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Connecting the Lab and the Field: Genome Analysis of *Phyllobacterium* and *Rhizobium* Strains and Field Performance on Two Vegetable Crops

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Abstract: The legume nodules are a rich source not only of rhizobia but also of endophytic bacteria exhibiting plant growth-promoting mechanisms with potential as plant biostimulants. In this work we analyzed the genomes of *Phyllobacterium endophyticum* PEPV15 and *Rhizobium laguerreae* PEPV16 strains, both isolated from *Phaseolus vulgaris* nodules. In silico analysis showed that the genomes of these two strains contain genes related to *N*-acyl-homoserine lactone (AHL) and cellulose biosynthesis, involved in *quorum sensing* and biofilm formation, which are essential for plant colonization. Several genes involved in plant growth promotion such as those related to phosphate solubilization, indole acetic acid production, siderophore biosynthesis and nitrogen fixation were also located in both genomes. When strains PEPV15 and PEPV16 were inoculated in lettuce and carrot in field assays, we found that both significantly increased the yield of lettuce shoots and carrot roots by more than 20% and 10%, respectively. The results of this work confirmed that the genome mining of genes involved in plant colonization and growth promotion is a good strategy for predicting the potential of bacterial strains as crops inoculants, opening new horizons for the selection of bacterial strains with which to design new, effective bacteria-based plant biostimulants.

Keywords: *Phyllobacterium*; *Rhizobium*; carrot; lettuce; bacterial endophytes; plant growth promotion; PGPB; genomics; microbial biostimulants; field assays

1. Introduction

New perspectives in agriculture include the use of technologies able to reduce its impact on the environment through a maximization of the efficiency in the use of resources. Within these technologies, crop inoculation with plant growth-promoting bacteria (PGPB) is one of the most attractive for sustainable agriculture [1,2]. In the last decade, several studies on the diversity of PGPB [3] and their potential to colonize plant roots [4] and to exhibit in vitro plant growth promotion mechanisms have been published [5]. These bacteria can inhabit the plant rhizosphere or the inner tissues of plants [6], plant endophytes being the most efficient inoculants [7]. For this reason, in the past year, several research studies have evaluated ways of increasing plant growth by using bacterial endophytes isolated from non-legume plants [8,9] and from legume nodules [10–18]. These nodules contain the

rhizobia responsible for their formation and for symbiotic nitrogen fixation as well as other bacterial endophytes with different plant growth promotion mechanisms [19–22].

Plant growth promotion mechanisms are related to the presence of several genes in the bacterial genomes whose analysis allows us to understand the adaptation of bacteria to plants [23,24], but few studies analyzing the genomes of PGPB endophytes inhabiting legume nodules have been carried out to date [12,17,18]. genome analysis can help to improve the selection process of PGPB and to commonly focus on the search for genes involved in metabolic pathways related to plant growth promotion [25,26]. However, the presence of these genes in the genome of a strain is not enough to ensure its effective performance on plants, and thus in silico genome mining should be accompanied by the analysis of plant effects, with field trials being the only way to verify the real potential of strains as crop biostimulants. Although this was the approach in some recent research on inoculating endophytic bacteria on non-legumes [27,28] and rhizobia on common bean [29], there are no studies relating in silico analysis and field performance for endophytic bacteria inhabiting legume nodules.

Therefore, the aims of this study were: (i) to perform a comparative in silico analysis of the whole genomes of two strains isolated from common bean nodules, *Phyllobacterium endophyticum* PEPV15 and *Rhizobium laguerreae* PEPV16 [30,31], which have similar in vitro plant growth patterns and are able to colonize lettuce and carrot roots and to promote the growth of the edible parts of these plants in pre-field studies carried out in microcosm conditions [32–35], and (ii) to carry out preliminary assays in commercial fields in order to evaluate the potential of these two strains as biostimulants of lettuce and carrot plants, two vegetables widely consumed worldwide in which the effects of the inoculation of rhizobia or legume nodule endophytic bacteria have not been studied to date.

2. Materials and Methods

2.1. Genome Analysis

To obtain genomic DNA, the strains *P. endophyticum* PEPV15 and *R. laguerreae* PEPV16 were grown on TY plates (Triptone Yeast Agar) [36] during 24 h at 28 °C. genomic DNA was obtained using the ZR Fungal/Bacterial DNA MiniPrep (Zymo Research, Irvine, CA, USA) following the manufacturer's protocol. Sequencing, upon preparation of paired-end libraries, was performed on an Illumina MiSeq sequencing platform (2 × 250 bp). Sequencing data were assembled using Velvet 1.2.10 [37]. The draft genome sequences of strains PEPV15 and PEPV16 were deposited in DDBJ/EMBL/GenBank under the BioProjects PRJNA562136 (accession number VSZT00000000) and PRJNA224116 (accession number VSZV00000000), respectively. Annotation was done using RAST 2.0 (Rapid Annotation using Subsystem Technology) [38,39] and the NCBI Prokaryotic genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/ (accessed on 15 March 2021)) [40,41]. KofamKOALA tool was used to annotate the genomes based on the KEGG database [42]. The circular genome map of the studied strains was generated using the CGView server (http://cgview.ca/ (accessed on 15 March 2021)) [43,44].

2.2. Quorum Sensing Assays

For the evaluation of the production of *quorum sensing* signals, the strains PEPV15 and PEPV16 were grown in 20 mL of YMB medium [45] during 48 h. Then 2 mL was transferred to 18 mL of YMB medium and was incubated during 24 h at 28 °C. The cultures were centrifuged 4 min at 4500× *g*, and the supernatants were filtered with a 0.22 µm pore filter. The strain *Agrobacterium tumefaciens* NTL4 (pZRL4) was employed as reporter of AHL (acylated homoserine lactone) *quorum sensing* molecules. This strain was inoculated in 20 mL of liquid AB medium [46] supplemented with 30 µg/mL gentamycin and was incubated for 24 h at 28 °C. Then, 10 mL of this culture was mixed with AB medium with agarose (1% *w*/*v*) at 43 °C and 150 µL of X-gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside) stock solution for a final concentration 60 µg/mL. Aliquots of 200 µL of this suspension were dispensed in each well of a 96-well microtiter plate. Upon medium

solidification, 10 µL of a solution containing 10 ng/µL of OHL (*N*-3-octanoyl homoserine lactone, Sigma Aldrich Co., St. Luis, MO, USA), filtered supernatants, and their serial dilutions was added. OHL was chosen as AHL positive control. After 24 h of incubation the digital image was acquired by a HP Scanjet 8200 flatbed scanner.

2.3. Field Experiments

The field experiment was conducted in Spain for two vegetable crops in one environment each. The soil characteristics of the experimental field are shown in Table 1 and the climatic conditions during the experiment in Table 2.

Table 1. Edaphic conditions corresponding to the fields from this study.

Location		**Remondo**	**Ciudad Rodrigo**
Crop		**Carrot**	**Lettuce**
Latitude **Longitude**		**41°20′00.9″ N** **4°29′30.7″ W**	**40°35′02.6″ N** **6°31′56.1″ W**
Texture (%)	Sand	93.1	30.6
	Silt	2.3	50.3
	Clay	4.6	19.1
pH 1:2	(soil: water)	7.0	7.6
Electric conductivity	(dS/m)	0.15	0.25
Organic matter	(%)	0.11	5.44
Total nitrogen *	(%)	0.01	0.35
Ratio C/N		4.3	9.0
Lime	(%)	0.29	3.64
P-Olsen	(mg kg^{-1})	33.0	280.0
K	(cmol (+) kg^{-1})	0.25	1.36
Ca	(cmol (+) kg^{-1})	4.10	23.6
Mg	(cmol (+) kg^{-1})	1.09	5.11
Na	(cmol (+) kg^{-1})	0.69	0.13

* Total N: organic + nitric + ammonia nitrogen.

Table 2. Climatic conditions corresponding to the field experiments in the year 2013. The climatic data were recorded at the Segovia and Saelices el Chico (Salamanca) weather stations.

Location	**Month**	**Temperatures (°C) ***					**Monthly Rainfall (mm)**
		Hmax (°C)	**Havg (°C)**	**Tavg (°C)**	**Lavg (°C)**	**Lmin (°C)**	
Remondo (Segovia station) (Carrot crop)	May	23.0	16.0	10.3	5.1	0.4	69.8
	June	31.0	23.9	16.7	9.5	3.3	7.0
	July	34.6	31.3	23.6	15.9	9.8	9.2
	August	35.8	30.3	22.4	14.4	8.8	0.4
	September	31.9	25.5	18.9	12.2	5.8	27.4
	October	25.4	19.6	14.4	9.2	0.7	32.4
Ciudad Rodrigo (Saelices el Chico station) (Lettuce crop)	June	34.0	26.5	18.5	10.5	8.8	18.2
	July	38.5	33.3	23.9	14.5	8.7	0.6
	August	38.9	32.6	23.2	13.9	8.7	0.0
	September	34.2	28.0	20.1	12.1	7.5	119

* Hmax: maximum high temperature) (°C); Havg: average high temperature (°C); Tavg: average mean temperature (°C); Lavg: average low temperature (°C); Lmin: minimum low temperature (°C).

The carrot (*Daucus carota* L.) experiment was conducted in Remondo (Segovia, Spain) (41°20′0.9″ N 4°29′30.7″ W). The Segovia province is the largest carrot production region in Spain. The agronomic practices were those commonly used in the carrot integrated production system. In brief, the tillage was minimal and consisted of two crossed passes with harrow before sowing. Fertilization consisted of 51 kg N, 68 kg P_2O_5, and 170 kg K_2O in the form of 850 kg ha^{-1} of the complex fertilizer 6-8-20, applied before sowing. The cultivar was "Nandrín", and the seeds were mechanically sowed (16 May 2013) to a

final density of 510,000 plants ha^{-1} in rows at 30 cm spacing. When seedlings showed three leaves, each carrot plant belonging to the inoculated treatments received 10 mL of a bacterial suspension with 1×10^7 cfu mL^{-1}. The crop was sprinkler irrigated. The soil was mechanically kept free from weeds. In order to prevent fungal diseases and pests, two treatments with sulphur 80% (water-dispersible granules) were carried out during the growing season, at a dose of 1 kg per ha.

The lettuce (*Lactuca sativa* L.) field experiment was conducted in Ciudad Rodrigo (Salamanca, Spain) (40°35′2.6″ N 6°31′56.1″ W), a region with a long tradition in seasonal vegetable production. The agronomic practices corresponded to an organic system; in brief, the tillage consisted of two passes with harrow in autumn, plus the seedbed preparation with a power harrow in spring. Fertilization was organic, with a dose of 10 t ha^{-1} of composted beef manure provided before transplanting. The lettuce cv. was "Romana". Seedlings were produced in a seedbed in individual pots with professional substratum (Pindstrup Plus Orange). Seedlings were grown in a greenhouse until the plants developed three leaves, and were then transplanted to the field (2 June 2013) to a final density of 20,000 plants ha^{-1} in rows separated by 60 cm. Three days after transplanting, each lettuce plant belonging to the inoculated treatments received 10 mL of a bacterial suspension with 1×10^7 cfu mL^{-1}. Irrigation was by sprinklers. Weeds were mechanically removed, and 500 g of chitosan (Poly-D glucosamine) diluted in 300 L water was sprayed per ha 21 days after transplanting with the purpose of improving plant natural resistance against pathogenic bacteria and fungi. No other phytosanitary treatment was carried out.

For the two crops, the experimental design consisted of a randomized complete block with three blocks. The experimental unit consisted of five rows of 10 plants per row in carrot and five rows of 5 plants per row in lettuce. To avoid microorganisms spreading in the soil, treatments were separated by five rows in carrot and one in lettuce, and blocks were separated by 2 m. For sampling, the 6 central plants of the three central rows in carrot, and the 3 central plants of the three central rows in lettuce were collected. Thus, 18 plants for carrot and 9 plants for lettuce per experimental unit were collected, weighted while fresh, and dried at 70 °C until constant weight for dry weight measurement and elemental analysis. The analysis of N, P, K, Ca, Fe, and Mg was performed at the Ionomic Service of CEBAS-CSIC (Spain). Univariate analysis of variance with block as a random factor and treatment as a fixed one was performed. The normality of standardized residuals was checked with Kolmogorov–Smirnov's test and the homoscedasticity with Levene's test. Mean values were compared with the Tukey post hoc test. Statistical analysis was conducted with SPSS v.26.

3. Results

3.1. Genome features

The characteristic of the whole genomes of the strains PEPV15 and PEPV16 are recorded in Table 3. Circular representation of the genome is shown in the Figure 1A,B, and functions classified in different categories of metabolism using RAST annotation service are shown in the Figure 1C,D. A similar distribution in annotated subsystems was observed in both strains (Figure 1C,D) and a similar number of RNA genes harbored each genome (Table 3); however, amino acid and derivative associated genes were more abundant in the genome of the strain PEPV16 than in that of the strain PEPV15 (Figure 1C,D).

Table 3. Genome characteristics of 1, *Phyllobacterium endophyticum* PEPV15^T; 2, *Rhizobium laguerreae* PEPV16.

Genome Data	1	2
DDBJ/EMBL/GenBank accession number	VSZT00000000	VSZV00000000
Sequence size (bp)	5,505,652	7,182,427
Number of contigs	15	356
GC content (%)	58.30	57.20
Shortest contig size	322	252
Longest contig size	2,119,450	557,230
N50 value	332,658	437,109
L50 value	5	74
Number of coding sequences	5152	7108
Number of RNAs (tRNAs, rRNAs, others RNAs)	52 (45, 3, 4)	53 (44, 5, 4)

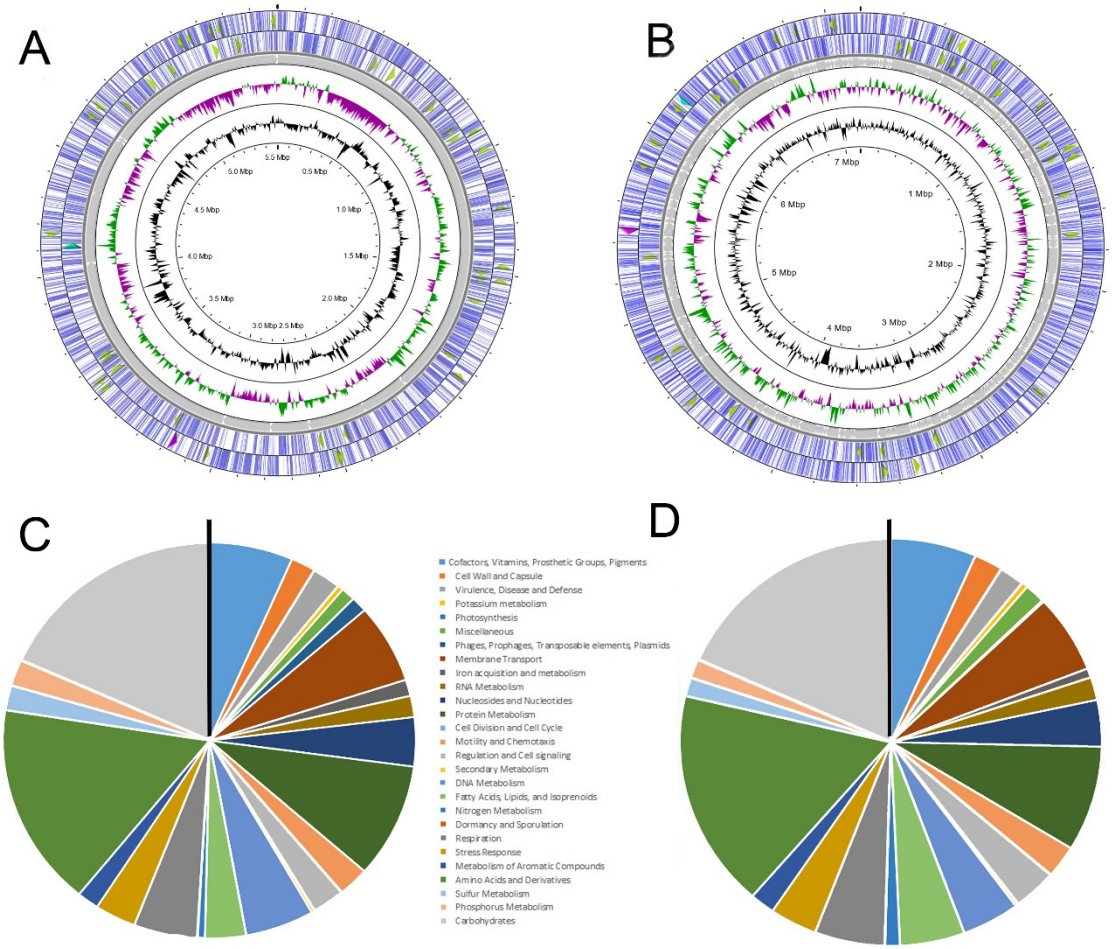

Figure 1. Genome circular representation and subsystems category distribution of annotated genes of the *Phyllobacterium endophyticum* PEPV15^T genome (**A**,**C**) and the *Rhizobium laguerreae* PEPV16 ^{T} (**B**,**D**). (**A**,**B**) From the outer to inner circles show coding sequences (CDSs; blue), rRNAs (violet), tmRNAs (turquoise), contigs (light grey), gC content (black), gC skew curves (+/−; green/purple). (**C**,**D**) Annotation of *Phyllobacterium endophyticum* PEPV15^T and *Rhizobium laguerreae* PEPV16 genomes via the RAST server, respectively. Subsystems are placed following the order of the legend, starting at the position of the black bar, from right to left.

3.2. Analysis of genes Involved in Quorum Sensing and Plant Colonization

The colonization of plant roots is essential for a beneficial plant–bacteria association with significant agronomic effects [29]. Together with bacterial cellulose, *quorum sensing* compounds are involved in biofilm formation and plant colonization [47,48]. The production of cellulose has already been shown for strains PEPV15 and PEPV16 [32,33], and as expected, their genomes contain the *bcsA* and *bcsB* genes that encode a UDP-forming cellulose synthase catalytic subunit and a cellulose biosynthesis cyclic di-GMP-binding regulatory protein, respectively, as occurs in other bacteria [47]. These two genes are enough to produce cellulose in vitro [47,49], but both genomes contain a third gene encoding an endonuclease, which has also been found in the genomes of other *Rhizobium* strains [50]. This enzyme (CelC2) has cellulase activity on CMC plates, is encoded by the *celC* gene in *Rhizobium* strains, and is involved in cellulose biosynthesis and biofilm formation [51].

Concerning to the *quorum sensing* compounds, in the case of rhizobia, they are related to the *N*-acyl-homoserine lactone groups (AHLs) [52,53]. In this study, we detected the production of AHL in both strains after dilutions 1:125 and 1:3126 for the strains PEPV15 and PEPV16, respectively. In agreement with these results, we found in the genome of the strain PEPV16 a gene encoding a *N*-acyl-L-homoserine lactone (AHL) synthase and in that of strain PEPV15 a gene encoding a GNAT family *N*-acetyltransferase involved in AHL biosynthesis [54].

Other genes related to plant colonization are those involved in motility, chemotaxis, and exopolysaccharide biosynthesis, which mediate biofilm formation and plant surface attachment [26]. The genes involved in plant colonization that are found in the genomes of strains PEPV15 and PEPV16 are recorded in Table S1.

3.3. Analysis of Genes Involved in Plant growth Promotion

Bacteria able to colonize plant roots can promote plant growth through several widely studied mechanisms, although some of them could remain unknown [5]. Several genes directly or indirectly related to plant growth promotion mechanisms can be mined in bacterial genomes [25] because good PGPB strains accumulate several of these genes [26]. The strains PEPV15 and PEPV16 showed similar patterns of in vitro plant growth promotion mechanisms that include phosphate solubilization, IAA production, and siderophore biosynthesis [32,33]; therefore, we searched the genes involved in these mechanisms in their genomes (Table S1).

An important skill in a PGPB is phosphate solubilization because phosphorous is, after nitrogen, the second limiting element in terrestrial ecosystems, and only a low percentage of this element present in soils is available for the plants [55]. Phosphorous can be released from soil organic compounds by several types of phosphatases and from inorganic ones through mineralization by organic acids originated during the bacterial growth, with the enzyme glucose dehydrogenase and the co-factor pyrroloquinoline quinone being involved in this process [56]. Both strains PEPV15 and PEPV16 harbored in their genomes different types of phosphatases that can carry out the solubilization of phosphate from organic compounds. In addition, the strain PEPV15 contains the *pqqB*, *pqqC*, *pqqD*, and *pqqE* genes, which are required for biosynthesis of pyrroloquinoline quinone and are markers for phosphate mineralization [25].

The production of the phytohormone indole acetic acid (IAA) is one of the most studied plant growth-promoting mechanisms in bacteria [57], with the indole-3-acetamide, the indole-3-pyruvic acid, and the indole-3-acetonitrile/indole-3-acetaldoxime being the most studied pathways involved in the production of IAA in bacteria. Bruto et al. [25] included in their research the indole-3-pyruvate decarboxylase/phenylpyruvate decarboxylase genes (*ipdC* and *ppdC*) involved in the IAA synthesis indole-3-pyruvate pathway, but these genes were not found in the genomes of strains PEPV15 and PEPV16. Other genes involved in IAA production via the indole-3-acetamide (IAM) pathway, such as *iaaM* and *iaaH* [58], and the enzyme acetaldoxime dehydratase involved in the conversion of IAN into IAA [59], were not found in the genomes of the strains PEPV15 and PEPV16. However, bacteria

can produce IAA through some alternative ways or by using other enzymes that have not been well studied [58]. It is interesting to note that in the PEPV15 genome, a nitrilase was found, and two subunits of a nitrile hydratase (*nthAB*) were found in both genomes. These enzymes participate in the last steps of the formation of IAA through the indole-3-acetonitrile/indole-3-acetaldoxime pathway from indole-3-acetonitrile (IAN) [59].

Siderophore production is considered within both direct and indirect plant growth promotion mechanisms [5]. The genome of strain PEPV15 contains an unnamed gene encoding a siderophore biosynthesis protein (accession in genProt PSH59744.1), while strain PEPV16 contains a gene encoding an acetyltransferase that is highly similar (higher than 92% similarity) to the *vbsA* gene that encodes a protein required for biosynthesis of vicibactin, a siderophore produced by *Rhizobium* [60].

These findings confirmed that in silico genome analyses are a good tool for detecting potential plant growth-promoting bacteria since the strains whose genome contains several genes involved in plant colonization and growth promotion mechanisms also display these mechanisms in vitro, and moreover, they showed good behavior in plant experiments carried out in microcosm conditions [26,32,33]. However, the incorporation of these strains in commercial inoculants requires conducting field experiments that have rarely been performed to date after the genome analysis of endophytic PGPB strains [27–29].

3.4. Field Trials

We performed field trials inoculating lettuces and carrots with strains PEPV15 and PEPV16 in commercial fields because these strains were able to colonize the roots of these two vegetables and to promote their plant growth in microcosm conditions [32,34,35]. However, only one environment per crop was analyzed, and the obtained results should be considered preliminary since this work was conducted in professional farmers' fields; nonetheless, it provides accurate information on the real effect of the MPB (microbial plant biofertilizer) in commercial situations.

The results obtained in the carrot field experiment showed that both fresh and dry root biomass significantly increased after inoculation with both strains of PEPV15 and PEPV16 with respect to the uninoculated plants (Table 4). The yield (t ha^{-1}) increased 22% with PEPV16 and 28% with PEPV15. No significant differences were found in the N and Fe concentration in the aerial biomass among treatments. Conversely, the P, Ca, and Mg concentration significantly increased as a result of inoculation, regardless of the strain. The concentration of Mg was significantly higher when strain PEPV16 was inoculated compared with the strain PEPV15 and with the uninoculated control (Table 5).

Table 4. Mean values and standard errors corresponding to the roots and shoots biomasses produced by carrot and lettuce crops, respectively, after the inoculation with the PEPV15 and PEPV16 strains and in the uninoculated controls in the field experiment. The values followed by the same letter did not significantly differ in the Tukey test. ANOVA was performed with blocks as a random factor and the treatment as a fixed factor (*p*-value: *** $p \leq 0.001$). Data refer to a single plant, and yield was calculated for an expected final density of 510,000 plants ha^{-1}.

Crop/Treatment	Biomass per Plant (g)				Expected Yield (t ha^{-1})
	Fresh		Dry		
Carrot (Roots)					
Uninoculated control	61.82 (±2.63)	b	6.40 (±0.25)	b	31.5
PEPV16	75.03 (±3.39)	a	7.57 (±0.32)	a	38.3
PEPV15	79.02 (±2.79)	a	8.01 (±0.24)	a	40.3
Mean square	4376.998		37.335		
F-value and significance	9.288	***	9.280	***	
Lettuce (shoots)					
Uninoculated control	1289 (±42)	b	65.4 (±2.1)	b	25.8
PEPV15	1432 (±44)	a	74.8 (±2.6)	a	28.6
PEPV16	1507 (±25)	a	82.8 (±2.3)	a	30.1
Mean square	331,459.370		2048.907		
F-value and significance	8.496	***	13.695	***	

Table 5. Mean values and standard errors corresponding to the mineral content in the roots and shoots produced by carrot and lettuce crops, respectively, after the inoculation with the strains PEPV15 and PEPV16 and in the uninoculated controls in the field experiment. The values followed by the same letter did not significantly differ in the Tukey test. ANOVA was performed with blocks as a random factor and the treatment as a fixed factor (p-value: *** $p \leq 0.001$; ** $0.001 < p \leq 0.01$; * $0.01 < p \leq 0.05$: ns, not significant).

	N (g/100g)		P (mg/kg)		K (mg/kg)		Mg (mg/kg)		Ca (mg/kg)		Fe (mg/kg)	
Carrot (roots)												
Uninoculated control	1.04 (±0.044)	a	2127 (±91)	b	24,547 (±923)	b	1058 (±46)	c	2742 (±154)	b	116 (±14)	a
PEPV15	1.10 (±0.008)	a	2529 (±27)	a	30,886 (±645)	a	1244 (±17)	b	3325 (±42)	a	78 (±5)	a
PEPV16	1.12 (±0.062)	a	2839 (±111)	a	27,909 (±966)	ab	1414 (±48)	a	3248 (±116)	a	116 (±11)	a
Mean Square	0.007		509,536.343		40,234,943.659		127,293.063		401,076.976		1967.711	
F value and significance	0.840	ns	17.957	***	13.705	**	19.920	***	7.761	*	4.128	ns
Lettuce (shoots)												
Uninoculated control	3.39 (±0.030)	a	3842 (±149)	a	50,659 (±2025)	a	2988 (±94)	ab	9604 (±397)	b	642 (±67)	a
PEPV15	3.43 (±0.059)	a	4238 (±168)	a	52,011 (±2210)	a	2877 (±49)	b	9949 (±123)	b	766 (±106)	a
PEPV16	3.62 (±0.083)	a	4243 (±113)	a	54,865 (±1597)	a	3385 (±140)	a	12,576 (±446)	a	1034 (±179)	a
Mean Square	0.058		212,160.780		18,436,322.965		285,011.620		10,572,050.235		160,557.838	
F value and significance	3.862	ns	2.515	ns	1.199	ns	6.883	**	21.334	***	2.517	ns

The results obtained in the lettuce field experiment showed a significant increase of fresh and dry aerial biomass inoculating with both strains PEPV15 and PEPV16, compared with the uninoculated controls (Table 4). The fresh yield (t ha^{-1}) increased by 11% and 16% in plants inoculated with strains PEPV15 and PEPV16, respectively, compared with the uninoculated controls (Table 4). No significant differences were found in the content of N, P, K, Mg, or Fe among treatments, whilst the contents of Ca were significantly higher in the plants inoculated with the strain PEPV16 compared with the rest of the treatments (Table 5).

To date, no field trials assays have been carried out in lettuce or carrots inoculating rhizobial strains or legume nodule endophytes, and only a few field trials assays have been performed in lettuce after inoculation with *Bacillus* strains, which showed increases ranging from 13% to 20% [61–63] and with *Pseudomonas* strains, which increased plant yield by 30% [64]. Therefore, this is the first field trial conducted with these two vegetables that demonstrated the biostimulant effect of strains of two genera belonging to the order Rhizobiales—namely, *Rhizobium* and *Phyllobacterium*, inhabiting legume nodules. Although the increases in the production were lower after the inoculation of these strains than in the case of *Pseudomonas* inoculation, rhizobial strains have particular advantages as biostimulants since they have been used for decades for legume inoculation without observing problems either for the environment or for plant, animal, or human health, which is essential for the inoculation of vegetables that can be consumed fresh, which occurs in the case of carrots and lettuce.

4. Conclusions

In the present work, a good agronomic performance upon the inoculation of *P. endophyticum* PEPV15 and *R. laguerreae* PEPV16 on lettuce and carrots was demonstrated, which can be explained by the presence of an assortment of genes related to root colonization ability and several PGP activities in their bacterial genomes. Thus, the genome mining of these genes qualifies as a sound strategy for predicting the potential of bacterial strains as crop inoculants and this work opens new horizons for the selection of bacterial strains with which to design new effective bacteria-based plant biostimulants.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11061124/s1, Table S1: genes involved in plant colonization and plant growth promotion found in the genomes of *Phyllobacterium endophyticum* PEPV15 and *Rhizobium laguerreae* PEPV16.

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Data Availability Statement: Genome data sequences are available in the genBank under the following accessions: PEPV15 (Bioproject: PRJNA562136; Assembly: ASM818021v1; Accession number: VSZT01000000) and PEPV16 (Bioproject: PRJNA562145; Assembly: ASM891945v1; Accession number: VSZV01000000).

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