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Bioprospecting of Plant Growth-Promoting Traits of *Pseudomonas* sp. Strain C3 Isolated from the Atacama Desert: Molecular and Culture-Based Analysis

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Abstract: Soil microorganisms that inhabit extreme environments have unique metabolic capacities and/or physical structures that allow them to survive in oligotrophic conditions. The bioprospecting of unknown bacteria in the context of current advances in genome mining is fundamental for the discovery of natural products with novel properties or applications. In this study, the plant growth-promoting and biocontrol traits of a *Pseudomonas* isolated from soil associated with plants from the Atacama Desert were characterized by whole-genome sequencing and in vitro assays. A high-quality genome draft of *Pseudomonas* sp. isolate C3 was obtained. An automated biosynthetic gene cluster analysis using antiSMASH 6.0 revealed the presence of a cluster of genes for the biosynthesis, regulation, and transport of the metabolite 2,4-diacetylphloroglucinol, which showed a high protein sequence identity (>89%) with a validated orthologous gene cluster from another *Pseudomonas*. In addition, via an in vitro assay, the biocontrol activity of *Pseudomonas* sp. isolate C3 against *Botrytis cinerea*, *Monilinia fructicola*, *Phytium* sp., *Alternaria* sp., *Geotrichum candidum*, and *Fusarium oxysporum* was corroborated. Finally, through KofamKOALA, the presence of genes involved in different metabolic pathways of plant growth-promoting traits was identified, which was corroborated by in vitro assays. This study provides information obtained from genomic analyses and culture tools on a bacterial isolate from the Atacama Desert characterized by plant growth-promoting capacities and biocontrol activity.

Keywords: plant growth promoting; extreme environments; Atacama Desert; 2,4-diacetylphloroglucinol; biocontroller

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1. Introduction

Plant-associated bacteria play several ecological roles and are generally enhanced in the rhizosphere [1,2]. They can increase the availability of nutrients in the soil, known as a direct mechanism [3], and/or confer defense against phytopathogenic microorganisms, which can indirectly provide benefits for plant growth and health [4]. These bacteria are characterized as plant growth-promoting (PGP) bacteria, and, in a biotechnological context, their metabolites are of great interest for the agricultural industry regarding their potential

applications for improving crop yields [5–7] through the replacement of chemical fertilizers and pesticides [8,9].

One of the metabolites produced by bacteria defined as PGP is indole acetic acid (IAA), a phytohormone of the auxin group that regulates vascular tissue differentiation and/or induces cell division and stem and root elongation [10]. Another compound produced by PGP is the enzyme ACC deaminase, which modulates ethylene synthesis by the plant, inactivating senescence processes in plant tissues in response to biotic or abiotic stress [11]. The ability of PGP to solubilize phosphate increases the availability of phosphate in the soil for metabolism by plants [12], similar to bacterium-mediated nitrogen fixation, which is also considered a PGP activity and depends on the presence of the enzyme nitrogenase, which fixes N_2 into ammonium (NH_3) [13]. Finally, siderophore production is also considered a PGP ability in bacteria because it increases the modification of iron hydroxide (Fe^{3+}) into ferrous hydroxide (Fe^{2+}), which is metabolically available to plants [14].

Antibiotic-producing PGP bacteria have been intensively studied, and special attention has been paid to bacteria of the *Pseudomonas* genus regarding the production of 2,4-diacetylphloroglucinol, a metabolite characterized by its ability to control a wide variety of diseases caused by plant pathogenic microorganisms, inducing systemic resistance mechanisms [15–20]. Bacteria with antimicrobial attributes have been widely studied [21–23]. Kumar et al. [24] demonstrated the low diversity and frequency of the reported antibiotic activity of bacteria of the genus *Pseudomonas*; however, they highlighted their potential as producers of 2,4-diacetylphloroglucinol, a potent broad-spectrum antibiotic, but the origins and evolutionary dynamics of the genes associated with this pathway remain undefined [25].

Two processes have been shown to be key for the application of these microorganisms: bacterial isolation and characterization as PGP [26]. However, if extreme environments are also used, there is the possibility of finding new, highly optimized molecules due to selection pressure, in accordance with the need for rapid evolution to survive [27].

The Atacama Desert is a hyper-arid region of Chile, which has been compared to Mars and termed "the dry edge of life" [28,29]. Specifically, the Talabre–Lejía transect (TLT) is in the western highlands of the Chilean Andes, between ~2500 and ~4500 m.a.s.l. (meters above sea level), bordering the active Lascar volcano and the saline Lejía Lake [30]. Despite vegetation in these areas being restricted by the temperature, pH, water availability, and radiation, among other factors [31,32], it is possible to observe vegetative patches along the entire location, from which bacteria with positive PGP capabilities have been isolated [33].

The aim of this study was to provide a broader view of PGP traits, not only through culture-based techniques, but also using different molecular tools to elucidate biosynthetic pathways associated with these beneficial attributes in plants. Thus, this study provides the first report of a bacterium of the genus *Pseudomonas* isolated from plant-associated soils in the Atacama Desert, characterized by genomic analysis and culture-based techniques as a bacterium with plant growth-promoting and biocontrol attributes, making it an excellent candidate for evaluation in in vivo or field assays.

2. Materials and Methods

2.1. Sampling Site Description

Pseudomonas sp. strain C3 is part of a microbial repository isolated in 2018 from the Talabre–Lejía transect, located in the Atacama Desert [34], where the most common plant species are *Calamagrostis crispa* and *Nassella nardoides* [31,32,35]. It has been deposited and is available in the microbial repository Colección Chilena de Recursos Genéticos Microbianos INIA (RGM, Chillán, Chile) under the internal code RGM2438.

2.2. Culture, DNA Isolation, and Whole-Genome Sequencing

Strain C3 was incubated in a stationary phase for 24 h at 30 °C in 2 mL of LB (Luria Bertani) culture medium, reaching an optical density (600 nm) of 1.3. DNA extraction was performed using the DNeasy Blood & Tissue (QIAGEN, Hilden, Germany) commercial kit

following the manufacturer's procedures. The isolated DNA was quantified by fluorometric analysis using the Broad-Range (BR) kit from Qubit (Invitrogen, Waltham, MA, USA) and sequenced using the NovaSeq system (Illumina, San Diego, CA, USA) by the Molecular Research DNA laboratory (Mr.DNA, Shallowater, TX, USA), with 2 × 250 bp paired-end sequencing and 4 million reads.

2.3. Genome Assembly and Annotation

De novo assembly was conducted using quality-filtered reads using the CLC Genomics Workbench (QIAGEN, Hilden, Germany) v12.0 with the default parameters [35]. The genome assembly was assessed by employing BUSCO v5.2.2 [36] in "genome" mode, "prodigal" v2.6.3 [37] was used for prediction and the genome representation was constructed using DNAPlotter [38]. The genome completeness was also evaluated with CheckM v1.1.2 [39]. Final gene prediction was performed using the NCBI Prokaryotic Genome Annotation Pipeline released in 2013 [40].

The 16S rDNA sequence used for the taxonomic classification of *Pseudomonas* sp. strain C3 has been deposited under GenBank code MT576541.1, and the genome sequence data have been deposited in DDBJ/ENA/GenBank under the accession number JAJNDW000000000 and linked to the National Center for Biotechnology Information (NCBI) under the BioProject accession number PRJNA783880.

2.4. Taxonomic Identification of the Complete Genome

The genome sequence data were uploaded and taxonomically analyzed by dDDH (digital DNA–DNA hybridization) using TYGS (Type Genome Server) [41]. The genome comparison was conducted using GBDP (Genome Blast Distance Phylogeny) [42]. The dDDH values and confidence intervals were calculated using the GGDC (Genome-to-Genome Distance Calculator) v3.0 [42,43]. The evolution tree was constructed with FASTME v2.1.6.1 [44] and supported with 100 pseudo-bootstrap replicates, rooted at the midpoint [45] and visualized with PhyD3 [46].

2.5. Identification of Metabolic Pathways and Specialized Metabolites

Genomic analyses were performed for the determination of metabolic pathways associated with PGP traits (nitrogen fixation, indole acetic acid (IAA) production, siderophore production, and iron and phosphate uptake) with KEGG (Kyoto Encyclopedia of Genes and Genomes) orthology [47] and complemented with KofamKOALA [48]. Additionally, the full genome of C3 was submitted to antiSMASH bacterial version v6.0.1 [49] for secondary metabolite biosynthetic gene cluster (BGC) detection with the strictness set to "relaxed". The optional parameters clusterblast and knownclusterblast [50] were called in order to compare the genetic diversity of the 2,4-diacetylphloroglucinol-producing BGC components against the antiSMASH database and the MIBiG 2.0 repository, respectively.

2.6. In Vitro Growth Inhibition of Phytopathogenic Fungi

The antagonism assay was performed according to the method described by Sepúlveda-Chavera et al. [51] Briefly, potato dextrose agar (PDA) plates were inoculated with the plant pathogenic fungi *Botrytis cinerea*, *Monilinia fructicola*, *Phytium* sp., *Alternaria* sp., *Geotrichum candidum*, and *Fusarium oxysporum* in the center of each plate, and 20 µL of *Pseudomonas* sp. strain C3 culture in a stationary phase at 2.5 cm from the center of the Petri dish culture. Plates only inoculated with fungi in the center were used as a control.

The inhibition of the mycelial radial growth (IMRG) of the fungi was calculated using the following equation:

$$\text{IMRG (\%)} = [(C - T)/C] \times 100 \quad (1)$$

where C represents the growth radius (mm) of each fungus on the control plate, and T is the fungal growth radius (mm) from the center of the Petri dishes under the treatment. The plates were incubated at room temperature until the control plates were fully covered with the phytopathogenic fungi.

2.7. In Vitro Identification of Plant Growth-Promoting Traits

The PGP assays were performed as described in Gaete et al. [33]. Briefly, specific culture media were used to determine four PGP attributes of *Pseudomonas* sp. strain C3. Siderophore production was assessed using CAS agar media [52]. A positive result for this colorimetric technique is a color change in the culture medium, from blue to an orange halo.

The phosphate solubilization and nitrogen-fixing assays were conducted according to the HIMEDIA Technical Data, using the PKV (Pikovskayas agar) [53] and NFM (Norris Glucose Nitrogen Free Medium) [54] culture media, respectively. Both methods are considered to show positive results when a transparent halo is observed in the plates. Finally, IAA production was measured using the Salkowski test following the suggestions described by Widawati [55], with a color change from yellow towards red being observed when IAA was synthesized.

2.8. Detection of In Vitro Hydrolytic Activity

The hydrolytic activity of *Pseudomonas* sp. strain C3 was evaluated using three specific culture media. For the chitinase activity assay, solid LB medium was supplemented with colloidal chitin [56]. A clear halo on a creamy background represents positivity for chitinase activity. The protease activity was evaluated using SMA (skim milk agar) media according to the protocol described by Bhowmik et al. [57]. The lipase activity was determined using a protocol described by Slifkin [58]. Again, for both assays, the appearance of a halo around the microorganism is indicative of a positive result.

3. Results

3.1. Genome Assembly and Annotation

The genome of *Pseudomonas* sp. strain C3 was assembled, obtaining completeness and contamination indexes of 99.38% and 0.1%, respectively, according to CheckM (Figure 1). This high-quality draft genome comprises 129 contigs with an N50 of 65,108 bp and a total length of 5,677,066 bp. In total, 5126 genes were predicted and analyzed with the BUSCO tool, obtaining 99.2% of the "bacterial" lineage markers in a single copy (Table 1).

Taxonomic identification based on the complete genome of *Pseudomonas* sp. strain C3 yielded 10 species grouped from other *Pseudomonas* genera, among which five (in addition to *Pseudomonas* sp. strain C3) formed a single paraphyletic clade (Figure 2). This phylogenetic tree was constructed based on the results obtained from the dDDH values of our strain (query strain) with each subject strain and its corresponding confidence interval (C.I., Table S1).

Table 1. Molecular features that determine the genome of *Pseudomonas* sp. strain C3.

Features	Chromosome
ID	C3
Marker lineage	f__Pseudomonadaceae (UID4490)
%Completeness	99.38
%Contamination	0.1
%Strain heterogeneity	0
N50	65,108
Sequences	129
%GC	61.37
Total length	5,677,066
Predicted genes	5126
CDS	5066
Proteins	4971
rRNA	2
tRNA	55
Genome Quality	High-quality draft

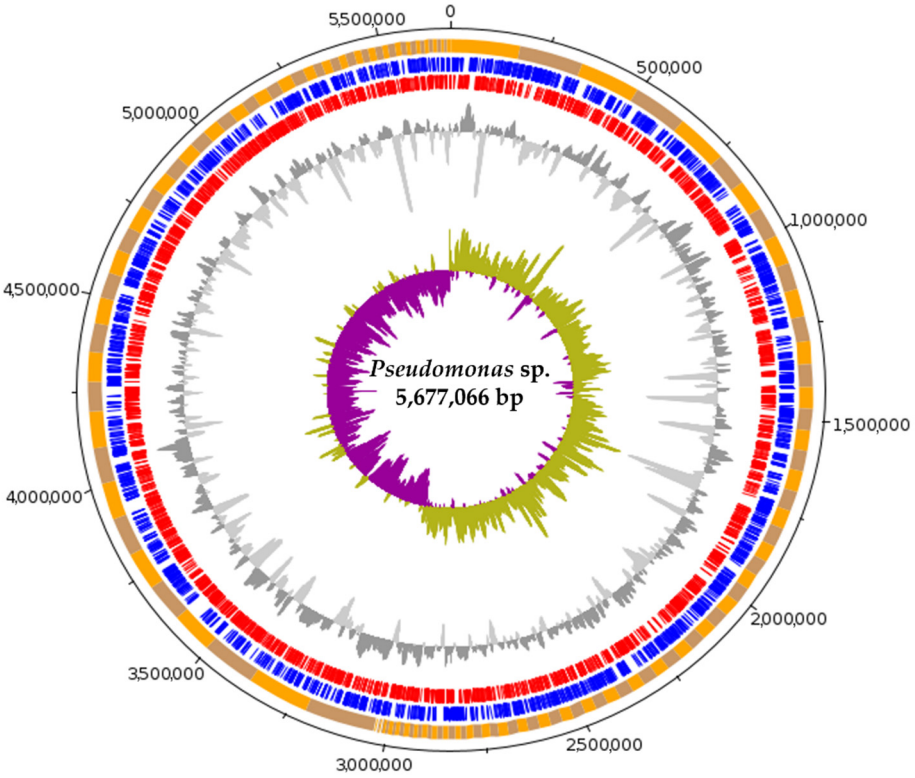

Figure 1. Whole-genome representation of *Pseudomonas* sp. strain C3 using DNAPlotter tool. Orange and gray represent scaffolds ordered by GC bias and length. Blue and red represent CDS in the forward and reverse directions, respectively. Gray represents GC content with respect to the mean. The innermost circle represents the GC bias with respect to the mean.

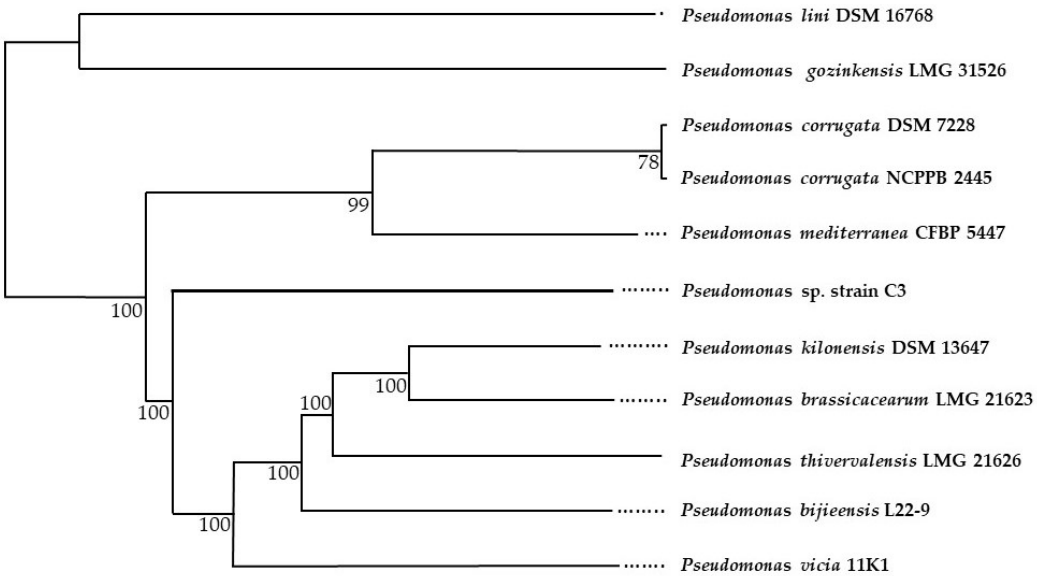

Figure 2. Phylogenetic analysis with taxonomic inference based on the genomic sequence of *Pseudomonas* sp. strain C3 for microbial species delimitation by dDDH (digital DNA-DNA hybridization). The dots in the figure represent evolutionary events for each bacterium.

3.2. Molecular Analysis of Metabolites and Metabolic Pathways

The genomic exploration of secondary metabolites in *Pseudomonas* sp. strain C3 predicted 13 regions, all hosting single candidate clusters (Table 2). Seven of them had no matches for the 'most similar known cluster' output, corresponding to the types NAGGN (N-acetylglutaminylglutamine amide) (region 15.1), butyrolactone (region 17.1), RiPP-like

(unspecified ribosomally synthesized and post-translationally modified peptide product) (region 31.1 and 46.1), NRPS-like (non-ribosomal peptide synthetase cluster) (region 59.1), lanthipeptide-class-ii (region 72.1), and siderophore (region 104.1) types. Five regions were partially matched with similarity scores ranging from 7% to 45% for the best hits: NRPS (non-ribosomal peptide synthetase cluster) (region 39.1) with crochelin A, betalactone (region 29.1) with fengycin, redox cofactor (region 30.1) with lankacidin C, NRPS-like (region 69.1) with fragin, and aryl polyene (region 12.1) with APE Vf (aryl polyene cluster), in that order. The T3PKS (type III polyketide synthase) was detected with 100% similarity to the BGC associated with 2,4-diacetylphloroglucinol production. After manual inspection, it was possible to confirm the presence of homologs for all the components of the *phl* gene cluster responsible for the biosynthesis, transport, and regulation of this metabolite (Figure 3).

Table 2. Secondary metabolites identified in *Pseudomonas* sp. strain C3.

Region	Type	From	To	Most Similar Know Cluster	Similarity
12.1	Arylpolyene	29,041	72,652	APE Vf	45%
15.1	NAGGN	31,762	46,460	–	–
17.1	Butyrolactone	54,893	68,324	–	–
29.1	Betalactone	7324	87,127	Fengycin	13%
30.1	Redox-cofactor	14,343	36,490	Lankacidin C	13%
31.1	RiPP-like	2417	14,033	–	–
39.1	NRPS	1	59,934	Crochelin A	7%
46.1	RiPP-like	1	6516	–	–
46.2	T3PKS	26,434	67,483	2,4-diacetylphloroglucinol	100%
59.1	NRPS-like	18,081	55,356	–	–
69.1	NRPS-like	1	27,536	Fragin	37%
72.1	Lanthipeptide-class-ii	25,424	48,519	–	–
104.1	Siderophore	5099	24,019	–	–

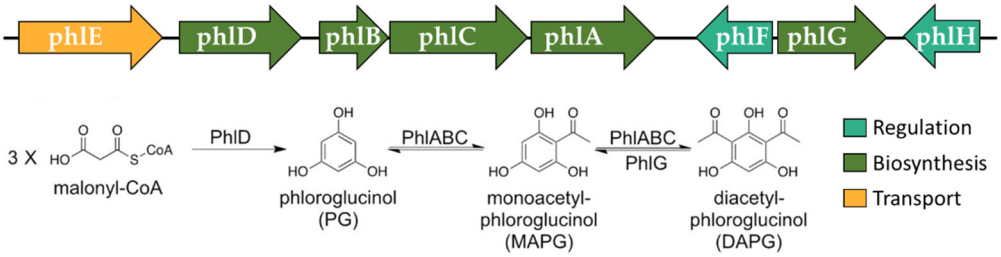

Figure 3. Genes involved in the 2,4-diacetylphloroglucinol pathway reported in the genome of *Pseudomonas* sp. strain C3.

A genetic diversity analysis based on the protein sequence similarity of the eight genes involved in the 2,4-diacetylphloroglucinol pathway showed almost no variation in the protein sequence, compared to corresponding orthologues in other *Pseudomonas* isolates (Table S2). The gene with the highest identity corresponded to PhlF, involved in the regulatory function of this metabolite, followed by the genes involved in biosynthesis (*phlB*, *phlC*, *phlD*, *phlG*, and *phlA*, in that order), and then the *phlE* and *phlH* genes, involved in transport and regulation, respectively.

In order to perform a more extensive screening of the genomic sequence of *Pseudomona* sp. strain C3 for possible PGP attributes, a search for specific metabolic pathways was performed. As detailed in the methodology section, PGP-related metabolic pathways were retrieved using the KEEG orthology and KofamKOALA databases (Table 3). This analysis determined the presence of genes involved in the phosphate and iron uptake pathways, indole acetic acid (IAA) production, the nitrate reduction pathway, the siderophore production pathway (enterochelin), and the ACC deaminase gene (Figure 4).

Table 3. *Pseudomonas* sp. strain C3 genes associated with plant growth-promoting and biocontrol traits discussed in this study.

	Metabolics Traits	Annotation Entry (KO)	Gene	Product Name
Plant growth promoting	Phosphate uptake	K02040	*pstS*	phosphate transport system substrate-binding protein
		K02038	*pstA*	phosphate transport system permease protein
		K02037	*pstC*	phosphate transport system permease protein
		K02036	*pstB*	phosphate transport system ATP-binding protein
		K01077	*phoA*	alkaline phosphatase
		K07636	*phoR*	phosphate regulon sensor histidine kinase PhoR
		K02039	*phoU*	phosphate transport system protein
		K07657	*phoB*	phosphate regulon response regulator PhoB
	Iron Uptake	K02015	*fhuB*	iron complex transport system permease protein
		K10829	*fhuC*	ferric hydroxamate transport system ATP-binding protein
		K02016	*fhuD*	iron complex transport system substrate-binding protein
		K04758	*feoA*	ferrous iron transport protein A
		K03711	*Fur*	Fur family transcriptional regulator, ferric uptake regulator
	IAA	K00274	*MAO*	monoamine oxidase
		K00138	*aldB*	aldehyde dehydrogenase
	Nitrate reduction	K02567	*napA*	nitrate reductase (cytochrome)
		K02568	*napB*	nitrate reductase (cytochrome), electron transfer subunit
		K00362	*nirB*	nitrite reductase (NADH) large subunit
		K00363	*nirD*	nitrite reductase (NADH) small subunit
	ACCd	K01505	*acdS*	1-aminocyclopropane-1-carboxylate deaminase
Biocontroler	Siderophore (Enterochelin)	K02362	*entD*	enterobactin synthetase component D
		K02363	*entE*	2,3-dihydroxybenzoate—[aryl-carrier protein] ligase
		K01252	*entB*	bifunctional isochorismate lyase/aryl carrier protein
		K02364	*entF*	L-serine—[L-seryl-carrier protein] ligase
	2,4-DAPG	K15431	*phlD*	phloroglucinol synthase
		K22840	*phlB*	2-acetylphloroglucinol acetyltransferase
		K22839	*phlC*	2-acetylphloroglucinol acetyltransferase
		K22838	*phlA*	2-acetylphloroglucinol acetyltransferase
		n.a.	*phlF*	TetR/AcrR family transcriptional regulator
		K23519	*phlG*	2,4-diacetylphloroglucinol hydrolase
		n.a.	*phlH*	TetR/AcrR family transcriptional regulator
	HCN	K10816	*hcnC*	hydrogen cyanide synthase
		K10815	*hcnB*	hydrogen cyanide synthase
		K10814	*hcnA*	hydrogen cyanide synthase
	Protease	K01438	*argE*	acetylornithine deacetylase
		K07678	*gacS*	sensor histidine kinase
		K07689	*gacA*	invasion response regulator

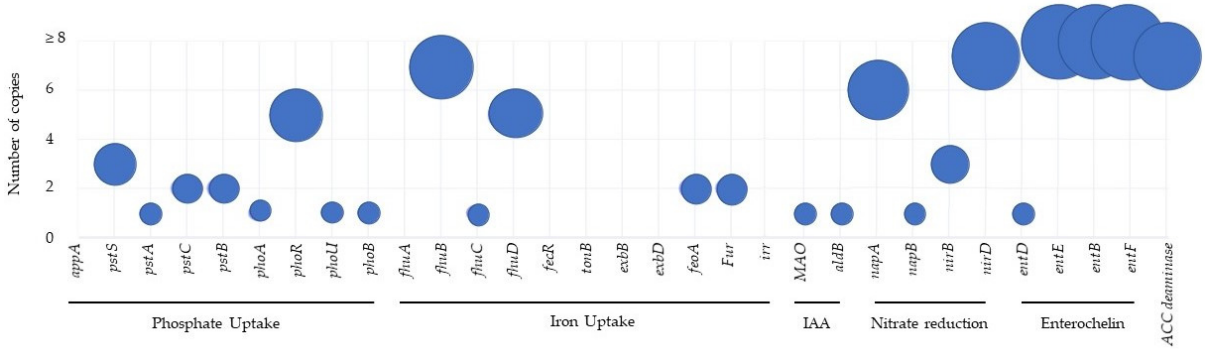

Figure 4. Different copies of genes involved in specific metabolic pathways associated with plant growth-promoting traits found in the genome of *Pseudomonas* sp. strain C3 through KEEG orthology and KofamKOALa databases.

Specifically, in the case of phosphate uptake, *appA* was the only gene that was not found in the genomic context of isolate C3, while the rest of the genes were present in a single copy (*phoA*, *pstA*, *phoU*, and *phoB*), two copies (*pstC* and *pstB*), three copies (*pstS*), and five copies (*phoR*). Genes associated with iron uptake were also examined, and was detected two copies of *fhuC*, three of *feoA* and *fur*, six of *fhuD*, and seven of *fhub*; however, six genes were not present (*fhuA*, *fecR*, *tonB*, *exbB*, *exbD*, and *irr*). Two genes involved

in an alternative metabolic pathway for IAA (tryptamine; TAM) were found, with only one copy of each (*MAO* and *aldB*). Regarding the nitrate reduction pathway, all the genes involved were present: *napB* in a single copy, *nirB* in three copies, and *napA* and *nirD* in six and seven copies, respectively. Four genes associated with enterochelin siderophore synthesis were detected, including one with one copy (*entD*) and three others with notably higher frequencies of 22, 11, and 23: *entE*, *entB*, and *entF*, respectively. Finally, the gene encoding ACC deaminase appeared to be present, with seven copies, in the genome of *Pseudomonas* sp. strain C3.

3.3. In Vitro Antifungal Activity of Pseudomonas sp. Strain C3

An in vitro assay involving six phytopathogenic fungi was performed to complement the molecular analysis that suggested a possible antifungal biocontrol activity attributed to the metabolite 2,4-diacetylphloroglucinol coded for in region 46.2. The exposure of *Pseudomonas* sp. strain C3 to these fungi evidenced its ability to control all of them, with different percentages of mycelial radial growth inhibition (%IMRG) (Figure 5). *Alternaria* sp. and *Geotrichum candidum* presented the highest inhibition rate (61.5%), followed, in descending order, by *Botrytis cinerea* (60%), *Monilinia fruticola* and *Fusarium oxysporum* (59.3%), and *Phytium* sp. (58.5%).

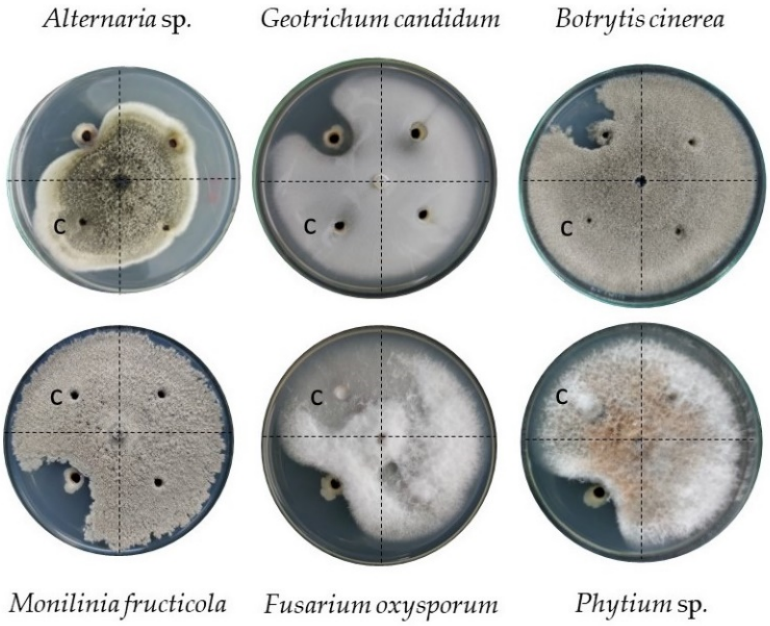

Figure 5. In vitro determination of biocontrol of *Pseudomonas* sp. strain C3 against six phytopathogenic fungi. The letter "C" in the Petri dish indicates the quadrant that corresponds to the control without bacteria.

Through in vitro assays (Figure 6), four positive PGP activities were confirmed in *Pseudomonas* sp. strain C3, such as siderophore production (using CAS agar medium), nitrogen fixation (NFM culture medium), phosphate solubilization (PVK culture medium), and IAA production (Salkowski test). Complementary to this, hydrolase activity was also detected, specifically for proteases through the SMA medium culture.

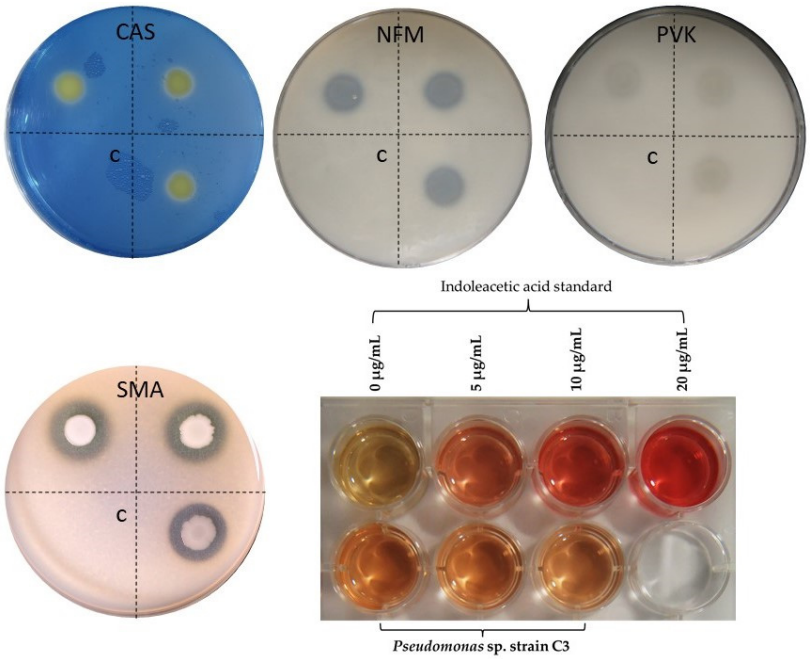

Figure 6. In vitro assays showing PGP traits and proteolytic activity of *Pseudomonas* sp. strain C3. Siderophore production (CAS). Nitrogen fixing (NFM). Phosphate solubilization (PVK). Protease activity (SMA) and IAA quantification assay (Salkowski test). The letter "C" on the Petri dishes indicates the negative control corresponding to the culture medium without bacteria.

4. Discussion

Considering the high level of completeness (99.38%) and a contamination percentage close to zero (0.1%), was reported here, a high-quality genome draft [59] of *Pseudomonas* sp. strain C3, which contains 5,677,066 bp, with a GC content of 61.37% (Figure 1). Similar results have been obtained by other researchers who sequenced *Pseudomonas wadenswilerensis* isolated from forest soils in 2014, achieving a high-quality draft of 5,966,942 bp with a GC content of 63.39% [60], while *Pseudomonas aeruginosa* isolated from a polluted industrial metalworking environment has also been drafted in high quality, yielding a genome 6,985,358 bp in length and with a GC content of 66.08% [61].

Some studies have indicated that secondary metabolism plays a significant ecological role in inter- and intra-specific communication among soil microorganisms, where its metabolites exhibit a broad range of biological activities (e.g., antibiotic and antifungal activities and siderophores) that could be relevant for plants, as they affect plant growth and defense responses [62]. The mechanisms of the action of biocontrol microorganisms include antibiosis, parasitism, or competition with the pathogen for nutrients and space. They may also induce disease resistance in the host plant, acting in different steps of the infection process [63]. Biocontrol activity is assisted by the production of different types of compounds, including siderophores, antibiotics, volatile organic compounds, and lytic enzymes [64].

In general, the results obtained using molecular tools (Figure 4, Tables 2 and 3) and culture-based techniques (Figures 5 and 6) were consistent. Using PVK culture medium was determined the phosphate-solubilizing activity of *Pseudomonas* sp. strain C3, and the genomic analyses showed that eight genes involved in phosphate uptake were present. These genes are involved in the solubilization of inorganic phosphate by modulating uptake and transport into the bacterial cell. The passive diffusion of the compound is facilitated by the hydrolysis of inositol-polyphosphate structures by *appA* [65]; however, in *Pseudomonas* sp. strain C3, no ortholog of this gene was detected. Nevertheless, diffusion can also occur by hydrolysis by the *phoA* gene, which was present along with the ABC phosphate-transporter-associated pstSCAB gene, which allows the selective internalization of inorganic phosphate [66]. An analysis performed by Blus-Kadosh et al. [67] in *Pseu-*

domonas aeruginosa explicitly based on the *pstS* gene responsible for phosphate uptake and the *phoB* gene responsible for phosphate-deficient regulation showed that both genes were essential in the phosphate-uptake pathway. Furthermore, relevant to this context, an assay with mutants of *Pseudomonas putida* revealed that the *pstSCAB* genes responsible for the transport system negatively regulated the *pho* regulon [68]. Therefore, phosphate uptake can be expected if the *pst* and *pho* cassettes are present.

Through the CAS agar culture medium assay, was observed siderophore production by *Pseudomonas* sp. strain C3, supported by the antiSMASH analysis' output. A siderophore-producing BGC was found in region 104.1 with no matches for the most similar known cluster against the MIBiG repository. In parallel, through genomic information using the KofamKOALA database for annotation analysis, it was determined that strain C3 contained the enterochelin synthesis gene cluster (entDEBF). Regarding the genes related to iron uptake, only five out of eleven genes were found. None of the genes classified by Clarke et al. [69] as essential for iron uptake, such as the transport-related *fhuA* gene or the *TonB–ExbD* system [70,71], were present. However, the absence of *fhuA* here does not limit iron uptake since the *fhuBCD* genes reported to be involved in the uptake of ferrichrome and ferrioxamine in other *Pseudomonas aeruginosa* strains were found to be present in the genome. An alternative way for bacteria to obtain iron from the environment is through the *feo* transporter [72]. Although there are not many studies based on this transporter, it has been proven that, if this gene is not present, iron absorption is reduced by 60%; thus, it has been described as essential for iron transport in bacteria [73]. The *Fur* gene is related to homeostasis control mechanisms in bacteria via iron storage and nutrient-dependent uptake [74]. A recent study of *fur*-deficient mutant lines demonstrated the effects of the *Fur* gene in the downregulation of more than a hundred genes, including the *TonB*-dependent and ABC-type transporters [75]. Both *feo* and *fur* were confirmed to be present in the genome of isolate C3.

Moreover, when IAA production was analyzed by means of the Salkowski test, a low presence of this hormone in the culture medium was observed. A manual genomic search revealed the presence of the *MAO* and *aldB* genes, both involved in this metabolite's synthesis. Multiple bacterial IAA synthesis pathways have recently been described by Duca et al., most of which use tryptophan as a precursor. An alternative pathway that uses tryptamine as a precursor is described in [76]. This tryptamine-involving pathway recruits an amine oxidase enzyme (*MAO*) to convert the primary substrate into indole acetaldehyde, and to subsequently obtain IAA through the effect of an aldehyde dehydrogenase (*aldB*). The function of *aldB* was characterized by mutagenesis assays in *Pseudomonas syringae;* the authors state that *aldB* is an alternative to *aldA* for IAA biosynthesis, and that *aldB* is directly related to a low production of IAA in the studied strain [77]. Additionally, previous work using in vitro assays demonstrated IAA production in two strains of the genus *Pseudomonas* together with plant-growth-promoting capabilities related to the germination percentage, shoot length, and root length, and increases in the vigor index in lentil *(Lens culinaris*) and barley (*Hordeum vulgare*) plants [78].

When in vitro atmospheric nitrogen fixation was evaluated using the PVK culture medium, *Pseudomonas* sp. strain C3 showed the expected halo around the inoculum indicative of a positive result. In addition to the manual gene search using the KofamKOALA tool, genes involved in the nitrate-reduction process were found. To this extent, Marzocchi [79] et al. and Huang et al. [80] recently highlighted the importance of the *napA* and *napB* genes in Gram-negative *Candidatus electronema* and of *nirB* and *nirD* in *Pseudomonas putida*, respectively, reported to be involved in the main reaction of nitrate reduction to ammonium and its assimilation/dissimilation rates, all of which were detected in the present study using the molecular tools detailed above. Moreover, previous work by Yan et al. [81] further elucidated the roles of *nirB* and *nirD* in nitrogen metabolism in unknown *Pseudomonas* sp. strain XS-18(). Finally, our genomic analysis for functional predictions indicated that isolate C3 encoded a specific BGC classified as T3PKS. Which exhibited a 100% similarity to the *phl* gene cluster (Table 2, Figure 3). Other studies that also included molecular

analysis using antiSMASH in their workflows have detected 2,4-diacetylphloroglucinol-producing gene clusters in different strains of the genus *Pseudomonas*, including *P. brassicacearum* [82], *P. protegens* [83], and *P. fluorescens* [84], and in bacteria belonging to other genera such as *Pseudogulbenkiania ferrooxidans* [85]. Interestingly, a recent molecular analysis of *P. putida* has shown that several strains could control phytopathogenic fungi without the 2,4-diacetylphloroglucinol gene cluster in their genome [86], suggesting that this biocontrol activity is linked to the production of the siderophore pyoverdine.

The diversity analysis of all the genes involved in the 2,4-diactetylphloroglucinol pathway detected by antiSMASH in isolate C3 (Table S2) revealed high identity and coverage with respect to ten other *Pseudomonas* strains that were obtained from agricultural soils, for example, potatoes [82,87], rice [88], soybeans [89,90], and wheat crops [91], as well as to two *Arabidopsis thaliana* strains obtained from rhizospheres [92] and groundwater [93] and two others from unreported origins. Interestingly, our strain was isolated from the western slopes of the Andes mountains, in the Atacama region, where a plant community can be found [29,31,32], suggesting that this potential metabolic capacity could be relevant in the interaction of *Pseudomonas* sp. strain C3 with plants.

Despite 2,4-diactetylphloroglucinol having been mainly associated with members of the *Pseudomonas* genus, a recent study reported the presence of six genes out of the eight participants in its biosynthetic pathway in three Betaproteobacteria species (*Pseudogulbenkiania ferrooxidans*, *Chromobacterium vaccini*, and *Chromobacterium piscinae)* [17]. In the three of them, the *phlG* (biosynthesis) and *phlH* (regulation) genes were absent. In *Pseudomonas* sp. strain C3, both genes had the lowest identity and coverage percentage with respect to other *Pseudomonas* strains. Overall, a low frequency of metabolites associated with antimicrobial molecules has been detected in *Pseudomonas*, including 2,4-diactetylphloroglucinol [24]. This could be related to the synthesis of 2,4-diacetylphloroglucinol, which can act as an elicitor of induced systemic resistance [94], an undesirable trait for a plant pathogen, which has been proposed as a counter-selection mechanism in pseudomonads [25].

The compound 2,4-diacetylphloroglucinol was initially a subject of interest to researchers due to its antibiotic properties until it was later described as a potent broad-spectrum antifungal, leading to an application that gave it practical relevance in the agricultural industry [95]. Here, was determined that *Pseudomonas* sp. strain C3 affected the growth in the culture plates of six phytopathogenic fungi—*Alternaria* sp., *Geotrichum candidum*, *Botrytis cinerea*, *Monilinia fruticola*, *Fusarium oxysporum*, and *Phytium* sp—which are of agronomic interest (Figure 5). Other studies have exposed some of these pathogens to 2,4-diacetylphloroglucinol, obtaining similar results, e.g., *Botrytis cinerea* and *Monilinia fructicola* [83], *Fusarium culmorum* and *Phytium* sp. [96], and *Alternaria* sp. and *Fusarium* sp. [97]. To the best of our knowledge, there are no previous reports of biological biocontrol for *Geotrichum candidum* using this metabolite. It should be noted that other pathogens have been controlled and were not included in this study, such as *Verticillium* sp. [98], *Ralstonia solanacearum* [99], *Magnaporthe oryzae* and *Rhizoctonia solani* [100], and *Pseudomonas syringae* [101]. However, it cannot rule out the biocontrol ability of C3 being linked with other C3 metabolic capacities, including hydrolase activity and nutrient competition [64]. In fact, proteolytic activity was detected in vitro for *Pseudomonas* sp. strain C3 using the MSA medium, a result that was supported by the search for genes related to this activity in our strain of interest. Thus, the *gacS* and *gacA* genes have been reported in *Pseudomonas fluorescens* as regulators of extracellular proteases [102] and suggested to be crucial for its biocontrol activity as revealed through assays of mutant bacteria with mutations in these genes [103,104].

Thus, *Pseudomonas* sp. strain C3 is an excellent candidate isolated from an extreme environment for bioprospecting about the urgent global interest in antimicrobial discovery and bacteria with PGP attributes. Future work should address the elucidation of how the metabolite 2,4-diacetylphloroglucinol participates in the biocontrol action of phytopathogenic fungi, including in vivo assays to determine if all the attributes reported here in *Pseudomonas* the sp. strain C3 generate a significant beneficial effect on plants.

5. Conclusions

To the best our knowledge, this is the first study on a plant-associated bacterium isolated from soils of the Atacama Desert characterized through molecular tools and culture-based techniques. The bacterium exhibited biocontrol capacities on six phytopathogenic fungi associated with agriculturally important crops, which could be attributed to the production of 2,4-diactetylphloroglucinol by *Pseudomonas* sp. strain C3. The genes involved in this metabolic pathway showed low gene diversity based on the protein sequences, with percentages close to 100% identity with respect to other *Pseudomonas* isolates. In addition, using genomic information, the presence of genes necessary for iron and phosphate uptake, nitrogen fixation, and indole acetic acid and siderophore production, which could contribute to soil biogeochemical processes and improve crop yields, was determined. Thus, *Pseudomonas* sp. strain C3 is an excellent candidate for the evaluation of its contribution as a biocontrol and plant growth-promoting agent in field assays.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d14050388/s1. Table S1: Pairwise comparison of genome sequences with 10 public genomes against *Pseudomonas* sp. strain C3; Table S2: Genetic diversity of 2,4-diacetylphloroglucinol in different *Pseudomonas* strains.

Author Contributions: A.G. conceived the study, designed the experiments, and wrote the first draft. J.E.M. performed the genome assembly. C.A.-G. analyzed the specific metabolites and participated in writing the draft. P.A.M.-T. evaluated the in vitro biocontrol assays. M.G. and G.F.S.-C. mainly funded the study. All the authors reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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