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Whole Genome Analysis of *Streptomyces* spp. Strains Isolated from the Rhizosphere of *Vitis vinifera* L. Reveals Their Role in Nitrogen and Phosphorus Metabolism

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Abstract: The rhizospheric microorganisms of agricultural crops play a crucial role in plant growth and nutrient cycling. In this study, we isolated two *Streptomyces* strains, *Streptomyces* sp. LM32 and *Streptomyces* sp. LM65, from the rhizosphere of *Vitis vinifera* L. We then conducted genomic analysis by assembling, annotating, and inferring phylogenomic information from the whole genome sequences. *Streptomyces* sp. strain LM32 had a genome size of 8.1 Mb and a GC content of 72.14%, while *Streptomyces* sp. strain LM65 had a genome size of 7.3 Mb and a GC content of 71%. Through ANI results, as well as phylogenomic, pan-, and core-genome analysis, we found that strain LM32 was closely related to the species *S. coelicoflavus*, while strain LM65 was closely related to the species *S. achromogenes* subsp. *achromogenes*. We annotated the functional categories of genes encoded in both strains, which revealed genes involved in nitrogen and phosphorus metabolism. This suggests that these strains have the potential to enhance nutrient availability in the soil, promoting agricultural sustainability. Additionally, we identified gene clusters associated with nitrate and nitrite ammonification, nitrosative stress, allantoin utilization, ammonia assimilation, denitrifying reductase gene clusters, high-affinity phosphate transporter and control of PHO regulon, polyphosphate, and phosphate metabolism. These findings highlight the ecological roles of these strains in sustainable agriculture, particularly in grapevine and other agricultural crop systems.

Keywords: *Streptomyces*; nitrogen metabolism; phosphorus metabolism; grapevine; sustainable agriculture; phylogenomic analysis; rhizospheric bacteria; core-genome

Citation: Montes-Montes, G.; González-Escobedo, R.; Muñoz-Castellanos, L.N.; Avila-Quezada, G.D.; Ramírez-Sánchez, O.; Borrego-Loya, A.; Ortiz-Aguirre, I.; Muñoz-Ramírez, Z.Y. Whole Genome Analysis of *Streptomyces* spp. Strains Isolated from the Rhizosphere of *Vitis vinifera* L. Reveals Their Role in Nitrogen and Phosphorus Metabolism. *Nitrogen* **2024**, *5*, 301–314. https://doi.org/10.3390/nitrogen5020020

Academic Editor: Tida Ge

Received: 18 February 2024
Revised: 24 March 2024
Accepted: 15 April 2024
Published: 16 April 2024

1. Introduction

The rhizosphere, which is the layer of soil surrounding plant roots, serves as an ecological niche where microorganisms interact with the host [1]. These interactions include bacteria that play a vital role in nitrogen and phosphorus metabolism, which are essential for soil health, productivity, and plant growth. These bacteria are involved in various processes such as denitrification, nitrate, and nitrite ammonification, as well as activities related to phosphate uptake, regulation, and utilization. They have the capability to efficiently utilize and recycle compounds, thereby enhancing plant development, nutrient uptake, and soil quality [2].

The grapevine (*Vitis vinifera* L.) is a perennial woody plant that plays a vital role in the global economy and society. Vineyards span approximately 7.5 million hectares worldwide and yield around 35.9 million tonnes of wine. In Mexico, viticulture and wine production have become a significant economic activity, resulting in an annual wine production of 36 million liters and involving the cultivation of approximately 73,000 tons of grapes [3].

Several studies have been conducted on the microbial communities associated with grapevines, specifically focusing on the microbial communities found in the endosphere, phyllosphere, and rhizosphere [4]. The rhizosphere is of particular importance due to the crucial role microorganisms play in soil biogeochemical processes, such as nitrogen and phosphorus metabolism [5,6]. The presence and activity of bacteria in the rhizosphere are essential for sustainable agriculture as they help reduce reliance on synthetic nitrogen and phosphorus fertilizers, thereby mitigating the negative environmental impacts associated with their production and usage, including water pollution and greenhouse gas emissions [7]. The *Streptomyces* bacterial genus plays a vital role in microbial dynamics and plant health. It promotes plant growth by participating in phosphorus and nitrogen metabolism [8]. Furthermore, it contributes to plant health by producing antimicrobial compounds [9,10] and other bioactive compounds involved in biological control [11–13]. Microbial nitrogen and phosphorus metabolism are crucial processes in the dynamics of biogeochemical cycles. Certain microorganisms possess the unique ability to convert nitrogen and phosphorus into chemically assimilable forms for living organisms. This process not only enhances plant growth but also influences soil fertility, thereby impacting the productivity and sustainability of terrestrial ecosystems [14,15].

In this context, genomic analyses applied to the study of biological nitrogen and phosphorus metabolism by *Streptomyces* spp. have provided a comprehensive understanding of the molecular mechanisms involved. These analyses have opened up new possibilities for enhancing agricultural sustainability and managing soil fertility [16–18]. These advanced tools enable a more precise comprehension of plant–microorganism interactions and offer opportunities to optimize the utilization of this crucial biological function for the benefit of agriculture and ecosystem health. Therefore, the objective of this study was to analyze the whole genome of two strains of *Streptomyces* spp. that were isolated from the rhizosphere of grapevines. To achieve this objective, we performed whole genome sequencing, assembly, annotation, phylogenomic analysis, as well as pan- and core-genome analysis to identify closely related species of *Streptomyces*. Additionally, gene functional annotation analysis was conducted to infer the functional capabilities of these *Streptomyces* strains in nitrogen and phosphorus metabolism.

2. Materials and Methods

2.1. Isolation of the Bacterial Streptomyces spp. Strains

The bacterial strains used in this study were obtained from a previous study, where a total of 122 bacteria were isolated from the rhizospheric soil of *Vitis vinifera* in Sacramento, Chihuahua, Mexico (28°50′04.9″ N, 106°15′25.1″ W). They were selected based on their demonstrated biological activities implicated in the production of agriculturally significant compounds. Soil samples were collected at a depth of 15–20 cm in the vineyard rhizosphere, and serial dilutions were streaked on Ashby mannitol agar. After 5–7 days of incubation at 30 ± 2 °C, individual bacterial colonies were further purified. The isolates were cryopreserved in 15% glycerol and stored at −80 °C.

2.2. Genomic DNA Extraction, Library Preparation, and Sequencing

Total DNA was extracted from each sample using a ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research, Irvine, CA, USA) following the manufacturer's instructions. The DNA quality and quantity were determined by using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) based on its A260/280 ratio, and observed in a 1.0% agarose gel electrophoresis. For genomic library preparation and sequencing, the total DNA was shipped to Illumina (San Diego, CA, USA). Briefly, 100 ng of total

DNA was processed following instructions of Illumina DNA prep (M) tagmentation kit (#Cat. 20018705). Because many samples were run in the same flow cell, specific indexes were added to each DNA library with IDT for Illumina DNA/RNA UD Indexes Set A Tagmentation (#Cat. 20027213). Library concentration was quantified with Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, Waltham, MA, USA) and the integrity of DNA libraries was assessed with the Bioanalyzer 2100 Agilent (NGS 1-6000 kit). Libraries were sequenced in an S4 flow cell in a 2 × 150 bp strategy on an Illumina NovaSeq 6000 sequencer (Illumina Inc., San Diego, CA, USA) [19].

2.3. Genome De Novo Assembly and Annotation

Illumina raw sequence reads of *Streptomyces* sp. strain LM32 and *Streptomyces* sp. strain LM65 were processed by trimming and filtering using a 4 bp Q20 sliding window in Trimmomatic v.0.39 [20]. To assess the quality of the obtained reads, FastQC v.0.11.9 [21] was employed and a de novo assembly was performed using SPAdes v.3.14.1 [22]. The assembled genomes were then evaluated using QUAST v.5.1.0rc1 [23]. To predict gene clusters related to nitrogen and phosphorus metabolism, the resulting complete genomes were annotated using Prokka v.1.14.5 [24] and RASTtk v.1.3.0 [25]. Finally, the gene function annotations were visualized as arrow diagrams using the ggplot2 library [26] and the gggenes library [27] in RStudio [28].

2.4. Genomes Selection and Phylogenomic Analysis of Streptomyces spp.

In order to conduct a comprehensive analysis of *Streptomyces* genomes, 1–4 genomes were downloaded for each species from the NCBI database (Table S1), resulting in a total of 307 genomes. Additionally, the genome of *Escherichia coli* str. K-12 substr. MG1655 (NC_000913.3) was downloaded as a reference outgroup for the phylogenetic tree. All statistical data (n = 308) were obtained using QUAST v.5.1.0rc1 [23]. Subsequently, a phylogenomic analysis was performed on 310 genome sequences, including the *Streptomyces* strains from this study, using the Virtual Analysis Method for Phylogenomic Fingerprint Estimation (VAMPhyRE v.2020; https://biomedbiotec.encb.ipn.mx/VAMPhyRE accessed on 5 January 2024), following the procedure described by Muñoz-Ramírez et al. [29]. Briefly, VAMPhyRE was used to determine Virtual Genome Fingerprints (VGF) from the bacterial genomes in our dataset, including both complete and draft forms. Virtual hybridization was conducted by identifying hybridization sites using a collection of 15,264 VAMPhyRE probes, each 13 nucleotides long (VPS-13), targeting both the positive (+) and negative (−) strands of the genomes, allowing for a single mismatch. Each genome's VGF is constituted by the assemblage of hybridization sites. Genomic distances were calculated by comparing all pairs of VGF and determining the number of shared homologous sites, resulting in a distance matrix using the methodology described by Nei and Li [30]. To ensure that only homologous sites shared within the distance metrics were considered, an extending-match technique was employed, where seven bases at both ends of the sites were extended, and homologous sites were determined by a minimum criterion of 27 base matches. The phylogenomic tree was constructed using the Neighbor-Joining method with the software MEGA 11 [31], and further refined and annotated using iTOL v.3 [32].

2.5. Average Nucleotide Identity Analysis

To gain a deeper understanding of the similarities between whole genomes and to determine whether two genomes share genomic identities above or below the species threshold, an Average Nucleotide Identity (ANI) analysis was performed. This analysis involved comparing *Streptomyces* sp. strain LM32, *Streptomyces* sp. strain LM65, and other reference strains of *Streptomyces* available in the NCBI database. Correlation indexes of tetra-nucleotide signatures (Tetra), ANIm, and FastANI values were calculated using the JspeciesWS web service [33] and FastANI [34]. The representation of visual reciprocal mappings between two pairs of *Streptomyces* genomes was plotted with a Python script (visualize.py) [35]. The ANI value, based on whole genome sequences, has been widely

accepted as a reliable method for determining whether organisms belong to the same species, with a typical threshold of ≥95% ANI [34].

2.6. Pan- and Core-Genome Analysis

An analysis of the pan- and core-genome was conducted for the genomes within the same cluster of strains LM32 and LM65. In the case of *Streptomyces* sp. strain LM32, the genomes of *S. coelicoflavus* strains DBR11, NBC_00465, NBRC 15399, and S3018 were included in the analysis. Conversely, for *Streptomyces* sp. strain LM65, the analysis was performed on the genome of *S. achromogenes* subsp. *achromogenes* NRRL B-2120 and included the genomes of *S. achromogenes* strains W4I19-2 and B2I10. Initially, annotation of the genomes was conducted using Prokka v.1.14.16 [24] using an e-value of 1×10^{-12}. The resulting GFF files from this annotation were then employed for the pan-genome calculation, carried out using the Panaroo pipeline v.1.4.2 [36] in 'strict' mode, with a 90% identity threshold for protein sequences and a 75% coverage cut-off for gene length. Subsequently, the presence and absence files generated were used to calculate a Venn diagram utilizing the ggVennDiagram library [37] in RStudio [28].

3. Results and Discussion

3.1. Genome De Novo Assembly

A total of 21,874,730 and 41,761,631 paired-end reads were obtained for *Streptomyces* sp. strain LM32 and *Streptomyces* sp. strain LM65, respectively, after processing the high-quality reads. The coverage for strain LM32 was approximately 70×, while for strain LM65 it was approximately 148×. The genome size and GC content for strain LM32 were 8.1 Mb and 72.14%, respectively, while for strain LM65 they were 7.3 Mb and 71%. The quality of the assemblies was assessed using the QUAST software v.5.1.0rc1, resulting in an N50 value of 150,500 and L50 of 18 for strain LM32, and an N50 value of 164,678 and L50 of 16 for strain LM65. *Streptomycetes* have unique genomic characteristics, including a lengthy linear chromosome ranging from 6 to 12 Mb, and encoding 5300 to 11,000 proteins, which distinguishes them from other bacterial genera [38,39]. In terms of annotation, a total of 7251 coding sequences (CDS), 83 tRNA, and 8 rRNA were identified in strain LM32, whereas in strain LM65, a total of 6440 CDS, 99 tRNA, and 4 rRNA were identified. The genome characteristics of both LM32 and LM65 strains are summarized in Table 1.

Table 1. Genome characteristics of *Streptomyces* sp. strain LM32 and *Streptomyces* sp. strain LM65.

Genome Characteristics	LM32	LM65
Genome size (Mb)	8.1	7.3
Contigs > 500 bp	112	92
G + C content (%)	72.14	71
N50	150,500	164,678
L50	18	16
CDS	7251	6440
rRNA genes	5,1,2 (5S,16S,23S)	2,1,1 (5S,16S,23S)
tRNA genes	83	99

3.2. Phylogenomic Analysis of Streptomyces Genomes

The whole genome sequences (Table S1) were analyzed using VAMPHyRe software v.2020 to identify specific genomic fingerprints of *Streptomyces* species. The phylogenomic analyses placed strain LM32 within the species *S. coelicoflavus*, whose genomes were recovered from soil samples. Similarly, strain LM65 was classified within the species *S. achromogenes* subsp. *achromogenes* (Figure 1), whose genome was also recovered from a soil sample [40]. It has been demonstrated that analyzing whole genome sequences instead of MLST/16S rRNA can result in better clustering and taxonomic assignment of bacterial strains. This is primarily due to the complexity associated with the vast amount of biological information analyzed with the VGF [29].

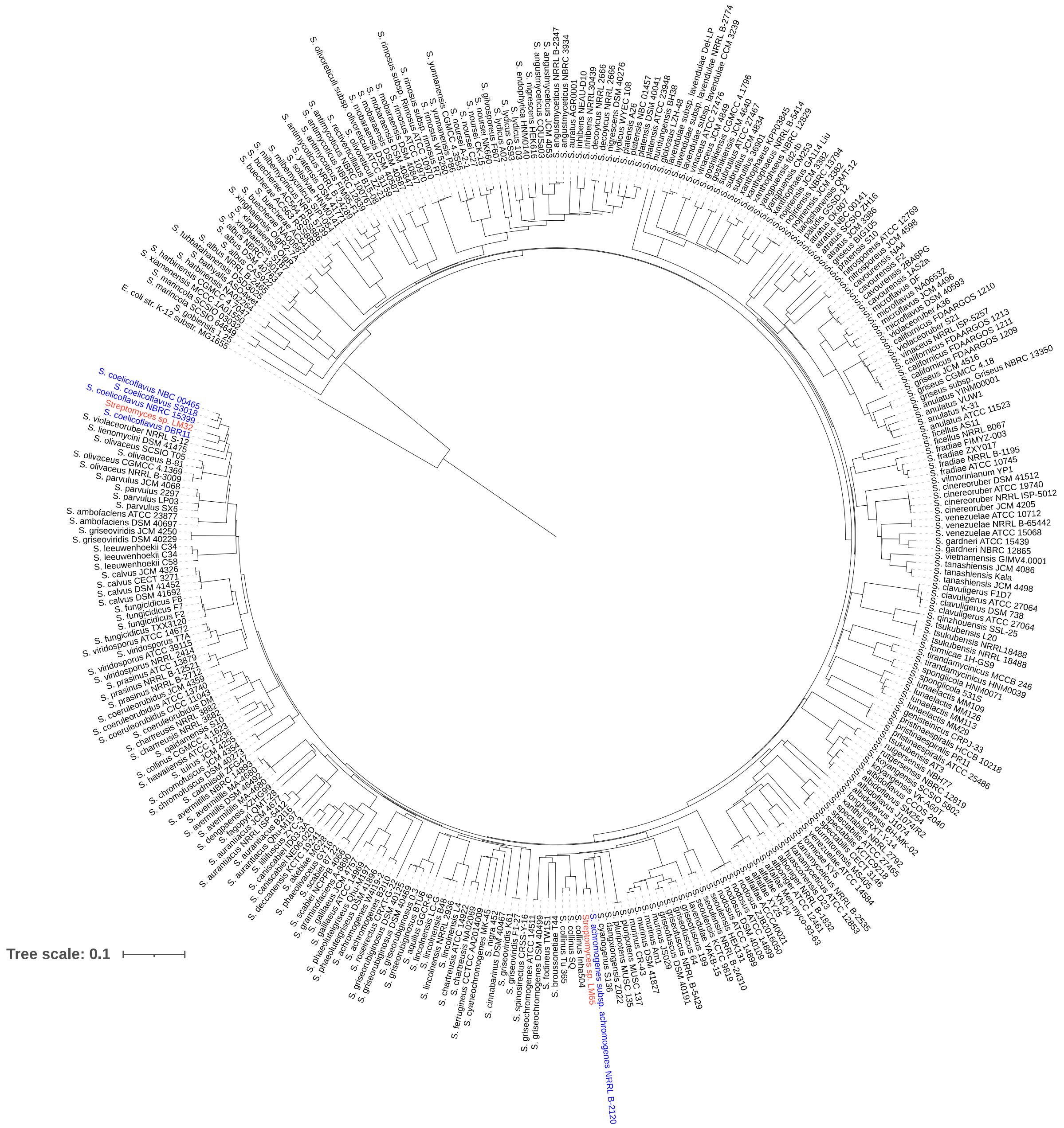

Figure 1. Phylogenomic analyses of 309 *Streptomyces* strains using whole-genome sequences analyzed with VAMPhyRE. The genome of the *E. coli* str. K-12 substr. MG1655 (NC_000913.3) was used to root the tree. The labels in blue indicate genomes that cluster within the same clade as the *Streptomyces* strains from this study. The labels in red identify the genomes of *Streptomyces* sp. strain LM32 and *Streptomyces* sp. strain LM65.

The species identification was reinforced using both phylogenomic analysis and ANI results (Figure 2). *Streptomyces* sp. strain LM32 exhibited a FastANI value of 95.61%, an ANIm value of 95.62%, and a Tetra correlation of 0.99966 with *S. coelicoflavus* strain DBR11, which was isolated from soil samples in Assam, India. Based on the cutoff threshold of >95%, the ANI values and phylogenomic analysis indicated a close relationship between strain LM32 and the species *S. coelicoflavus*. Similarly, *Streptomyces* sp. strain LM65 strain showed cutoff values >95% with FastANI (98.87%), ANIm (98.93%), and Tetra correlation (0.99983) with *S. achromogenes* subsp. *achromogenes* strain NRRL B-2120, which was isolated from soil samples in Tokyo, Japan [40]. This result suggests a close relationship between strain LM65 and the compared species [33,34]. It is noteworthy that in the case of *S. achro-*

mogenes, within the results of the phylogenomic analysis, two of the deposited genomes (strains B2I10 and W4I19-2) clustered into a distinct clade from that of *S. achromogenes* subsp. *achromogenes* and *Streptomyces* sp. strain LM65. Upon proceeding with the ANI analyses, the results for these two strains exhibited ANI values significantly lower than 95% (Table S2). Upon further investigation into these two strains, their taxonomy check status on the NCBI page appears inconclusive, indicating a potential misassignment.

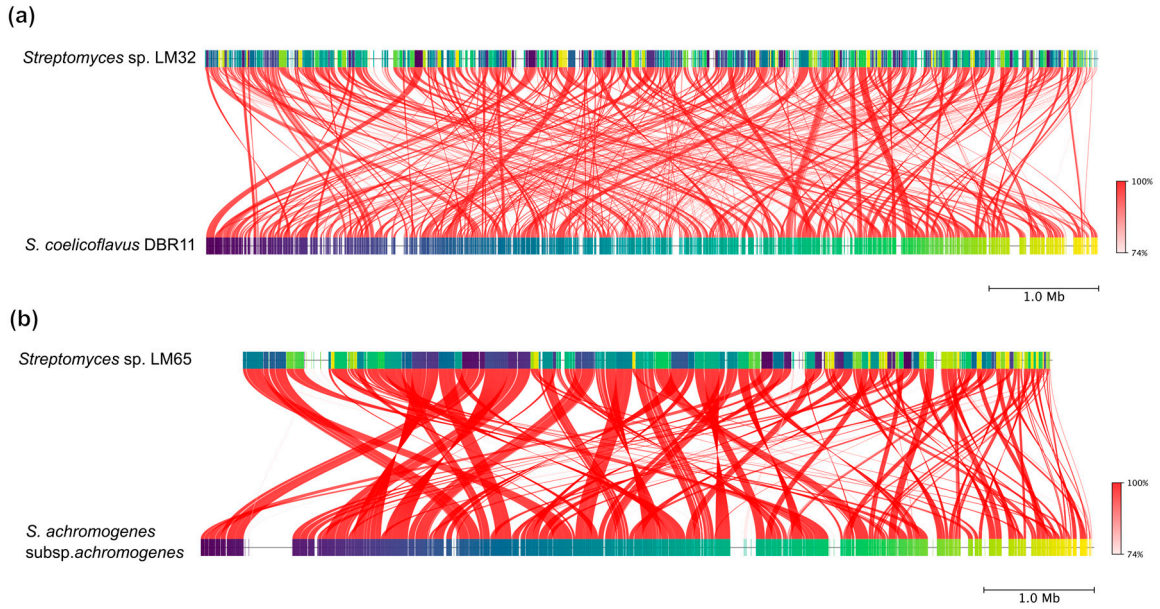

Figure 2. Detailed visualization of reciprocal mappings between two pairs of *Streptomyces* genomes calculated with FastANI. (**a**) Comparison between *Streptomyces* sp. strain LM32 and *Streptomyces coelicoflavus* DBR11. (**b**) Comparison between *Streptomyces* sp. strain LM65 and *Streptomyces achromogenes* subsp. *achromogenes*. The red lines denote conserved genomic regions with the color intensity indicating a high value of ANI.

3.3. Pan- and Core-Genome

The pan- and core-genome analysis presented in the Venn diagram (Figure 3) reveals patterns of conservation and genetic diversity within the examined *Streptomyces* species. The core-genome, represented by the central intersections of the diagram, underscores a set of essential genes shared among the strains, reflecting their evolutionary heritage and fundamental biological functions, and the pan-genome is the set of all genes that are present in the analyzed dataset [41]. Instead, the strain-specific genes, located on the outer portions of the diagram, suggest genomic adaptability possibly linked to survival and specialization in various ecological niches [42]. Notably, the genome of *Streptomyces* sp. strain LM32 shares most of its genes with the other included genomes, exhibiting a core-genome of 53% (5,308 genes), which implies that *Streptomyces* sp. strain LM32 maintains a close evolutionary connection with its congeners.

On the other hand, the genome of *Streptomyces* sp. strain LM65 shares 25% of its genetic content with *S. achromogenes* subsp. *achromogenes* NRRL B-2120, indicative of a potential closer phylogenetic relationship. Conversely, the comparison of *Streptomyces* sp. strain LM65 with *S. achromogenes* W4I19-2 and *S. achromogenes* B2I10 shows less than 1% genetic overlap with each, suggesting a significant genetic divergence. Moreover, the comparison between *S. achromogenes* W4I19-2 and *S. achromogenes* B2I10 demonstrates a 36% shared genetic content, denoting a close relationship as corroborated by the phylogenetic tree (Figure 1), distinctly separate from the clade comprising *Streptomyces* sp. strain LM65 and *S. achromogenes* subsp. *achromogenes* NRRL B-2120. In addition, the pan and core genome analysis enabled the identification of genes detected as unique to the strains in this study, with some implicated in the metabolism of nitrogen. In the case of *Streptomyces* sp.

strain LM32, a nitrite reductase [NAD(P)H] was detected as unique, while in *Streptomyces* sp. strain LM65, genes encoding the respiratory nitrate reductase alpha, beta, and delta chains were identified. These genes and their products are of significance in addressing extreme nutrient and energy limitations through the efficient utilization of nitrate as an energy source. This unique genetic advantage provides them with a competitive edge, particularly in environments rich in nitrate, maintaining their metabolic activity as a key survival strategy [43].

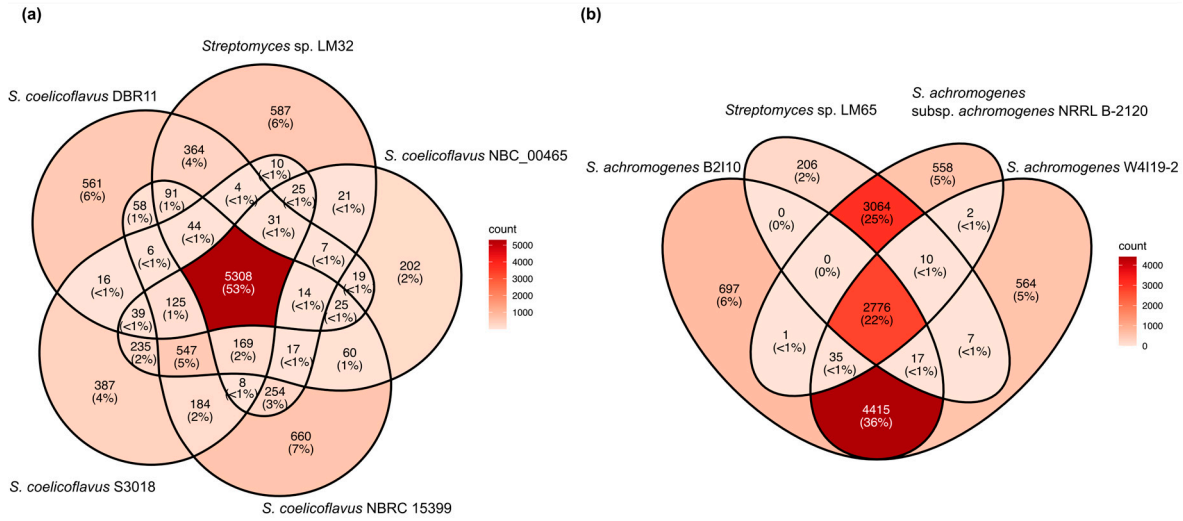

Figure 3. Comparative pan- and core-genome analysis of *Streptomyces* species. (**a**) Comparison between *Streptomyces* sp. strain LM32 and *Streptomyces coelicoflavus*. (**b**) Comparison between *Streptomyces* sp. strain LM65 and *Streptomyces achromogenes*. Each ellipse shows in sum the total number of genes of one strain. Intersections indicate predicted shared content. The color gradient represents the density of shared genes across the genomes.

In general, there are no studies describing the involvement of *S. coelicoflavus* and *S. achromogenes* in nitrogen and phosphorus metabolism. Existing studies have instead focused on investigating their microbial capabilities in biocontrol through the production of secondary metabolites. For example, *S. coelicoflavus* has been utilized as a biological control agent [44], demonstrating its ability to inhibit quorum sensing [45] and produce extracellular enzymes such as peroxidase, laccase [46], and cellulases [47]. On the other hand, biosynthetic genes have been identified in the genome of *S. achromogenes* [48], some of which have been reported as potential sources of bioactive metabolites with antioxidant and anticancer activities [49]. Additionally, *S. achromogenes* has been shown to exhibit antifungal action against pathogens like *Alternaria alternata*, *Mucor fragilis*, and *Fusarium brachygibbosum*. Furthermore, it has been highlighted for its growth-stimulating activities when interacting with tomato plants [50].

3.4. Gene Functional Annotation

The functional categories of genes encoded in both *Streptomyces* strains LM32 and LM65 were annotated using the RAST server (Figure 4). This server predicted functional subsystems that included genes involved in various cellular activities, such as nitrogen and phosphorus metabolism. The nitrogen and phosphorus metabolism subsystem encompasses a range of biochemical pathways and enzymes that are responsible for the uptake, assimilation, and utilization of nitrogen and phosphorus compounds by microorganisms. Some of these pathways are involved in processes like nitrate and nitrite ammonification, nitrosative stress, allantoin utilization, ammonia assimilation, denitrifying reductase gene clusters, high-affinity phosphate transporter, and control of PHO regulon, polyphosphate, and phosphate metabolism. Among the *Streptomyces* genomes reported in the GenBank, the

majority have been isolated from terrestrial environments, such as soil and land plants [39]. This preference for inhabiting terrestrial settings is due to *Streptomyces*' significant role in nitrogen and phosphorus metabolism, which is facilitated by the metabolic pathways described above [51,52].

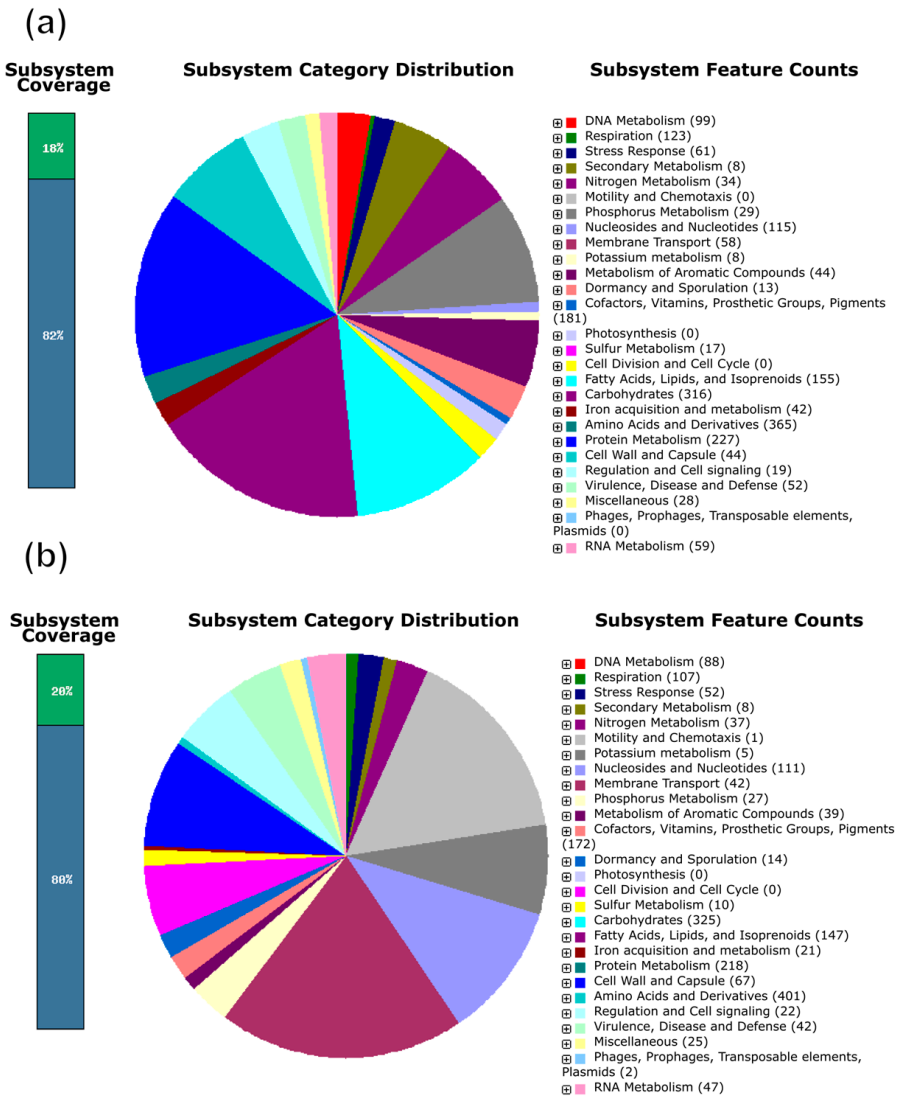

Figure 4. Pie charts showing an overview of RAST subsystems assigned to genes in (**a**) *Streptomyces* sp. strain LM32 and (**b**) *Streptomyces* sp. strain LM65.

In order to identify the genes responsible for nitrogen and phosphorus metabolism, we conducted analyses using RAST software (Figure 5 and Table 2). The results showed that *Streptomyces* sp. strain LM32 and *Streptomyces* sp. strain LM65 encode gene clusters involved in denitrification. These gene clusters include the nitrate reductase complex, which consists of *narG*, *narI*, *narX*, and *narH*. In *S. coelicolor*, it has been demonstrated that this complex catalyzes the reduction of nitrate to nitrite, coupling this process to energy conservation under anoxic conditions, allowing the bacteria to survive and remain metabolically active [43,53]. Additionally, the activation of genes associated with pathways related to nitrate reduction has been observed in various bacterial groups. Among these genes, *narH* encodes a peptide with multiple sites for the binding of cofactors, *narG* is involved in the reduction in dinitrogen and ammonia, and *nasD* is involved in the reduction in nitrate in the cytoplasm [54].

Table 2. Predicted genes related to nitrogen and phosphorus metabolism activities in *Streptomyces* spp.

Nitrogen Metabolism	Gene Name	Gene Annotation	Reference
Denitrifying reductase gene clusters	*narG*	Respiratory nitrate reductase alpha chain	[55]
	narI	Respiratory nitrate reductase gamma chain	
	narX	Respiratory nitrate reductase delta chain	
	narH	Respiratory nitrate reductase beta chain	
Nitrosative stress	*NsrR*	Nitrite-sensitive transcriptional repressor	[56]
Ammonia assimilation	*gln-1*	Glutamine synthetase type II	[55,57,58]
	gltD	Glutamate synthase [NADPH] small chain	
	glnB	Nitrogen regulatory protein P-II	
	gltB	Glutamate synthase [NADPH] large chain	
	glnD	[Protein-PII] uridylyltransferase	
	amtB	Ammonium transporter	
	glnE	Glutamate-ammonia-ligase adenylyltransferase	
	glnA	Glutamine synthetase type I	
Nitrate and nitrite ammonification	*narG*	Respiratory nitrate reductase alpha chain	[43,54]
	narX	Respiratory nitrate reductase delta chain	
	narH	Respiratory nitrate reductase beta chain	
	nasD	Nitrite reductase [NAD(P)H] large subunit	
	nasE	Nitrite reductase [NAD(P)H] small subunit	
	narI	Respiratory nitrate reductase gamma chain	
Allantoin utilization	*alc*	Allantoicase	[59]
	gcl	Glyoxylate carboligase	
	glxK	Glycerate kinase	
	allB	Allantoinase	
	garR	2-hydroxy-3-oxopropionate reductase	
Phosphorus metabolism	**Gene name**	**Gene annotation**	
High-affinity phosphate transporter and control of PHO regulon	*phoU*	Phosphate transport system regulatory protein	[60]
	phoR	Phosphate regulon sensor protein	
	phoB	Phosphate regulon transcriptional regulatory protein	
	ppk1	Polyphosphate kinase	
Polyphosphate	*ppgk*	Polyphosphate glucokinase	[61]
	ppx	Exopolyphosphatase	
	ppk1	Polyphosphate kinase	
Phosphate metabolism	*phoH*	Phosphate starvation-inducible protein PhoH, predicted ATPase	[61–63]
	pitB	Probable low-affinity inorganic phosphate transporter	
	PhoU	Phosphate transport system regulatory protein	
	HWU94	Phosphate transport regulator (distant homolog of PhoU)	
	phoL	Predicted ATPase related to phosphate starvation-inducible protein	
	ppx	Exopolyphosphatase	
	ppk1	Polyphosphate kinase	
	hppA	Pyrophosphate-energized proton pump	
	phoB	Phosphate regulon transcriptional regulatory protein	
	pntB	NAD(P) transhydrogenase subunit beta	
	PhoR	Phosphate regulon sensor protein	
	ppa	Inorganic pyrophosphatase	

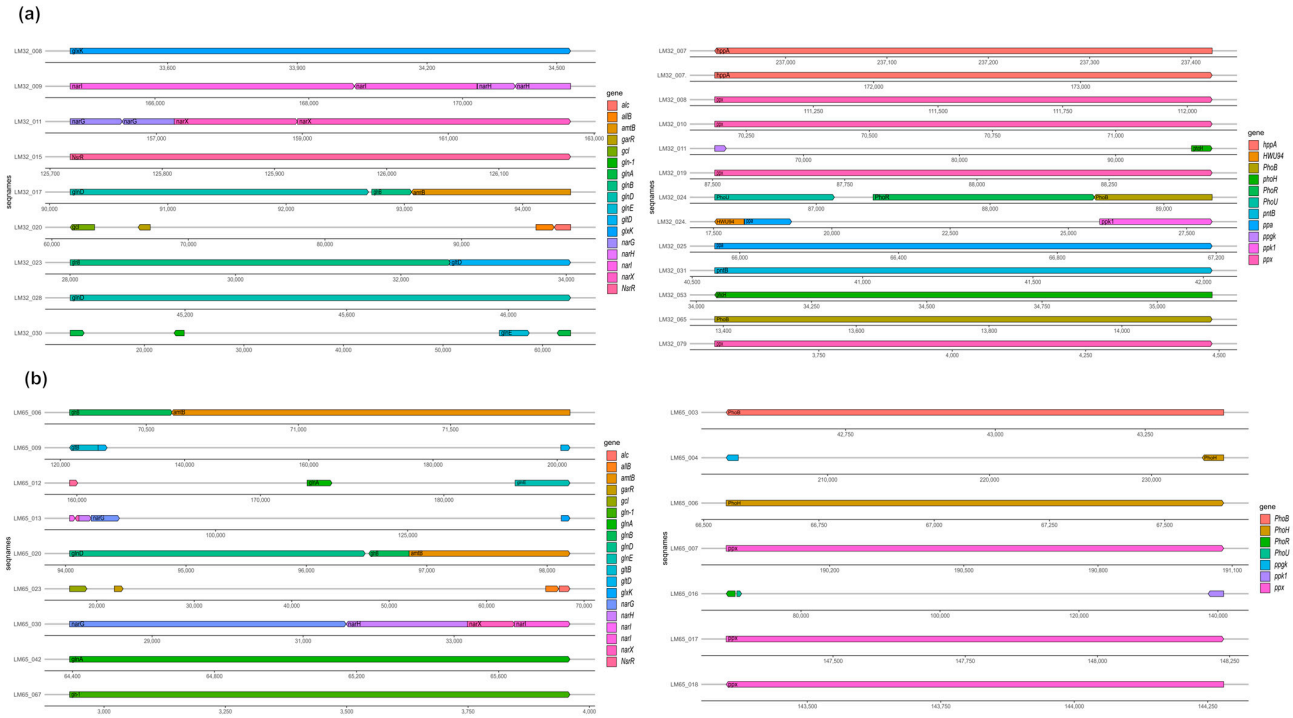

Figure 5. Predicted gene clusters of nitrogen and phosphorus metabolism in (**a**) *Streptomyces* sp. strain LM32 and (**b**) *Streptomyces* sp. strain LM65.

Regarding the assimilation of ammonia, it plays a crucial role in the overall nitrogen metabolism of organisms. The process is regulated by the glutamine synthetase complex, specifically through the involvement of *glnA* and *glnD*, which control nitrogen metabolism transcriptionally in response to nitrogen concentration [55,57,58,64]. On the other hand, the utilization of allantoin is of significant importance in various organisms as it serves as a nitrogen and carbon source. Allantoin, derived from purine metabolism, is a valuable nutrient for bacteria like *S. coelicolor*. In this organism, the assimilation of purines follows a pathway regulated by genes such as *alc* and *gcl*, which are distributed across different regions. This suggests that both genes are involved in the regulation of purine catabolism and the production of antibiotics. Furthermore, it is possible that the enzymes generated by this pathway are also involved in the specific regulation of antioxidants and the disruption of oxidative homeostasis [59]. Additionally, in response to stress caused by reactive nitrogen species compounds, *NsrR* plays a regulatory role in reducing the concentration of nitric oxide. This mechanism is attributed to the loss of an iron–sulfur center in the structure [56,65].

In relation to phosphorus metabolism, genes that are known to have crucial roles in the uptake, regulation, and efficient utilization of phosphate through its metabolism have been identified. These genes include coding genes for both high- and low-affinity phosphate transporters, transcriptional regulators of the PHO regulon, enzymes involved in polyphosphate synthesis and degradation, as well as proteins involved in energy generation from inorganic pyrophosphate. Specifically, genes such as *PhoR* and *PhoB* in *S. coelicolor* have been found to be necessary for the full induction of the pho regulon, which is important for phosphate regulation. Additionally, these genes indirectly participate in nitrogen and carbon metabolism through interactions with their main regulators [61]. Other important genes that have been identified include those that encode for polyphosphate kinase, exopolyphosphatase, and inorganic pyrophosphatase. These genes have been reported in genera of the Actinomycetota phylum, such as *S. albulus* [62], *Corynebacterium glutamicum* [63], and *Streptomyces alfalfae* [66]. These genes are involved in assembly processes by catalyzing the synthesis of polyphosphate from inorganic phosphate or the reverse process, and they also play a role in recycling processes through the hydrolysis of polyphosphates

and inorganic pyrophosphate. This leads to the release of inorganic phosphate, contributing to the overall functioning of phosphate cycling and nutrient dynamics in the soil.

4. Conclusions

In summary, this study has developed de novo complete genome assemblies for two strains of *Streptomyces*. This has allowed for a deeper understanding of the genomic composition, genetic similarity, and the genes related to nitrogen and phosphorus metabolism. Through phylogenomic analysis, ANI results, as well as pan- and core-genome analysis, it was determined that the LM32 strain was closely related to the species *S. coelicoflavus*, while the LM65 strain was closely related to the species *S. achromogenes* subsp. *achromogenes*. The functional annotation of genes in both strains revealed their involvement in nitrogen and phosphorus metabolism. Specifically, genes related to nitrate reduction, ammonia assimilation, and allantoin utilization were identified. Additionally, genes associated with phosphate metabolism, such as phosphate transporters and enzymes involved in polyphosphate synthesis and degradation, were also discovered. These findings are crucial for understanding the ecological roles of these strains in the rhizospheric soil of *Vitis vinifera* L.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nitrogen5020020/s1, Table S1: Details and statistics of the 308 genome sequences downloaded. Table S2: ANI results.

Author Contributions: Conceptualization, R.G.-E. and Z.Y.M.-R.; methodology, G.M.-M., G.D.A.-Q., O.R.-S., I.O.-A., A.B.-L., L.N.M.-C. and Z.Y.M.-R.; validation, R.G.-E. and Z.Y.M.-R.; formal analysis, G.M.-M., R.G.-E. and Z.Y.M.-R.; investigation, R.G.-E. and Z.Y.M.-R.; resources, G.D.A.-Q. and L.N.M.-C.; data curation, G.M.-M. and Z.Y.M.-R.; writing—original draft preparation, R.G.-E. and Z.Y.M.-R.; writing—review and editing, G.M.-M., R.G.-E., G.D.A.-Q., O.R.-S., A.B.-L., I.O.-A., L.N.M.-C. and Z.Y.M.-R.; visualization, G.M.-M. and Z.Y.M.-R.; supervision, Z.Y.M.-R. and R.G.-E.; project administration, G.D.A.-Q. and L.N.M.-C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The datasets generated and/or analyzed during the current study are available in the NCBI Bioproject database (PRJNA1075334) under the accession number SAMN39912921 (*Streptomyces* sp. strain LM32) and SAMN39912940 (*Streptomyces* sp. strain LM65).

Acknowledgments: We thank the National Council of Humanities, Sciences, and Technologies (CONAHCYT-Mexico) for the financial support provided to G.M.-M. throughout their master's fellowship and to Z.Y.M.-R. throughout their postdoctoral fellowship. We thank the three anonymous reviewers for their valuable comments on the manuscript, which have helped enhance its clarity and quality.

Conflicts of Interest: The authors declare no conflicts of interest. Author O.R.-S. has been involved as a consultant and expert in bioinformatics.

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