis* RA62c-B2 was compared with those of three phylogenetically closest strains: *P. marginalis* ICMP 3553 (GCF_003700725.1), *P. pergaminensis* 1008T (GCF_024112395.2), and *P. lurida* LMG 21995 (GCF_002563895.1). To visualize the distribution of shared and strain-specific orthologous gene clusters, a Venn diagram was constructed using OrthoVenn3 [40]. Structural annotation included identification of transfer RNA (tRNA) genes with tRNAscan-SE v 2.0 [41] and prediction of ribosomal RNA (rRNA) genes using RNAmmer v 1.2 [42]. To compare the results of antiSMASH RA62c-B2 with those of other *Pseudomonas marginalis* strains, the genomes of *Pseudomonas* strains PS92 (GCA_034011435.1), MGMM3 (GCA_029916865.1), Qb1D2 (GCA_057396095.1), and M13 (GCA_045037965.1) were analyzed.

2.4. In Vitro Characterization of PGPB Traits

The growth under nitrogen-deficient conditions of the isolate was evaluated using Ashby agar plates. Following incubation at 26 °C for 72 h, growth on the medium was interpreted as

a positive indication of nitrogen acquisition. Phosphate solubilization activity was assessed in Pikovskaya's medium containing tricalcium phosphate as the insoluble phosphorus source. Under identical incubation conditions (26 °C, 72 h), the development of a transparent halo around the colonies was considered a positive result. Potassium solubilization potential was tested on Alexandrovsky agar. The culture plates were incubated at 26 °C for 96 h; a distinct halo zone around the colonies was interpreted as a positive result. Sulfur oxidation capacity was examined using modified Thiosulfate Agar (TSA) supplemented with Bromcresol Purple as a pH indicator. Following a 48 h incubation at 26 °C, the formation of a yellow halo around the growth zone was interpreted as a positive result.

To detect phytohormones, a 50 mL sample of an overnight culture of *P. marginalis* RA62c-B2 grown in R2B medium was processed to obtain a homogeneous suspension. First, the sample was homogenized using an analytical mill (IKA A11 basic; IKA Werke GmbH & Co. KG, Staufen, Germany). Cell disruption was achieved by ultrasonic treatment using a Sonicator Q55 (QSonica, Newtown, CT, USA) for 5 min. Phytohormones were extracted with ethyl acetate at a 1:2 ratio (100 mL solvent per 50 mL culture) under continuous stirring on a heated magnetic stirrer (40 °C, 40 min). The mixture was centrifuged at 3500 rpm for 5 min in 50 mL Falcon tubes. The upper ethyl acetate phase was collected with an automatic pipette and transferred to a 250 mL flask. The extract was concentrated to 3–4 mL using a rotary evaporator, then aliquoted into 1.5 mL tubes and dried in a concentrator at 40 °C. The dry residue was redissolved in 1 mL methanol and purified by solid-phase extraction with a Discovery® DSC 18 SPE tube (50 mg; Supelco, Bellefonte, PA, USA). Phytohormone analysis was performed by HPLC-DAD on an LC 20 AD XR system (Shimadzu, Kyoto, Japan).

To test strain RA62c-B2 as a biological control agent, plant pathogens such as *Alternaria alternata*, *Erwinia billingiae*, and *Fusarium avenaceum* were used. Preparation for testing is described in Ananev et. al., 2024 [27]. Antibacterial activity was assessed using the agar plate method [43] and fungicidal activity was evaluated using the dual-culture method [44]. All experiments were carried out independently in three biological replicates.

2.5. Biometric Response of Plants to Seed Inoculation

Seeds of the rice cultivar *Oryza sativa* 'Dubrava' were surface-sterilized following the protocol described for grapevine leaf (see Section 2.1, Isolation and Identification of the Bacterial Strain). The overnight culture was obtained by inoculating strain RA62c-B2 into 50 mL of liquid R2A medium [45]. The culture was then incubated in an orbital shaker at 130 rpm and 23 °C for 48 h. The inoculum was prepared by centrifuging an overnight culture of *P. marginalis* RA62c-B2 at 3000 rpm for 5 min to obtain a cell pellet. The supernatant was removed, and the resulting cell pellet was resuspended in sterile water to achieve a final concentration of 3×10^8 CFU/mL. Sterilized seeds were immersed in 50 mL of the bacterial suspension and incubated for 1 h; excess liquid was then carefully removed, and seeds were air-dried. The control group underwent identical procedures, except that sterile water was used instead of the bacterial suspension.

The impact of *P. marginalis* RA62c-B2 on plant development was assessed using two distinct experimental setups. In the first setup, 7-day-old seedlings were cultivated in Petri dishes on double layers of filter paper saturated with 6 mL of sterile water. The second setup involved 30-day-old plants grown in pots. Throughout the experiment, all plant materials were kept in a climate chamber under standardized conditions: temperature at 25 °C, a 16 h light/8 h dark photoperiod, 70% relative humidity, and a light intensity of 250 μmol $m^{-2}s^{-1}$. At the designated time points, root and shoot lengths were measured (coleoptile length was recorded for seedlings). Drying of the plant samples was carried out at 30 °C over 3 days. For the 30-day-old plants, roots and shoots were separated prior to dry biomass determination.

The study included three independent biological replicates, with ten technical repeats per biological replicate. The results are expressed as the mean ± standard error (SE). Statistical significance was determined by ANOVA followed by Tukey's test; a p value of less than 0.05 was regarded as statistically significant.

3. Results

3.1. Observable Phenotypic Traits Associated with PGPB Functionality

To validate the plant growth-promoting functions encoded in the genome of the studied strain, phenotypic assays were carried out. Growth on solid media in Petri dishes was used to assess key metabolic traits, while HPLC-MS analysis provided insights into the production of bioactive compounds. The *P. marginalis* RA62c-B2 strain is rod-shaped with rounded ends; it is Gram-negative, possesses a bacterial capsule, and is equipped with a flagellum, with cell dimensions of 1.57 ± 0.1 µm (Figure S1).

The *P. marginalis* RA62c-B2 strain demonstrated growth on Ashby medium, indicating its potential nitrogen (N) acquisition (Figure 1a). Furthermore, the formation of a transparent halo around the colonies on Pikovskaya's and Alexandrovsky media suggests the strain's ability to solubilize inorganic phosphorus and potassium compounds (Figure 1b,c). The presence of a yellow halo indicates sulfur (S) oxidation activity (Figure 1d). The size of the halo zone observed during testing of battery cell mobilization in three replicates, along with their standard deviation, is presented in Table S3.

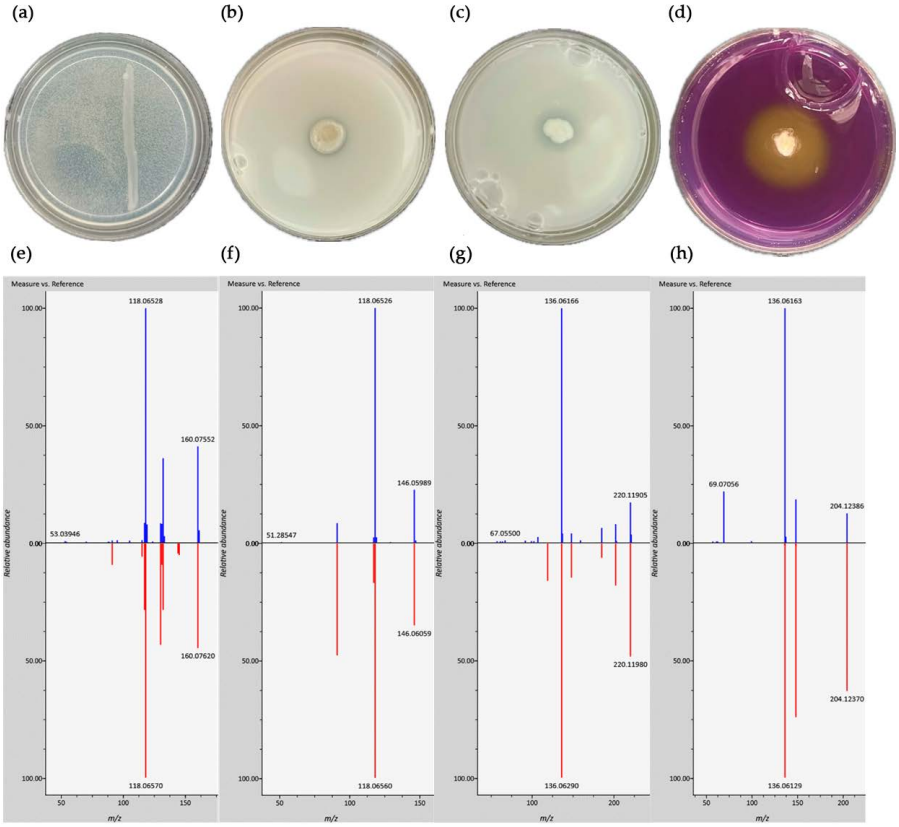


Figure 1. Evaluation of plant growth-promoting properties of *Pseudomonas marginalis* RA62c-B2 *in vitro*: (**a**) nitrogen acquisition on Ashby's medium; (**b**) detection of phosphate solubilization on Pikovskaya's medium; (**c**) detection of potassium solubilization on Alexandrovsky's medium; (**d**) detection of sulfur oxidation on TSA medium; MS/MS detection of: (**e**) indole-3-acetoaldehyde, (**f**) indole-3-carboxaldehyde, (**g**) *trans*-zeatin, and (**h**) N^6-isopentenyladenine. The mass spectra of the substances found in the *P. marginalis* RA62c-B2 extract are shown in blue for (**e**–**h**); the reference mass spectra are indicated in red.

In addition, HPLC-MS analysis revealed the presence of tryptophan-derived metabolites: indole-3-acetaldehyde and indole-3-carboxaldehyde (Figure 1e,f). Moreover, two cytokinins—*trans*-zeatin and N^6-isopentenyladenine—were detected in the extract of the *P. marginalis* RA62c-B2 bacterial culture (Figure 1g,h).

3.2. Impact of Pseudomonas marginalis RA62c-B2 Inoculation on Biometric Parameters and Dry Biomass of Oryza sativa

P. marginalis RA62c-B2 did not exhibit antagonistic properties against plant phytopathogens, specifically *Alternaria alternata*, *Erwinia billingiae*, and *Fusarium avenaceum* (Figure S2).

To assess the plant growth-promoting potential of *P. marginalis* RA62c-B2, we examined its effect following seed inoculation in the rice cultivar 'Dubrava'. The bacterial treatment consistently enhanced growth across both systems under standard conditions, with a particularly strong stimulatory effect on root elongation and development (Figures 2 and 3).

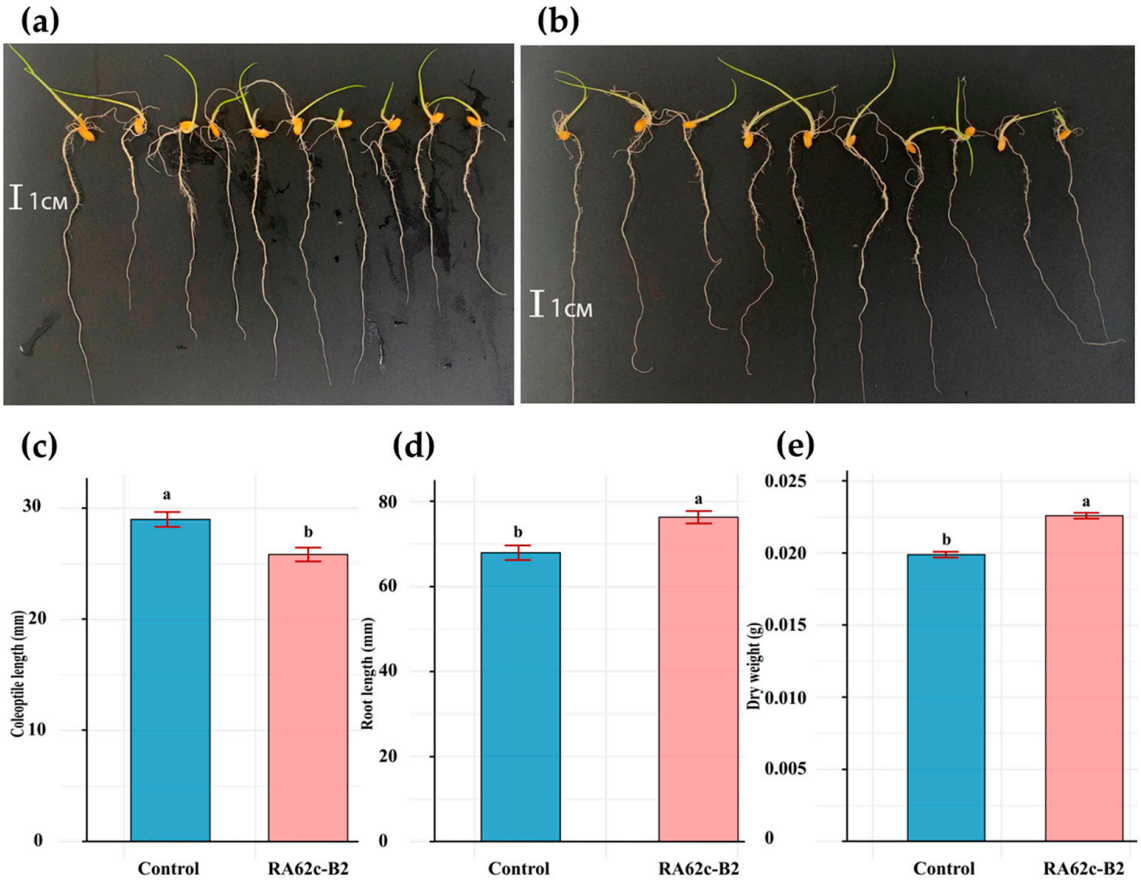


Figure 2. Influence of *Pseudomonas marginalis* RA62c-B2 inoculation on morphological parameters of 7-day-old *Oryza sativa* seedlings. (**a**) Control; (**b**) seedlings inoculated with *P. marginalis* RA62c-B2; (**c**) coleoptile length; (**d**) root length; (**e**) total dry weight of seedlings. Values are expressed as means; groups marked with the same letter are not significantly different (ANOVA followed by Tukey's test, $p < 0.05$).

On day 7 after inoculation with the RA62c-B2 strain, a statistically significant decrease in coleoptile length was recorded, amounting to 11.5% relative to the control group (Figure 2a). Conversely, root length exhibited a significant 10% increase in the RA62c-B2-treated samples (Figure 2b). Moreover, inoculation with RA62c-B2 led to a 17.4% rise in the dry weight of the seedlings (Figure 2e), indicating enhanced biomass accumulation under the treatment.

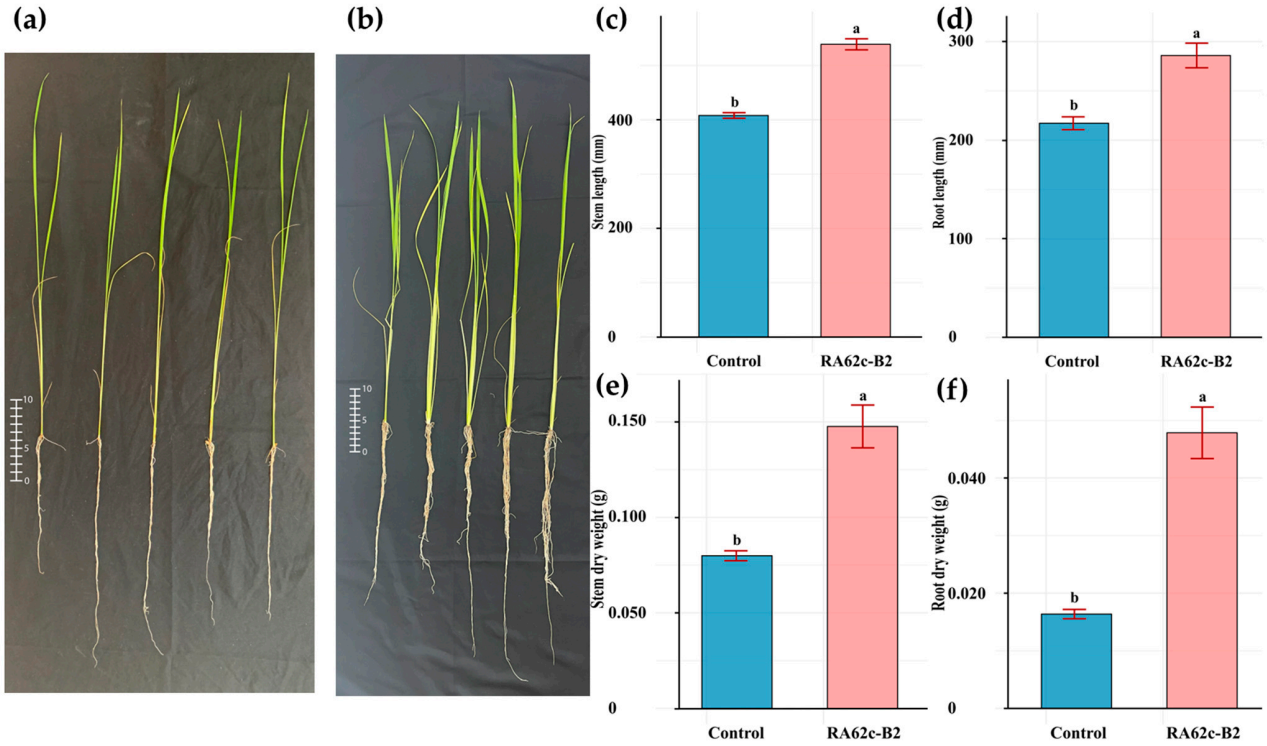


Figure 3. The effect of *Pseudomonas marginalis* RA62c-B2 inoculation on 30-day-old *Oryza sativa* plants. (**a**) Control; (**b**) plants inoculated with *P. marginalis* RA62c-B2; (**c**) stem length; (**d**) root length; (**e**) stem dry weight; (**f**) root dry weight. Values are expressed as means; groups marked with the same letter are not significantly different (ANOVA followed by Tukey's test, $p < 0.05$).

Inoculation of rice seeds with *P. marginalis* RA62c-B2 resulted in a significant 24.5% increase in shoot length (Figure 3c) in 30-day-old plants. The root length of RA62c-B2-treated rice plants was significantly greater by 24.13% compared to the control (Figure 3d). Furthermore, the dry weight of the shoots was elevated by 44.2% under RA62c-B2 treatment (Figure 3e), while the dry weight of the roots showed an even more pronounced increase of 63.8% (Figure 3f).

3.3. Phylogenetic Identification

The analysis of phylogenetic relatedness (ANI) and divergence degree (dDDH), performed using the eight closest species, revealed that RA62c-B2 shows the greatest similarity to *Pseudomonas marginalis* ICMP3553, with an ANI value of 98.3% (Figure 4) and a dDDH value of 85.4% (Table 1). Based on the phylogenetic analysis (Figure 4a) and the ANI and dDDH values, it is established that RA62c-B2 belongs to *P. marginalis*.

Table 1. Comparison of the bacterial endophyte *Vitis amurensis* RA62c-B2 with representatives of the genus *Pseudomonas*, performed using dDDH.

Strain	dDDH (%)
P. marginalis ICMP 3553	85.4
P. pergaminensis 1008T	42.4
P. lurida LMG 21995	42.1
P. azotoformans LMG 21611	40.5
P. extremorientalis LMG 19695	40.3
P. allii MAFF 301514	40.3
P. simiae CCUG 50988	39.7
P. canadensis 2-92	39.4

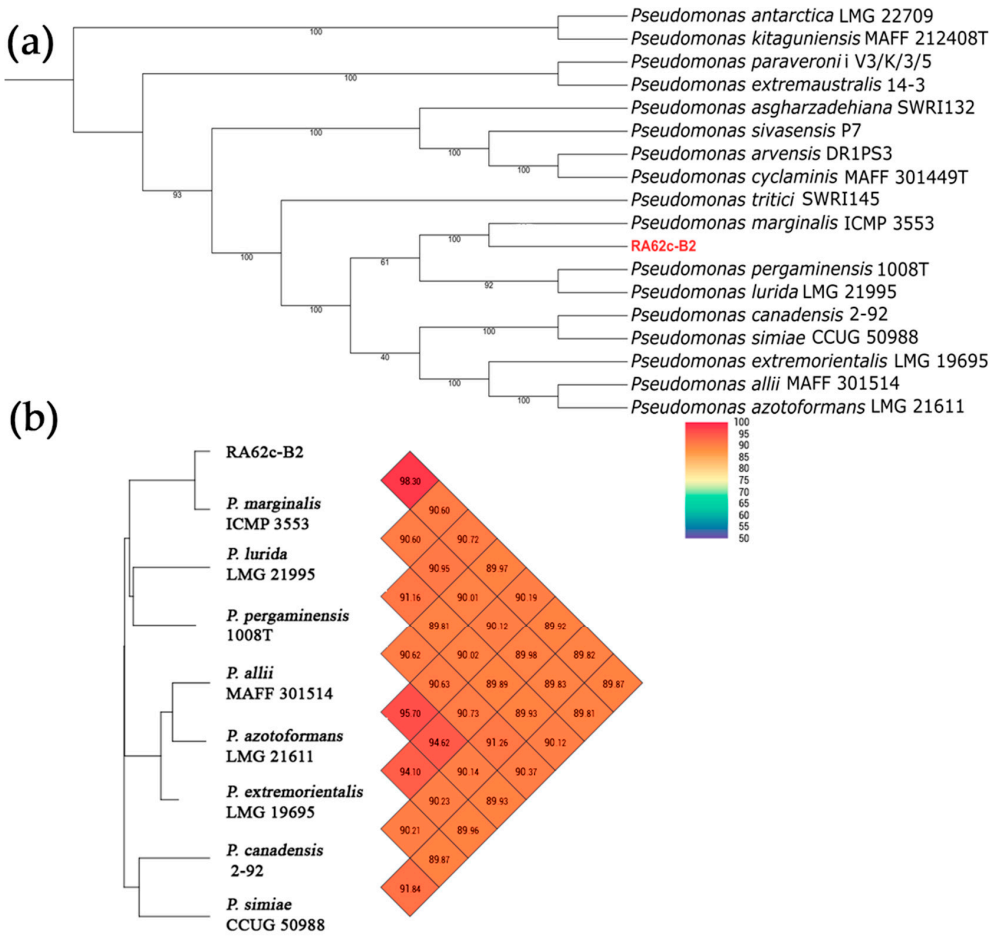


Figure 4. The phylogenetic analysis of *Pseudomonas marginalis* RA62c-B2: (**a**) Phylogenetic tree reconstructed with FastME 2.1.6.1 using GBDP distances computed from genome sequences. Branch lengths correspond to the GBDP distance formula d5. NCBI RefSeq assembly numbers are indicated next to each species name and are also listed in Table S1. The phylogenetic position of strain RA62c-B2 is highlighted in red. (**b**) oANI analysis was conducted with the Orthologous Average Nucleotide Identity Tool.

3.4. Genomic Characteristics and Comparative Genetic Analysis

The genome of *P. marginalis* RA62c-B2 comprises 6,620,267 bp, assembled into 81 scaffolds, with an average GC content of 60.6%. It encodes 6545 protein-coding sequences, 61 tRNA genes, nine rRNA genes, and three prophage-encoding sites (Table 2). Genome completeness and purity analysis revealed that the RA62c-B2 genome exhibits 100% completeness and a contamination level of 0.43% (Table 2).

Functional gene annotation using GO nomenclature allowed us to characterize 5471 genes, which accounts for 83.59% of the total number of genes. The most represented term in the GO sub-ontology «biological process» is DNA transcription (435 genes); in the «cellular component» sub-ontology, it is the plasma membrane (911 genes); and in the «molecular function» sub-ontology, it is ATP binding (559 genes) (Figure 5b). Using KEGG, 5957 genes were characterized and divided into 256 categories. The most frequently occurring categories were metabolic pathways (1092 genes), biosynthesis of secondary metabolites (441 genes), microbial metabolism in various environments (367 genes), two-component system (275 genes), and ABC transporters (247 genes) (Figure 5c). Using the COG nomenclature, 6401 genes were annotated. Among the annotated genes, the largest proportion consisted of sequences assigned to the «unknown function» category (1562 genes). In the group of genes with an established functional assignment, the highest number of sequences belonged to amino acid transport

and metabolism (602 genes), transcription (565 genes), transport and metabolism of inorganic ions (415 genes), signal transduction mechanisms (367 genes), and cell wall structure and biogenesis (345 genes) (Figure 5d).

Table 2. Genomic features of the *Pseudomonas marginalis* RA62c-B2 and related members of the *Pseudomonas* genus.

	RA62c-B2	ICMP 3553	LMG 21995	1008T
GeneBank acc. number	PRJNA1517745	GCA_030815455.1	GCA_002563895.1	GCA_024112395.2
Genome size (bp)	6,620,267	6,571,914	6,311,648	6,609,381
G+C content (mol%)	60.6	60.4	60.6	60.5
Number of Contigs	2	200	1	1
Protein-coding genes (CDS)	6545	5807	5621	5876
tRNA	61	60	60	70
rRNA	9	4	4	16
Prophage	3	1	2	1

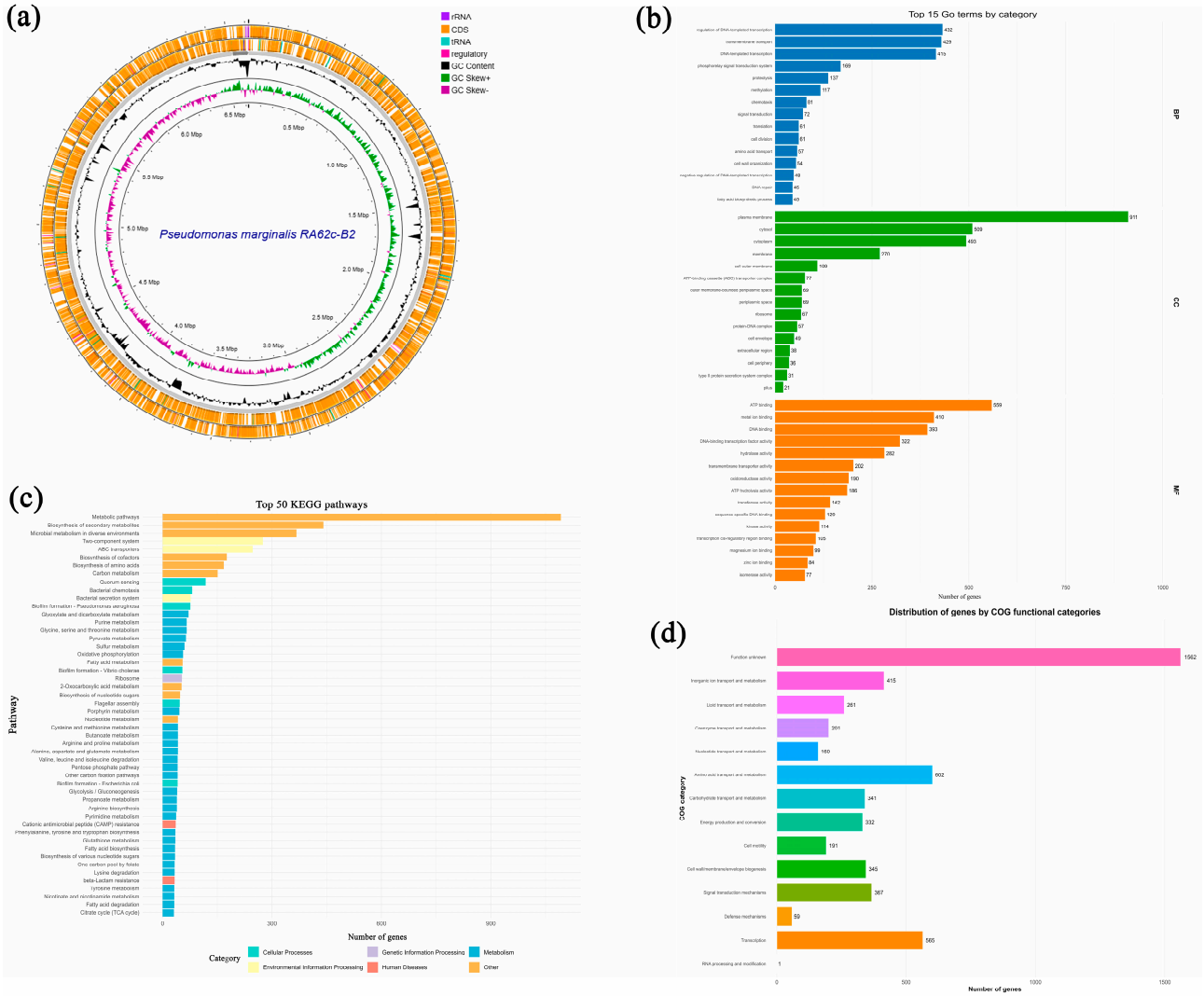


Figure 5. Functional annotation and map of the whole genome of *Pseudomonas marginalis* RA62c-B2. (**a**) Circular genome map. (**b**) GO function classification. BP—biological process, CC—cellular component, MF—molecular function. (**c**) KEGG function classification. (**d**) COG function classification.

A comparative analysis of the protein clusters in four *Pseudomonas* strains revealed that these strains share 4133 common protein clusters (Figure 6a). The highest number of shared clusters for *P. marginalis* RA62c-B2 was observed with *P. lurida* LMG 21995, amounting to 4908 clusters. Additionally, RA62c-B2 harbored 10 unique protein clusters (Figure 6a). Characterization of the unique clusters using the GO database showed that the smallest number of clusters was associated with DNA repair (GO:0006281). Furthermore, the clusters were evenly distributed (30% each) across the following functional categories: organic acid metabolism (GO:0006082), metabolic process (GO:0008152), and biological process (GO:0008150) (Figure 6b).

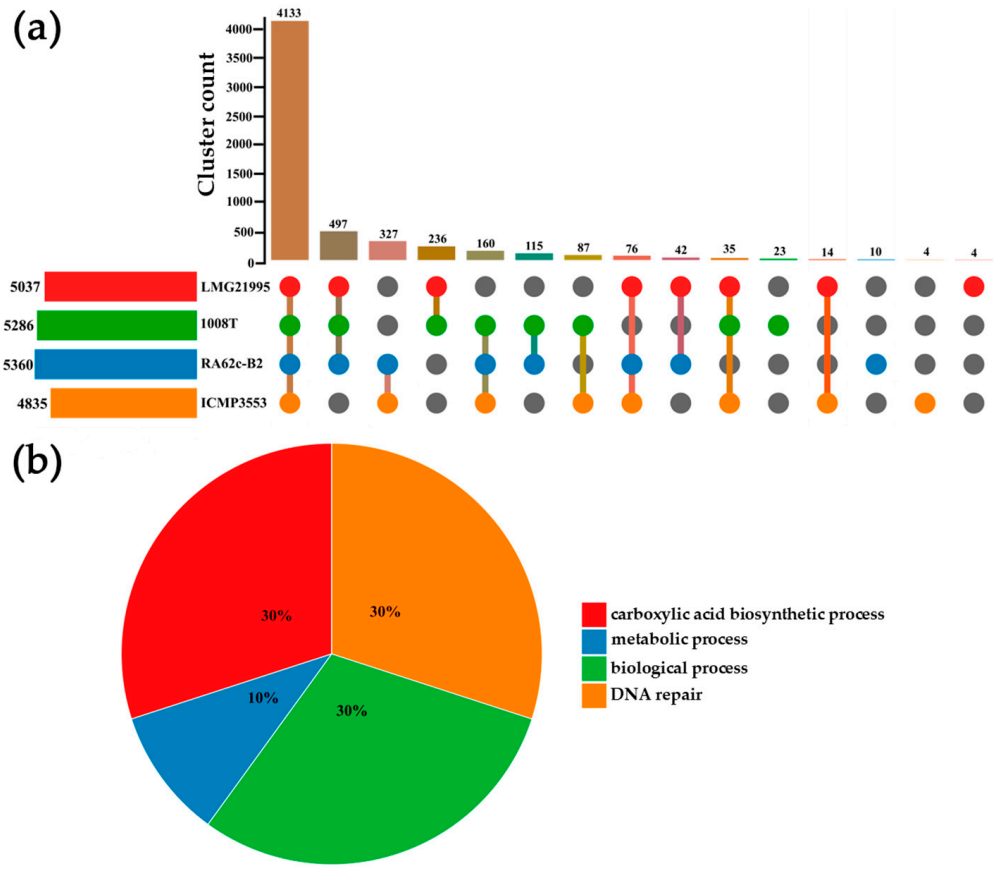


Figure 6. The UpSet plot of the bacterial strains *Pseudomonas marginalis* RA62c-B2, *Pseudomonas marginalis* ICMP 3553, *Pseudomonas pergaminensis* 1008T, and *Pseudomonas lurida* LMG 21995, generated using the OrthoVenn3 software (**a**). Classification of the unique clusters of *P. marginalis* RA62c-B2 according to the Gene Ontology database (**b**).

Analysis of the *P. marginalis* RA62c-B2 genome using antiSMASH revealed 11 clusters of secondary metabolism genes. Among these, the gene clusters for arylpolyene, beta-lactone, non-ribosomal peptide synthetase (NRPS), NAGGN, and redox cofactor biosynthesis were conserved across all compared strains (Table S4). Hydrogen cyanide was detected in the RA62c-B2, M13, PS92, and Qb1D2 strains. Viscosin and histicorrugatin were identified in RA62c-B2 and Qb1D2. Notably, the gene cluster responsible for icosaside A biosynthesis was unique to RA62c-B2. Sequence similarity analysis showed high conservation of genes of viscosin (100%) and histicorrugatin (94%) biosynthesis within the RA62c-B2 genome (Table S4).

3.5. Genes of Pseudomonas marginalis RA62c-B2 Associated with Plant Growth Promotion

In the genome of *P. marginalis* RA62c-B2, a number of plant growth-promoting genes (PGPGs) were identified. Using PGPT-Pred PlaBase, it was found that 31% of the PGPGs were related to direct mechanisms of plant growth promotion and included 10% of genes associated with phytohormone synthesis, 13% with increased bioavailability of nutrients, and 8% with bioremediation. Genes indirectly associated with plant growth promotion accounted for 69% of all those detected with PGPT-Pred; among them, 26% were linked to colonization of plant systems, 20% to the control of biotic and abiotic stresses, 22% to competitive exclusion, and 1% to stimulation of the plant immune response (Figure 7; Table S5).

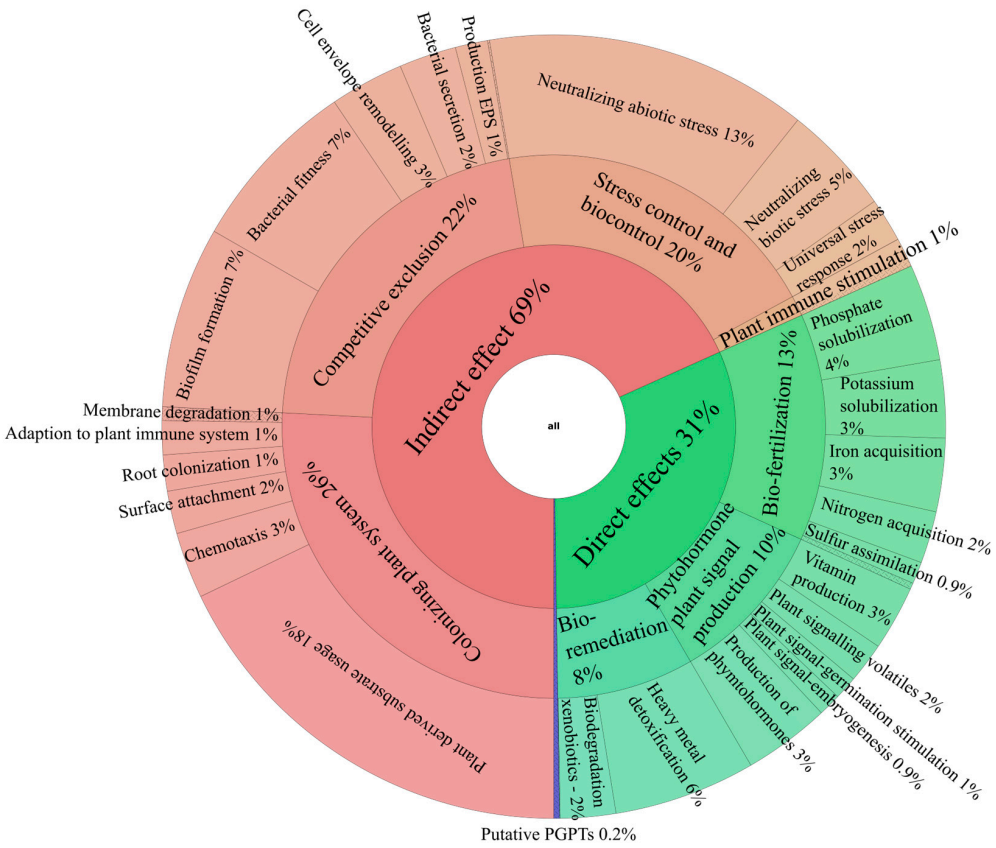


Figure 7. Genomic characterization based on PGPT-Pred analysis.

Of particular interest are the genes responsible for IAA biosynthesis, which in the *P. marginalis* RA62c-B2 genome are represented by the IAM pathway genes *iaaM* (tryptophan 2-monooxygenase) and *iaaH* (indole-3-acetyl-asparagine hydrolase) (Table 3); additionally, genes for cytokinin biosynthesis (*BacCYPs*, *miaAB*, *fni*, and *ispDEFU*) were detected. The genome also harbors genes enhancing the bioavailability of various nutrients—including phosphorus (*phoU* and *pstABCS*), potassium (*kdpABCDE*, *kup*, *kefCF*, *nhaP*, *chaA*, *mrpD*, and *trkAH*), sulfur (*cysAWTPNDHJIB*, *sfnG*, *iscSRUA*, *hscAB*, *fdx*, *iscX*, *dsrEF*, *sseA*, and *ssuABCDE*), iron (*fpvA*, *fhuA*, *fpuC*, and *cntO*), and nitrogen (*glnA*, *napA*, and *nasD*)—as well as genes involved in the biosynthesis of organic acids such as gluconic acid (*pqqABCDE* and *gcd*), fumaric acid (*aspA* and *fumAC*), and succinic acid (*sucCD*) (Table 3). Furthermore, the genome contains genes contributing to plant tolerance to osmotic (*betABIT*, *opuAA*, *opuAB*, and *prop*) and oxidative (*katBE*, *sodB*, *ahpCF*, *msrAB*, and *gshAB*) stress, along with genes for xylene degradation (*xylABFGHR*) and the catechol pathway (*pcaGHBKRF*) (Table 3). The

presence of annotated genes (in particular *phoU*, *katB*, *cysA*, *miaB*, and *opuA*) was confirmed by PCR analysis of some of the identified plant growth-promoting genes (Figure S3).

Table 3. Genes associated with plant growth promotion in *Pseudomonas marginalis* RA62c-B2.

Function	Genes
Biosynthesis of IAA	*iaaM*, *iaaH*
Cytokinin biosynthesis	*BacCYPs*, *PpnN*, *miaAB*, *dxr*, *fni*, *ispDEFU*
Biosynthesis of organic acids	*pqqABCDE*, *gcd*, *aspA*, *fumAC*, *sucCD*, *pta*
Metabolism of N	*glnA*, *napA*, *nasD*
Mobilization of P	*phoU*, *pstABCS*
Mobilization of K	*kdpABCDE*, *kup*, *kefCF*, *nhaP*, *chaA*, *mrpD*, *trkAH*
Assimilation of Fe	*fpvA*, *fhuA*, *fpuC*, *cntO*
Metabolism of S	*cysAWTPNDHJIB*, *sfnG*, *iscSRUA*, *hscAB*, *fdx*, *iscX*, *dsrEF*, *sseA*, *ssuABCDE*
Resistance to osmotic stress	*betABIT*, *opuAA*, *opuAB*, *prop*
Resistance to oxidative stress	*katBE*, *sodB*, *ahpCF*, *msrAB*, *gshAB*
Bioremediation	*antABC*, *pcaGHBKRF*, *hcaB*, *xylABFGHR*

4. Discussion

The data obtained in this study demonstrate a consistent alignment between the genomic potential of *P. marginalis* RA62c-B2 and its experimentally confirmed plant growth-promoting activity.

Growth on Ashby medium indicates the strain's potential for nitrogen acquisition; no nitrogen fixation genes were detected. Growth under nitrogen-deficient conditions may be supported by alternative utilization pathways or synergistic factors that promote the strain's growth. Transparent halos on Pikovskaya's and Alexandrovsky media confirm the strain's capacity to solubilize phosphorus and potassium, consistent with the identified transport genes (*phoU*, *pstABCS*, *kdpABCDE*, *kup*, *trkAH*) and organic biosynthesis genes (*pqqABCDE*, *gcd*, *aspA*, *fumAC*, *sucCD*, *pta*). These results align with prior findings on *Pseudomonas* isolates' nutrient-solubilizing activity [46] and data for the *P. marginalis* EU-MRN-08 strain, which improved maize growth via potassium solubilization from minerals [47]. The yellow halo on solid media indicates sulfur oxidation activity, corroborated by the presence of sulfur-metabolizing genes. This is consistent with reports of sulfur compound oxidation in other *Pseudomonas* strains (e.g., PRK786, C27) [48,49]. Overall, the data confirm and extend the known nutrient-solubilizing potential of *Pseudomonas* strains.

Genomic analysis of the RA62c-B2 strain revealed the presence of two key genes involved in IAA biosynthesis via the IAM pathway: *iaaM* and *iaaH*. At the same time, HPLC-MS analysis did not detect IAA in the extracts of *P. marginalis* RA62c-B2. The absence of IAA in the analyzed extracts may be explained by the lack of expression of these genes under laboratory conditions. It is likely that specific conditions are required for their activation, such as the presence of plants, a particular medium composition, or sufficient tryptophan availability [6,50]. In our study, HPLC-MS detected tryptophan derivatives—indole-3-acetaldehyde and indole-3-carboxaldehyde—in the bacterial extracts. Their presence suggests that IAA biosynthesis may be limited by the availability of tryptophan as a substrate. IAA production has been previously reported for *P. marginalis* strains IT-357P (MI5) [51] and BMH-2007, the latter also showing stimulation of pepper (*Capsicum annuum*) development without phytopathogenicity [52]. The identification of mature cytokinins (*trans*-zeatin and N^6-isopentenyladenine) and the presence of a gene set capable of supporting cytokinin biosynthesis demonstrate the strain's ability to produce several types of cytokinins, which likely contributes to enhanced root development and biomass accumulation. Cytokinin production may also support plant defence against pathogens, as shown for *P. fluorescens*

G20-18 in suppressing *P. syringae* in *Arabidopsis* [53]; however, this protective role warrants further investigation.

The inoculation experiments with *O. sativa* confirmed the pronounced plant growth-promoting effect of *P. marginalis* RA62c-B2. A transient reduction in coleoptile length on day 7 was accompanied by a significant increase in root length and seedling dry weight, indicating a shift in resource allocation towards root development. In 30-day-old plants, the treatment led to substantial increases in shoot and root lengths, as well as in dry weights, with root dry weight rising notably by 63.8%. This pattern reflects not only enhanced total biomass but also a more robust root system, which can improve nutrient and water uptake. Such biometric improvements are likely driven by a combination of phytohormone production, nutrient solubilization, and organic acid synthesis (e.g., gluconic, fumaric, and succinic acids). These phenotypic effects align with the genomic potential of RA62c-B2: PGPT-Pred PlaBase analysis revealed that 31% of its plant growth-promoting genes (PGPGs) are linked to direct mechanisms (phytohormone synthesis, nutrient bioavailability, bioremediation), while 69% are associated with indirect ones (stress tolerance, colonization, competitive exclusion, immune response modulation). This distribution underscores the multifunctional nature of the strain, combining direct growth stimulation with mechanisms that enhance plant fitness under stress. In addition, strain RA62c-B2 possesses a complete cluster of genes involved in the biosynthesis of viscosin and histicorrugatin, an antimicrobial substance [54,55]. However, *in vitro* testing using phytopathogens showed that it lacked antagonistic properties against them. The identification of viscosin and histicorrugatin gene clusters in *P. marginalis* RA62c-B2 points to key ecological functions. Viscosin's antimicrobial and surfactant properties are expected to support competitive exclusion of other microbes and efficient colonization of plant-associated habitats (e.g., rhizosphere, phyllosphere) [56,57]. Such lipopeptides may also shape plant–microbe relationships by modulating host immunity, either by attenuating defence responses or by triggering systemic resistance, depending on context and dosage [58]. Additionally, surfactant-mediated biofilm formation may improve stress tolerance (e.g., to desiccation and UV exposure), further supporting niche adaptation [59].

Notably, RA62c-B2 harbors genes related to osmotic and oxidative stress tolerance (*katBE*, *sodB*, *ahpCF*, *msrAB*, *gshAB*), xylene degradation, and the catechol pathway (*xylABFGHR*, *pcaGHBKRF*), indicating its potential not only for promoting plant growth but also for contributing to bioremediation. Together with the demonstrated capacity for nitrogen acquisition, phosphorus and potassium solubilization, sulfur oxidation, and phytohormone biosynthesis, these traits highlight RA62c-B2 as a multifaceted PGP bacterium. While the current data consistently link the strain's genomic features to its observed activity, the precise contribution of individual mechanisms to the overall growth-promoting effect requires further investigation.

5. Conclusions

This study characterized the RA62c-B2 strain isolated from *Vitis amurensis*, confirming its identity as *Pseudomonas marginalis* via phylogenetic analysis. The strain exhibits multiple plant growth-promoting traits: it synthesizes mature phytohormones such as *trans*-zeatin and N^6-isopentenyladenine, enhances the bioavailability of key nutrients (P and K), and carries genes supporting stress tolerance and bioremediation (e.g., xylene degradation, catechol pathway). Experimental data showed that a single application of the RA62c-B2 suspension significantly stimulated *O. sativa* growth, though the underlying mechanisms require further elucidation. It is important to note that these results were obtained in a controlled setting with a single treatment, and direct extrapolation to field performance should be avoided. These findings lay the groundwork for developing biologics based on

this strain; future work will focus on assessing its effects on grapevine growth and fruit quality in industrial vineyards.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae12111327/s1: Table S1: Primers used to validate the annotation; Table S2: Genomes of the strains included in the set of genomes; Table S3: Mobilization of nutrients by *Pseudomonas marginalis* RA62c-B2; Table S4: Secondary metabolism gene clusters in certain strains of *Pseudomonas marginalis*, including RA62c-B2; Table S5. Sequences detected in PGPT-Pred analysisin *Pseudomonas marginalis* RA62c-B2; Figure S1: Morphological characteristics of strain RA62c-B2; Figure S2: Testing of strain RA62c-B2 for antagonistic activity; Figure S3: PCR of certain plant growth-promoting genes in RA62c-B2.

Author Contributions: O.A.A. and A.A.A. contributed to the research design, experimental implementation, data analysis, and manuscript preparation. Material collection was carried out by A.R.S., O.A.A. and A.A.A.; A.R.S. conducted the HPLC analysis, while O.A.A. performed DNA isolation for NGS. Bioinformatic analysis and data visualization were executed by N.N.N. and A.A.A. The writing, critical review, and editing of the manuscript were performed by O.A.A., A.A.A. and K.V.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was carried out within the state assignment of the Ministry of Science and Higher Education of the Russian Federation (theme no. 124012200181-4).

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author(s). The complete genome sequence for *P. marginalis* RA62c-B2 can be found in the NCBI database "BioProject PRJNA1517745".

Acknowledgments: We are grateful to Evgeniya S. Martynenko (Laboratory of Soil Science and Soil Ecology, Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences) for her valuable contribution to the bacterial morphological analysis.

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

References

1. Sharma, P.; Sharma, P.; Thakur, N. Sustainable Farming Practices and Soil Health: A Pathway to Achieving SDGs and Future Prospects. *Discov. Sustain.* **2024**, *5*, 250. [CrossRef]
2. Kassawmar, T.; Desta, G.; Tadesse, M.; Teferi, E.; Kaba, M.; Suryabhagavan, K.V.; Shiferaw, A. Editorial: Evidences (States and Experiences) of Land Management and Food/Nutrition (in)Security in Mixed Farming Systems: A Global Perspective. *Front. Sustain. Food Syst.* **2025**, *9*, 1561449. [CrossRef]
3. Kumari, E.; Kumari, S.; Das, S.S.; Mahapatra, M.; Sahoo, J.P. Plant Growth-Promoting Bacteria (PGPB) for Sustainable Agriculture: Current Prospective and Future Challenges. *AgroEnviron Sustain.* **2023**, *1*, 274–285. [CrossRef]
4. Brito-Lopez, C.; van der Wielen, N.; Barbosa, M.; Karlova, R. Plant Growth–Promoting Microbes and Microalgae-Based Biostimulants: Sustainable Strategy for Agriculture and Abiotic Stress Resilience. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2026**, *380*, 20240251. [CrossRef] [PubMed]
5. Wang, Z.; Lu, K.; Liu, X.; Zhu, Y.; Liu, C. Comparative Functional Genome Analysis Reveals the Habitat Adaptation and Biocontrol Characteristics of Plant Growth-Promoting Bacteria in NCBI Databases. *Microbiol. Spectr.* **2023**, *11*, e05007–e05022. [CrossRef] [PubMed]
6. Spaepen, S.; Vanderleyden, J.; Remans, R. Indole-3-Acetic Acid in Microbial and Microorganism-Plant Signaling. *FEMS Microbiol. Rev.* **2007**, *31*, 425–448. [CrossRef] [PubMed]
7. Tang, J.; Li, Y.; Zhang, L.; Mu, J.; Jiang, Y.; Fu, H.; Zhang, Y.; Cui, H.; Yu, X.; Ye, Z. Biosynthetic Pathways and Functions of Indole-3-Acetic Acid in Microorganisms. *Microorganisms* **2023**, *11*, 2077. [CrossRef] [PubMed]
8. Frébortová, J.; Frébort, I. Biochemical and Structural Aspects of Cytokinin Biosynthesis and Degradation in Bacteria. *Microorganisms* **2021**, *9*, 1314. [CrossRef] [PubMed]
9. Nett, R.S.; Montanares, M.; Marcassa, A.; Lu, X.; Nagel, R.; Charles, T.C.; Hedden, P.; Rojas, M.C.; Peters, R.J. Elucidation of Gibberellin Biosynthesis in Bacteria Reveals Convergent Evolution. *Nat. Chem. Biol.* **2017**, *13*, 69–74. [CrossRef] [PubMed]

10. Fortt, J.; González, M.; Morales, P.; Araya, N.; Remonsellez, F.; Coba de la Peña, T.; Ostria-Gallardo, E.; Stoll, A. Bacterial Modulation of the Plant Ethylene Signaling Pathway Improves Tolerance to Salt Stress in Lettuce (*Lactuca sativa* L.). *Front. Sustain. Food Syst.* **2022**, *6*, 768250. [CrossRef]
11. Guo, D.-J.; Singh, R.K.; Singh, P.; Li, D.-P.; Sharma, A.; Xing, Y.-X.; Song, X.-P.; Yang, L.-T.; Li, Y.-R. Complete Genome Sequence of *Enterobacter roggenkampii* ED5, a Nitrogen Fixing Plant Growth Promoting Endophytic Bacterium With Biocontrol and Stress Tolerance Properties, Isolated From Sugarcane Root. *Front. Microbiol.* **2020**, *11*, 580081. [CrossRef] [PubMed]
12. Rodríguez, H.; Fraga, R.; Gonzalez, T.; Bashan, Y. Genetics of Phosphate Solubilization and Its Potential Applications for Improving Plant Growth-Promoting Bacteria. *Plant Soil* **2006**, *287*, 15–21. [CrossRef]
13. Chen, X.; Zhao, Y.; Huang, S.; Peñuelas, J.; Sardans, J.; Wang, L.; Zheng, B. Genome-Based Identification of Phosphate-Solubilizing Capacities of Soil Bacterial Isolates. *AMB. Express* **2024**, *14*, 85. [CrossRef] [PubMed]
14. Cronin, M.; Zomer, A.; Fitzgerald, G.; Sinderen, D. van Identification of Iron-Regulated Genes of *Bifidobacterium breve* UCC2003 as a Basis for Controlled Gene Expression. *Bioengineered* **2012**, *3*, 159–169. [CrossRef] [PubMed]
15. Latorre, M.; Quenti, D.; Travisany, D.; Singh, K.V.; Murray, B.E.; Maass, A.; Cambiazo, V. The Role of Fur in the Transcriptional and Iron Homeostatic Response of *Enterococcus faecalis*. *Front. Microbiol.* **2018**, *9*, 1580. [CrossRef] [PubMed]
16. Cánovas, D.; Vargas, C.; Kneip, S.; Morón, M.-J.; Ventosa, A.; Bremer, E.; Nieto, J.J. Genes for the Synthesis of the Osmoprotectant Glycine Betaine from Choline in the Moderately Halophilic Bacterium *Halomonas elongata* DSM 3043The EMBL Accession Number for the Sequence Reported in This Paper Is AJ238780. *Microbiology* **2000**, *146*, 455–463. [CrossRef] [PubMed]
17. Gamalero, E.; Glick, B.R. Mechanisms Used by Plant Growth-Promoting Bacteria. In *Bacteria in Agrobiology: Plant Nutrient Management*; Maheshwari, D.K., Ed.; Springer: Berlin/Heidelberg, Germany, 2011; pp. 17–46.
18. Zamanzadeh-Nasrabadi, S.M.; Mohammadiapanah, F.; Hosseini-Mazinani, M.; Sarikhan, S. Salinity Stress Endurance of the Plants with the Aid of Bacterial Genes. *Front. Genet.* **2023**, *14*, 1049608. [CrossRef] [PubMed]
19. Satognon, F.; Akuku, V. Mechanisms of *Pseudomonas* spp. as Plant Growth Promoting Rhizobacteria: Plant Nutrition and Biocontrol. *Antonie Van Leeuwenhoek* **2026**, *119*, 141. [CrossRef] [PubMed]
20. Ghadamgahi, F.; Tarighi, S.; Taheri, P.; Saripella, G.V.; Anzalone, A.; Kalyandurg, P.B.; Catara, V.; Ortiz, R.; Vetukuri, R.R. Plant Growth-Promoting Activity of *Pseudomonas aeruginosa* FG106 and Its Ability to Act as a Biocontrol Agent against Potato, Tomato and Taro Pathogens. *Biology* **2022**, *11*, 140. [CrossRef] [PubMed]
21. Deshwal, V.K.; Kumar, P. Plant Growth Promoting Activity of *Pseudomonads* in Rice Crop. *Int. J. Curr. Microbiol. Appl. Sci.* **2013**, *2*, 152–157. [CrossRef] [PubMed]
22. Biessy, A.; Cadieux, M.; Mc Duff, F.; Deshaies, A.; Khun, K.; Lafond-Lapalme, J.; Vigneault, P.; Filion, M. Single-Strain and Consortium Inoculations with Plant-Beneficial *Pseudomonas* spp. Promote Lettuce Growth under Field Conditions. *Microbiol. Spectr.* **2026**, *14*, e03509-25. [CrossRef] [PubMed]
23. Gutiérrez-Gamboa, G.; Liu, S.-Y.; Sun, X.; Fang, Y. Oenological Potential and Health Benefits of Chinese Non-*Vitis vinifera* Species: An Opportunity to the Revalorization and to Breed New Varieties. *Food Res. Int.* **2020**, *137*, 109443. [CrossRef] [PubMed]
24. Wu, J.; Zhang, Y.; Zhang, H.; Huang, H.; Folta, K.M.; Lu, J. Whole Genome Wide Expression Profiles of *Vitis amurensis* Grape Responding to Downy Mildew by Using Solexa Sequencing Technology. *BMC Plant Biol.* **2010**, *10*, 234. [CrossRef] [PubMed]
25. Liu, L.; Li, H. Review: Research Progress in Amur Grape, *Vitis amurensis* Rupr. *Can. J. Plant Sci.* **2013**, *93*, 565–575. [CrossRef]
26. Aleynova, O.A.; Nityagovsky, N.N.; Ananev, A.A.; Suprun, A.R.; Ogneva, Z.V.; Dneprovskaya, A.A.; Beresh, A.A.; Tyunin, A.P.; Dubrovina, A.S.; Kiselev, K.V. The Endophytic Microbiome of Wild Grapevines *Vitis amurensis* Rupr. and *Vitis coignetiae* Pulliat Growing in the Russian Far East. *Plants* **2023**, *12*, 2952. [CrossRef] [PubMed]
27. Ananev, A.A.; Ogneva, Z.V.; Nityagovsky, N.N.; Suprun, A.R.; Kiselev, K.V.; Aleynova, O.A. Whole Genome Sequencing of *Bacillus velezensis* AMR25, an Effective Antagonist Strain against Plant Pathogens. *Microorganisms* **2024**, *12*, 1533. [CrossRef] [PubMed]
28. Echt, C.S.; Erdahl, L.A.; McCoy, T.J. Genetic Segregation of Random Amplified Polymorphic DNA in Diploid Cultivated Alfalfa. *Genome* **1992**, *35*, 84–87. [CrossRef] [PubMed]
29. Prjibelski, A.; Antipov, D.; Meleshko, D.; Lapidus, A.; Korobeynikov, A. Using SPAdes De Novo Assembler. *Curr. Protoc. Bioinform.* **2020**, *70*, e102. [CrossRef] [PubMed]
30. Simão, F.A.; Waterhouse, R.M.; Ioannidis, P.; Kriventseva, E.V.; Zdobnov, E.M. BUSCO: Assessing Genome Assembly and Annotation Completeness with Single-Copy Orthologs. *Bioinformatics* **2015**, *31*, 3210–3212. [CrossRef] [PubMed]
31. Schwengers, O.; Jelonek, L.; Dieckmann, M.A.; Beyvers, S.; Blom, J.; Goesmann, A. Bakta: Rapid and Standardized Annotation of Bacterial Genomes via Alignment-Free Sequence Identification. *Microb. Genom.* **2021**, *7*, 000685. [CrossRef] [PubMed]
32. Cantalapiedra, C.P.; Hernández-Plaza, A.; Letunic, I.; Bork, P.; Huerta-Cepas, J. eggNOG-Mapper v2: Functional Annotation, Orthology Assignments, and Domain Prediction at the Metagenomic Scale. *Mol. Biol. Evol.* **2021**, *38*, 5825–5829. [CrossRef] [PubMed]

33. Patz, S.; Gautam, A.; Becker, M.; Ruppel, S.; Rodríguez-Palenzuela, P.; Huson, D. PLaBAse: A Comprehensive Web Resource for Analyzing the Plant Growth-Promoting Potential of Plant-Associated Bacteria. *BioRxiv* **2021**. Available online: https://www.researchgate.net/publication/357065129_PLaBAse_A_comprehensive_web_resource_for_analyzing_the_plant_growth-promoting_potential_of_plant-associated_bacteria (accessed on 15 October 2026). [CrossRef]
34. Blin, K.; Shaw, S.; Vader, L.; Szenei, J.; Reitz, Z.L.; Augustijn, H.E.; Cediel-Becerra, J.D.D.; de Crécy-Lagard, V.; Koetsier, R.A.; Williams, S.E.; et al. antiSMASH 8.0: Extended Gene Cluster Detection Capabilities and Analyses of Chemistry, Enzymology, and Regulation. *Nucleic Acids Res*. **2025**, *53*, W32–W38. [CrossRef] [PubMed]
35. Ye, J.; Coulouris, G.; Zaretskaya, I.; Cutcutache, I.; Rozen, S.; Madden, T.L. Primer-BLAST: A Tool to Design Target-Specific Primers for Polymerase Chain Reaction. *BMC Bioinform*. **2012**, *13*, 134. [CrossRef] [PubMed]
36. Ondov, B.D.; Treangen, T.J.; Melsted, P.; Mallonee, A.B.; Bergman, N.H.; Koren, S.; Phillippy, A.M. Mash: Fast Genome and Metagenome Distance Estimation Using MinHash. *Genome Biol*. **2016**, *17*, 132. [CrossRef] [PubMed]
37. Lefort, V.; Desper, R.; Gascuel, O. FastME 2.0: A Comprehensive, Accurate, and Fast Distance-Based Phylogeny Inference Program. *Mol. Biol. Evol*. **2015**, *32*, 2798–2800. [CrossRef] [PubMed]
38. Lee, I.; Ouk Kim, Y.; Park, S.-C.; Chun, J. OrthoANI: An Improved Algorithm and Software for Calculating Average Nucleotide Identity. *Int. J. Syst. Evol. Microbiol*. **2016**, *66*, 1100–1103. [CrossRef] [PubMed]
39. Meier-Kolthoff, J.P.; Auch, A.F.; Klenk, H.-P.; Göker, M. Genome Sequence-Based Species Delimitation with Confidence Intervals and Improved Distance Functions. *BMC Bioinform*. **2013**, *14*, 60. [CrossRef] [PubMed]
40. Sun, J.; Lu, F.; Luo, Y.; Bie, L.; Xu, L.; Wang, Y. OrthoVenn3: An Integrated Platform for Exploring and Visualizing Orthologous Data across Genomes. *Nucleic Acids Res*. **2023**, *51*, W397–W403. [CrossRef] [PubMed]
41. Chan, P.P.; Lin, B.Y.; Mak, A.J.; Lowe, T.M. tRNAscan-SE 2.0: Improved Detection and Functional Classification of Transfer RNA Genes. *Nucleic Acids Res*. **2021**, *49*, 9077–9096. [CrossRef] [PubMed]
42. Lagesen, K.; Hallin, P.; Rødland, E.A.; Stærfeldt, H.-H.; Rognes, T.; Ussery, D.W. RNAmmer: Consistent and Rapid Annotation of Ribosomal RNA Genes. *Nucleic Acids Res*. **2007**, *35*, 3100–3108. [CrossRef] [PubMed]
43. Leska, A.; Nowak, A.; Szulc, J.; Motyl, I.; Czarnecka-Chrebelska, K.H. Antagonistic Activity of Potentially Probiotic Lactic Acid Bacteria against Honeybee (*Apis mellifera* L.) Pathogens. *Pathogens* **2022**, *11*, 1367. [CrossRef] [PubMed]
44. Mirsam, H.; Aqil, M.; Azrai, M.; Efendi, R.; Muliadi, A.; Sembiring, H.; Azis, A.I. Molecular Characterization of Indigenous Microbes and Its Potential as a Biological Control Agent of *Fusarium* Stem Rot Disease (*Fusarium verticillioides*) on Maize. *Heliyon* **2022**, *8*, e11960. [CrossRef] [PubMed]
45. Reasoner, D.J.; Geldreich, E.E. A New Medium for the Enumeration and Subculture of Bacteria from Potable Water. *Appl. Environ. Microbiol*. **1985**, *49*, 1–7. [CrossRef] [PubMed]
46. Bakki, M.; Banane, B.; Marhane, O.; Esmaeel, Q.; Hatimi, A.; Barka, E.A.; Azim, K.; Bouizgarne, B. Phosphate Solubilizing *Pseudomonas* and *Bacillus* Combined with Rock Phosphates Promoting Tomato Growth and Reducing Bacterial Canker Disease. *Front. Microbiol*. **2024**, *15*, 1289466. [CrossRef] [PubMed]
47. Negi, R.; Sharma, B.; Yadav, N.; Kaur, N.; Yadav, A.; Puri, P.; Singh, S.; Yadav, A.N. Novel Potassium Solubilizing Bacterium *Pseudomonas marginalis* (EU-MRN-08) and Its Role in Plant Growth Promotion of Maize (*Zea mays* L.). *Biologia* **2025**, *80*, 2183–2190. [CrossRef]
48. Pothana, S.A. International Journal of Advanced Research. *Int. J. Adv. Res*. **2014**, *2*, 714–722.
49. Xu, X.-J.; Chen, C.; Guo, H.; Wang, A.; Ren, N.; Lee, D.-J. Characterization of a Newly Isolated Strain *Pseudomonas* sp. C27 for Sulfide Oxidation: Reaction Kinetics and Stoichiometry. *Sci. Rep*. **2016**, *6*, 21032. [CrossRef] [PubMed]
50. Patten, C.L.; Glick, B.R. Bacterial Biosynthesis of Indole-3-Acetic Acid. *Can. J. Microbiol*. **1996**, *42*, 207–220. [CrossRef] [PubMed]
51. Todorović, I.; Abrouk, D.; Kyselková, M.; Rey, M.; López-Mondéjar, R.; Raičević, V.; Jovičić-Petrović, J.; Moënne-Loccoz, Y.; Muller, D. Fluorescent *Pseudomonas* spp. from Suppressive and Conducive Soils Share Genomic and Functional Traits Relevant to *Fusarium graminearum* Disease Suppression. *BMC Genom*. **2025**, *27*, 57. [CrossRef] [PubMed]
52. Serrano-Gamboa, J.; Tavarez-Arriaga, J.; Iza Arteaga, M.; Ide-Pérez, M.; Tapia-Vázquez, I.; Folch-Mallol, J. Phylogenomic Insights into a Psychrotolerant, Plant Growth-Promoting Strain of *Pseudomonas marginalis* Isolated from *Xinantécatl volcano*. *Gene Rep*. **2025**, *41*, 102356. [CrossRef]
53. Großkinsky, D.K.; Tafner, R.; Moreno, M.V.; Stenglein, S.A.; García de Salamone, I.E.; Nelson, L.M.; Novák, O.; Strnad, M.; van der Graaff, E.; Roitsch, T. Cytokinin Production by *Pseudomonas fluorescens* G20-18 Determines Biocontrol Activity against *Pseudomonas syringae* in *Arabidopsis*. *Sci. Rep*. **2016**, *6*, 23310. [CrossRef] [PubMed]
54. Oni, F.E.; Geudens, N.; Adiobo, A.; Omoboye, O.O.; Enow, E.A.; Onyeka, J.T.; Salami, A.E.; De Mot, R.; Martins, J.C.; Höfte, M. Biosynthesis and Antimicrobial Activity of Pseudodesmin and Viscosinamide Cyclic Lipopeptides Produced by *Pseudomonads* Associated with the Cocoyam Rhizosphere. *Microorganisms* **2020**, *8*, 1079. [CrossRef] [PubMed]
55. De Vleeschouwer, M.; Van Kersavond, T.; Verleysen, Y.; Sinnaeve, D.; Coenye, T.; Martins, J.C.; Madder, A. Identification of the Molecular Determinants Involved in Antimicrobial Activity of Pseudodesmin A, a Cyclic Lipopeptide From the Viscosin Group. *Front. Microbiol*. **2020**, *11*, 646. [CrossRef] [PubMed]

56. Rather, M.A.; Gupta, K.; Mandal, M. Microbial Biofilm: Formation, Architecture, Antibiotic Resistance, and Control Strategies. *Braz. J. Microbiol.* **2021**, *52*, 1701–1718. [CrossRef] [PubMed]
57. Guan, Y.; Bak, F.; Hennessy, R.; Herms, C.; Elberg, C.; Dresbøll, D.; Winding, A.; Sapkota, R.; Nicolaisen, M. The Potential of *Pseudomonas fluorescens* SBW25 to Produce Viscosin Enhances Wheat Root Colonization and Shapes Root-Associated Microbial Communities in a Plant Genotype-Dependent Manner in Soil Systems. *mSphere* **2024**, *9*, e00294-24. [CrossRef] [PubMed]
58. Kumar, D.; Sattiraju, K.S.; Reddy, M.S. A Systems Approach to Endophyte-Mediated Plant Holobiont and Microbiome Dynamics. *Plants* **2026**, *15*, 802. [CrossRef] [PubMed]
59. Alsohim, A.S.; Taylor, T.B.; Barrett, G.A.; Gallie, J.; Zhang, X.-X.; Altamirano-Junqueira, A.E.; Johnson, L.J.; Rainey, P.B.; Jackson, R.W. The Biosurfactant Viscosin Produced by *Pseudomonas fluorescens* SBW25 Aids Spreading Motility and Plant Growth Promotion. *Environ. Microbiol.* **2014**, *16*, 2267–2281. [CrossRef] [PubMed]

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