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Draft genome sequence of *Agrobacterium pusense* strain CMT1: A promising growth-promoting bacterium isolated from nodules of soybean (*Glycine max* L. Merrill) crops for the One Health approach in Paraguay

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ABSTRACT

Strain CMT1 was isolated from nodules of non-inoculated Roundup Ready (RR) soybean plants (*Glycine max* L. Merrill), which were collected in fields in Itauguá, Paraguay. The genome of this strain had 338,984,909 bp; 59.2 % G + C content; 377648 bp N50; 5 L50; 55 contigs; 51 RNAs and 5,272 predicted coding DNA sequences (CDS) distributed in 327 subsystems. Based on overall genome-relatedness indices (OGRIs), this strain was taxonomically affiliated with *Agrobacterium pusense*. Based on genome mining, strain CMT1 is a promising plant growth-promoting bacterium that could be validated in agricultural fields for increasing soybean yield and quality, diminishing the economic, environmental, and health costs of non-sustainable food production.

Soybean (*Glycine* max) is an annual herbaceous plant belonging to the Fabaceae family (Lim, 2012). It is a crop in high demand due to its oil and protein content, which caused an expansion of the cultivation area in South America, representing an important pillar of the Paraguayan economy (Morínigo et al., 2018). For example, in the 2021/2022 agricultural season, Paraguay produced 4,380,736 tons and was worldwide ranked as the sixth soybean-producing country and the third exporting country according to the Paraguayan Chamber of Exporters and Marketers of Cereals and Oilseeds (CAPECO, 2023). The importance of the soybean sector lies in the different uses of this oilseed as it is part of oil processing, grain milling and bakery, human food, beverage, and other food processing as well as fodder for cattle feed. Currently, soybean cultivation represents approximately 50 % of the world's total oilseed production (Morínigo et al., 2018).

A relevant characteristic of the soybean plant is the formation of specialized structures that are nitrogen-fixing root nodules that create a suitable microenvironment in symbiosis with soil microorganisms (Graham et al., 2004). Nodules are very important in plants as they have the function of reducing gaseous nitrogen to an ammonium compound that can be exploited by the plant to meet its nutritional requirements for growth and development (Méndez et al., 2014). This metabolism diminishes the high application of synthetic nitrogen fertilizer and enhances the One-Health approach in soybean production because inorganic fertilizers contribute to soil, water, and air pollution, which damages the environment, animals, vegetation, and human health (Ajmal et al., 2018).

Thus, rhizobial and non-rhizobial bacteria have been found present in the root nodules of leguminous plants, although the ecological functions of the latter are not yet understood (Etesami, 2022). Among the most named genera of non-rhizobial bacteria present in nodules are *Bacillus, Acinetobacter, Enterobacter, Agrobacterium, Mycobacterium, Pseudomonas,* and other enterobacterial species (Deng et al., 2011). In this sense, the bacterial genus *Agrobacterium* constitutes a diverse group of gram-negative bacteria found in soil and associated with plants (Farrand et al., 2003; Matthysse, 2006). The presence of *Agrobacterium* strains in bean and soybean nodules has been reported which could contribute to plant growth (Delamuta et al., 2020). Understanding the genomic bases of bacterial species allows us to know the capabilities they possess (Betancor et al., 2008). In this sense, it is possible to analyze complete genome sequences and mining to identify promising beneficial

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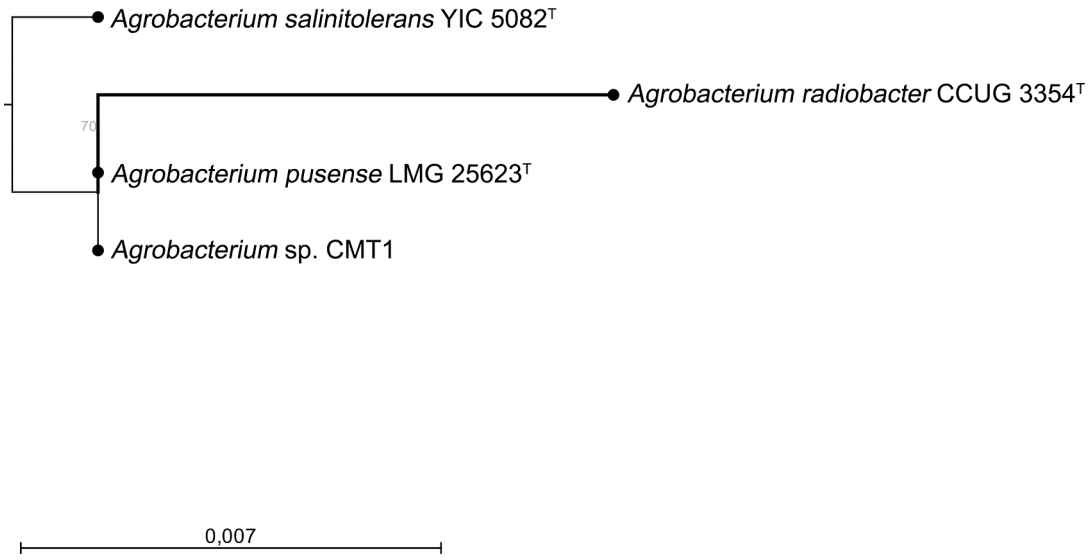

Fig. 1. Phylogenetic relation between *Agrobacterium* sp. CMT1 and closely related species, based on the 16S rRNA gene.

bacteria to the agricultural sector (Bistue, 2018; Gan and Savka, 2018), which is needed to migrate to sustainable agriculture based on a One-Heatlh approach.

In this context, during the year 2022 in the agricultural season, active nodules from non-inoculated Roundup Ready (RR) soybean plants were collected from soybean plants (Somasegaran et al., 1994 and Tokgöz et al., 2020) in the experimental field of the Faculty of Agricultural Sciences of the Universidad Columbia del Paraguay (UCP), located in Itauguá, Central Department of Paraguay (25°21′40.8 "S 57°22′23.8 "W). These plants were harvested at the R5 phenological stage and transported refrigerated to the Biotechnology Laboratory of the Multidisciplinary Center for Technological Research (CEMIT). Then, Yeast Mannitol Agar (YMA) culture medium (medium 79) with the following composition: 10 % K_2HPO_4 1 ml/L, 10 % $Mg_2SO_4{\cdot}7H_2O$ 2 ml/L, 10 % NaCl 1 ml/L, mannitol 10 g/L, yeast extract 0.4 g/L, agar 15 g/L, distilled water, Congo red dye 5 ml/L, and a pH of 6.8, was used as a nutrient medium for bacterial isolation. For this, the collected nodules were disinfected with 70 % alcohol for a few minutes, then squeezed into 50 ml Falcon tubes with 20 mL of physiological solution consisting of sterile sodium chloride solution, and serial dilutions were performed until a concentration of 10^{-5}. These dilutions were inoculated on Petri dishes containing YMA, and incubated at 28 °C for 4 to 5 days. Subsequently, successive purifications were performed until axenic cultures were obtained (López et al. 2017). Here, one of the most abundant colonies (CMT1) showed the following morphological traits: bacillary morphology, rod-shaped, aerobic, non-spore formation, and Gram-negative bacterium (Lacroix & Citovsky, 2022).

This strain was cryopreserved at -80 °C by using Nutrient Broth (NB) and 30 % glycerol, in the Colección de Microorganismos Edáficos y Endófitos Nativos (COLMENA, www.itson.edu.mx/COLMENA) (de los Santos-Villalobos et al., 2018; 2021).

Strain CMT1 was studied to explore its genomic background. Thus, high-quality extraction of genomic DNA from strain CMT1 was carried out, grown at 30 °C for 24 h in 30 mL of nutrient broth, shaking at 121

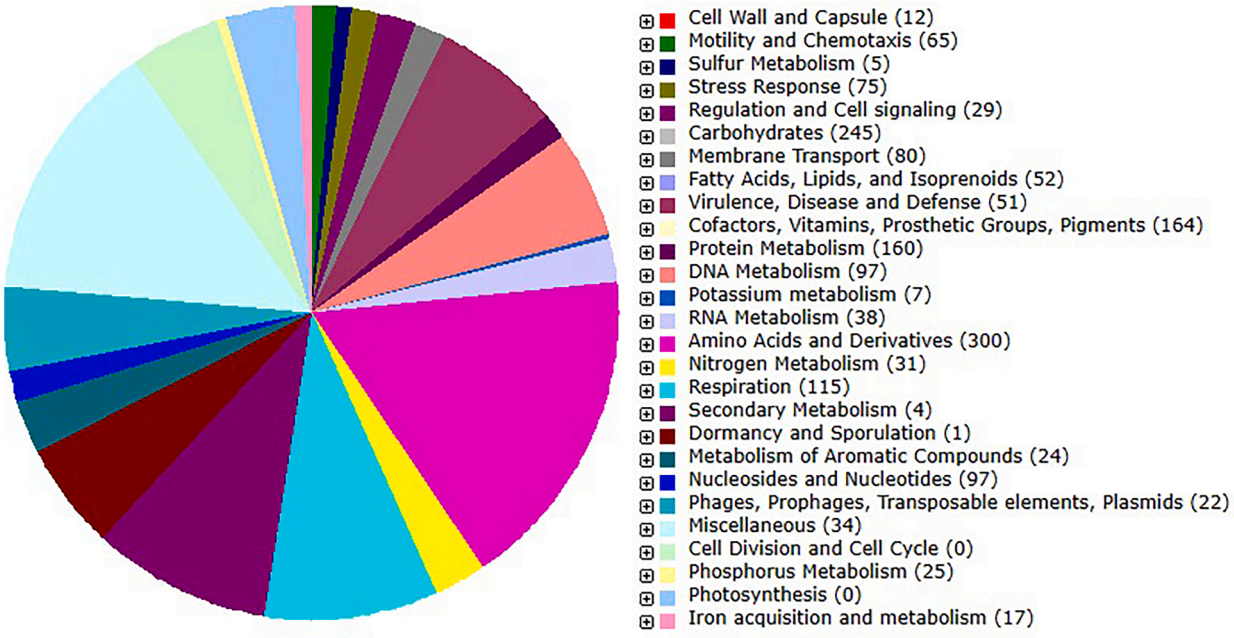

Fig. 2. Genome annotation of *Agrobacterium* sp. CMT1 by Rapid Annotation Using Subsystem Technology (RAST) server version 2.0 (http://rast.nmpdr.org).

Table 1
Number of CDS associated with different subsystems of *Agrobacterium* sp. CMT1 compared to other species.

Subsystem feature counts	*Agrobacterium* sp. CMT1	*Agrobacterium pusense* LMG 25623	*Agrobacterium salinitolerans* YIC 5082	*Agrobacterium radiobacter* CCUG 3354
Amino Acids and Derivatives	298	296	307	307
Carbohydrates	243	235	256	256
Cell Wall and Capsule	12	28	27	30
Cofactors, Vitamins, Prosthetic Groups, Pigments	161	165	172	166
DNA Metabolism	97	105	83	96
Dormancy and Sporulation	1	1	1	1
Fatty Acids, Lipids, and Isoprenoids	52	52	54	66
Iron acquisition and metabolism	16	16	20	17
Membrane Transport	80	84	90	103
Metabolism of Aromatic Compounds	24	29	26	27
Miscellaneous	34	35	35	32
Motility and Chemotaxis	64	62	56	58
Nitrogen Metabolism	31	31	12	32
Nucleosides and Nucleotides	97	94	91	98
Phages, Prophages, Transposable elements, Plasmids	21	22	25	21
Phosphorus Metabolism	25	26	26	27
Potassium metabolism	7	9	7	9
Protein Metabolism	160	54	156	191
Regulation and Cell signaling	29	33	24	29
Respiration	114	124	105	112
RNA Metabolism	39	41	37	43
Secondary Metabolism	4	4	4	4
Stress Response	76	74	77	79
Sulfur Metabolism	5	6	3	5
Virulence, Disease, and Defense	51	42	49	47

rpm, obtaining 1×10^6 Colony Forming Units (CFU)/mL (Valenzuela-Aragón et al., 2018). NanoDrop spectrophotometer (Thermo Fisher Scientific) and agarose electrophoresis (2 %) were used to determine the quantity and quality of the extracted bacterial DNA (OD 260/280 = 1.8–2.0, total amount of DNA ≥ 1 μg, concentration ≥ 20 ng/μL). The extracted DNA was subsequently sequenced by using the Illumina Miseq platform (2 × 300 bp), and the Next Generation Sequencing library preparation was carried out by using the TruSeq DNA Nano Kit for Illumina® Platforms, following the manufacturer's specified protocols. A total of 338,984,909 total reads were obtained [2 × 300 base pairs (bp)], and coverage of 50x.

The quality of the raw reads obtained in sequencing was analyzed using FastQC v 0.11.5 (Andrés, 2010). Low-quality adapters and bases were removed using Trimmomatic v 0.32 (Bolger et al., 2014), where 5.93 % was dropped. SPAdes v 3.15.4 was subsequently used to perform a *de novo* assembly (Bankevich et al., 2012), using the "–careful" parameter to correct errors in reads. The assembled sequenced reads (contigs) were ordered with the Mauve contig Mover tool v 2.4.0 (Darling et al., 2004; Rissman et al., 2009) using *Agrobacterium pusense* LMG 25,623 (accession number jgi.1102370) based on the highest similarity (100 %) of the 16S rRNA gene sequence. Based on the 16S rRNA gene (Fig. 1), using the EzBiocloud database (https://www.ezbioc loud.net/) (Yoon et al., 2017; Chun et al., 2018), strain CMT1 was taxonomically affiliated with the genus *Agrobacterium*, which also corresponds to its macro and microscopic morphology as mentioned before. *Agrobacterium* is one of the most important genera for biotechnology due to its capacity to be used as a vector to improve crops by transferring DNA into plant cells (De Saeger et al., 2021).

The genome annotation of *Agrobacterium* sp. CMT1 was carried out using the Rapid Annotation Using Subsystem Technology (RAST) server version 2.0 (http://rast.nmpdr.org) (Aziz et al., 2008; Overbeek et al., 2014), by the RASTtk pipeline. This strain consists of a total of 51 RNAs, and 5272 predicted Coding DNA Sequences (CDS) distributed into 327 subsystems. The most abundant subsystem was Amino Acids and Derivatives (300 CDS), followed by Carbohydrates (245 CDS), Cofactors, Vitamins, Prosthetic Groups, Pigments (164 CDS), Protein Metabolism (160 CDS), Respiration (115 CDS), and Nucleosides and Nucleotides (97 CDS) (Fig. 2).

Based on the detected subsystems, strain CMT1 could benefit our efforts to achieve the One-Health approach in Paraguay, for example, CDS associated with nitrogen metabolism (Table 1). Strain CMT1 has 31 CDS related to nitrogen metabolism, which are beneficial because biological nitrogen fixation is crucial for agricultural sustainability as it provides an ecological and economical option (Castro et al., 2016). Nitrogen-fixing bacteria-based biofertilizers are a safe and harmless alternative for food production and simultaneously reduce the increased use of chemical fertilizers (Mendoza et al., 2020). Additionally, there are 17 CDS related to iron acquisition and metabolism, with 7 CDS associated with siderophores. These molecules help promote plant growth by supplying iron to the plants (Kramer et al., 2020). Siderophores have a high affinity for the Fe+ ion, thus preventing its availability to other microorganisms like *Fusarium, Pythium, Rhizoctonia,* and *Phytophthora,* making them effective biocontrol agents against significant diseases (Blanco and Castro, 2021). In addition, 51 CDS related to virulence and disease include 2 CDS associated with bacteriocins, which are antibacterial peptides, that are used as natural bio-preservatives in the food industry, while other types of bacteriocins help treat infectious diseases, making them relevant compounds in the world facing antimicrobial resistance issues (Londoño et al., 2015).

Strain CMT1 also has **virulence, disease**, and defense (51 CDS), i.e., resistance to antibiotics and toxic compounds (29 CDS), invasion and intracellular resistance (20 CDS), and bacteriocins and ribosomally synthesized antibacterial peptides (2 CDS), which exert their antibacterial effects and inhibit the growth of closely or non-closely related bacterial strains; **iron acquisition and metabolism** (17 CDS), i.e., siderophores (7 CDS). Siderophores are small molecules used by prokaryotic microorganisms to sequester extracellular iron (Rondon et al., 2004).; and **secondary metabolism** (4 CDS), i.e., auxin biosynthesis, which plays an important role in shaping plant organogenesis, tropic responses, and plant morphogenesis in general (Kaur et al., 2022). Furthermore, subsystems related to bacterial resilience, such as the **stress response** (75 CDS), i.e., osmotic stress (21 CDS) and oxidative stress (42 CDS), and **Nitrogen Metabolism** (31 CDS), i.e., Denitrification (20 CDS) and Nitrogen Metabolism - no subcategory (11 CDS)

Table 2
OGRIs values of *Agrobacterium* sp. CMT1 and closely related *Agrobacterium* species obtained different algorithms.

Agrobacterium species	Strain name	Accession number	Ortho ANIu value (%)	ANIb (%)	ANIm (%)	GGDC (%)
Agrobacterium pusense	LMG 25623^T	jgi.1102370.1	98.37	98.05	98.4	85.80 %
Agrobacterium salinitolerans	YIC 5082^T	GCA_002008225.1	87.35	86.70	88.38	33,60 %
Agrobacterium radiobacter	CCUG 3354^T	GCA_008801385.1	87.84	87.03	88.63	34,30 %

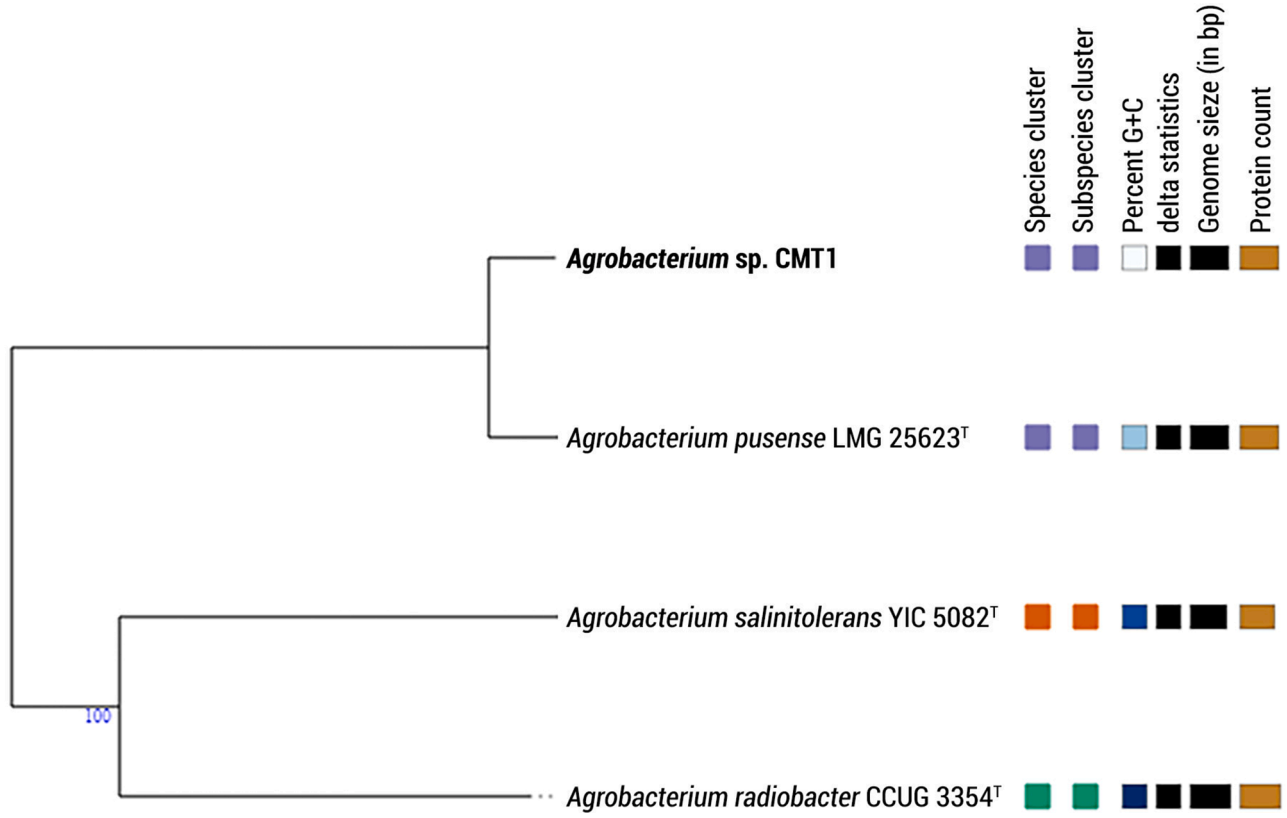

Fig. 3. Phylogenomic relationship of strain CMT1 and closely related *Agrobacterium* species by using Type (Strain) Genome Server (TYGS).

(Fig. 2).

On the other hand, overall genome-relatedness indices (OGRIs) were used for a correct taxonomic affiliation of the studied strain. The average nucleotide identity (ANI) was calculated by the OrthoANI (Yoon et al., 2017) algorithm, while the pair-based algorithms on BLAST (ANIb) and MUMmber (ANIm) were obtained by the JSpeciesWS (Richter et al., 2016) server to determine the average percentage of an orthologous gene nucleotide sequence shared by two genomes using the cut-off value between 95 and 96 % (Goris et al., 2007; Meier- Kolthoff et al., 2013). Strain CMT1 showed the highest ANI values (average of orthoANI, ANIb, and ANIm) of 98.27 % with *Agrobacterium pusense* LMG 25623^T, 87.83 % with *Agrobacterium radiobacter* CCUG 3354^T and 87.47 % with *Agrobacterium salinitolerans* YIC 5082^T (Table 2).

Furthermore, the calculation of the distance between the studied strains was obtained using the genome-to-genome distance calculator (GGDC) based on BLAST version 2.1, where formula 2 is considered for incompletely sequenced genomes (Meier-Kolthoff et al., 2013). To delimit a prokaryotic taxonomic affiliation at the species level, the GGDC value $\geq$ 70 % is used, which was obtained with 85.80 % with *Agrobacterium pusense* LMG 25623^T, 34.30 % with *Agrobacterium radiobacter* CCUG 3354^T and 33.60 % with *Agrobacterium salinitolerans* YIC 5082^T (Table 2).

On the other hand, a phylogenomic tree was built with the genomic relationships established between the strain under study and the genome sequences of the three closely related *Agrobacterium* species (Table 2) by using the Type (Strain) Genome Server (TYGS) tool (Meier-Kolthoff and Göker, 2019), a high-performance web server for genome-based taxonomy of prokaryotes. *Agrobacterium* sp. CMT1 clustered with the *Agrobacterium pusense* LMG 25623^T strain into a different species group compared to the other closely related species (Fig. 3 and Table 2), indicating that strain CMT1 belongs to *Agrobacterium pusense*.

Finally, the values obtained by ANI, GGDC (Table 2), and phylogenomic relationship (Fig. 3) of the studied strain compared with the genomes of the strains closely strongly confirm that strain CMT1 belongs to *Agrobacterium pusense*.

In conclusion, strain CMT1 belongs to the *Agrobacterium pusense*, and based on genome mining, it has the potential for its use in agriculture to promote the growth of crops, due to several action modes. Thus, this strain should be studied in depth to validate its genomic background, and thus, promote its use in soybean production sustainability to reach the One-Health approach in Paraguay.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number JBBCAR000000000. The version described in this paper is JBBCAR010000000 under BioSample accession SAMN40283865 and BioProject number PRJNA1084830. Raw data have been deposited in NCBI SRA under accession number SRR28290933.

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References

Ajmal, M., Ali, H.I., Saeed, R., Akhtar, A., Tahir, M., Mehboob, M.Z., Ayub, A., 2018. Biofertilizer as an alternative to chemical fertilizers. J. Agric. Allied Sci. 7 (1), 1–7.

Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc. (Consulted November 2023).

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A., Dvorkin, M., et al., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477.

Betancor, L., Gadea, M., Flores, K., 2008. Genética bacteriana. Instituto De Higiene, Facultad de Medicina (UDELAR). Temas de Bacteriología y Virología Médica, 3ra Ed. Oficina del Libro FEFMUR, Montevideo, pp. 65–90.

Bistue, A.S., 2018. Hacia la reprogramación de crecimiento bacteriano mediante edición genómica: la bioinformática puesta a prueba. Química Viva 2, 13–22.

Blanco, E.L., Castro, Y., 2021. Antagonismo de rizobacterias sobre hongos fitopatógenos, y su actividad microbiana con potencial biofertilizante, bioestimulante y biocontrolador. Revista Colombiana de Biotecnología 23 (1), 6–16. https://doi.org/10.15446/rev.colomb.biote.v23n1.84808.

Bolger, A., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 30, 2114–2120.

Cámara Paraguaya de Exportadores y Comercializadores de Cereales y Oleaginosas (CAPECO), 2023. *Estadísticas Ranking Mundial.* Cámara Paraguaya de Exportadores y Comercializadores de Cereales y Oleaginosas. Asunción. Disponible en. https://capeco.org.py/ranking-mundial-es/.

Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahal, D.R., et al., 2018. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int. J. Syst. Evol. Microbiol. 68, 461–466.

Darling, A., et al., 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res. 14, 1394–1403.

Deng, Z.S., Zhao, L.F., Kong, Z.Y., Yang, W.Q., Lindström, K., Wang, ET y, Wei, G.H, 2011. Diversidad de bacterias endofíticas dentro de nódulos de *Sphaerophysa salsula* en diferentes regiones de la meseta de Loess en China: bacterias endofíticas *Sphaerophysa salsula*. Ecología de microbiología FEMS 76 (3), 463–475.

de los Santos-Villalobos, S., Parra-Cota, F.I., Herrera-Sepúlveda, A., Valenzuela-Aragón, B., Estrada-Mora, J.C., 2018. Colmena: colección de microorganismos edáficos y endófitos nativos, para contribuir a la seguridad alimentaria nacional. Rev. Mex. Ciencias Agrícolas 9 (1), 191–202. https://doi.org/10.29312/remexca.v9i1.858.

de los Santos-Villalobos, S., Díaz-Rodríguez, A., Ávila-Mascareño, M., Martínez-Vidales, A., Parra-Cota, F., 2021. COLMENA: a culture collection of native microorganisms for harnessing the agro-biotechnological potential in soils and contributing to food security. Diversity. (Basel) 13, 337. https://doi.org/10.3390/d13080337.

De Saeger, J., Park, J., Chung, H.S., Hernalsteens, J.P., Van Lijsebettens, M., Inzé, D., Depuydt, S., 2021. Agrobacterium strains and strain improvement: present and outlook. Biotechnol. Adv. 53, 107677.

Delamuta, J.R.M., Scherer, A.J., Ribeiro, R.A., Hungria, M., 2020. Genetic diversity of *Agrobacterium* species isolated from nodules of common bean and soybean in Brazil, Mexico, Ecuador, and Mozambique, and description of the new species *Agrobacterium* fabacearum sp. nov. Int. J. Syst. Evol. Microbiol. 70 (7), 4233–4244.

Castro, I.V., Fareleira, P., Ferreira, E., 2016. Nitrogen fixing symbiosis in a sustainable agriculture. Plant Soil Microb. 55–91 https://doi.org/10.1007/978-3-319-27455-3_4.

Etesami, H., 2022. Nódulos radiculares de leguminosas: un nicho ecológico adecuado para aislar bacterias no rizobias con potencial biotecnológico en agricultura. Investigación actual en biotecnología 4, 78–86. https://doi.org/10.1016/j.crbiot.2022.01.003.

Farrand, S.K., van Berkum, P.B., Oger, P., 2003. *Agrobacterium* is a definable genus of the family Rhizobiaceae. Int. J. Syst. Evol. Microbiol. 53 (5), 1681–1687. https://doi.org/10.1099/ijs.0.02445-0.

Gan, H.M., Savka, M.A., 2018. One more decade of agrobacterium taxonomy. Curr. Top. Microbiol. Immunol. https://doi.org/10.1007/82_2018_81.

Goris, J., Konstantinidis, K.T., Klappenbach, J.A., Coenye, T., Vandamme, P., et al., 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int. J. Syst. EvolMicrobiol. 57 (Pt 1), 81–91.

Graham, M., Silverstein, K., Cannon, S., Vandenbosch, K., 2004. Computational identification and characterization of novel genes from legumes. Plant Physiol. 135, 1179–1197. https://doi.org/10.1104/pp.104.037531.

Kaur, J., Mudgal, G., Chand, K., Singh, G.B., Perveen, K., Bukhari, N.A., Singh, G., 2022. An exopolysaccharide-producing novel *Agrobacterium pusense* strain JAS1 isolated from a snake plant enhances plant growth and soil water retention. Sci. Rep. 12 (1), 21330.

Kramer, J., Ozkaya, O., Kümmerli, R., 2020. Bacterial siderophores in community and host interactions. Nat. Rev. Microbiol. 18, 152–163. https://doi.org/10.1038/s41579-019-0284-4.

Lim, T.K., 2012. Glycine max. Edible Medicinal And Non-Medicinal Plants: Volume. Fruits 2, 634–714.

Londoño, N.A., Taborda, M.T., López, C.A., Acosta, L.V., 2015. Bacteriocinas producidas por bacterias ácido lácticas y su aplicación en la industria de alimentos. Alimentos hoy 23 (36), 186–205.

López, J., Lépiz, R., González, D., Rodríguez, R., López, E., Olalde, V., 2017. Caracterización morfológica y bioquímica de cepas de *Rhizobium* colectadas en frijol común silvestre y domesticado. Rev Fitotec Mex 40 (1), 73–81.

Matthysse, A.G., 2006. The genus *Agrobacterium*. Prokaryotes 5, 91–114.

Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.P., Göker, M., 2013. Genomesequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14 (1), 60.

Meier-Kolthoff, J.P., Göker, M., 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat. Commun. 10 (1), 2182.

Méndez, C., Trujillo, L., Ramírez, R., Enriquez, G., Hernández, L., González-Peña, D., et al., 2014. Production of a liquid *Bradyrhizobium japonicum* inoculant with high impact on the mechanized sowing of soybean in Cuba. Biotecnol. Apl. 31 (2), 111–120.

Mendoza, V.V., García, J.A., Narvaréz, J.V., Cuevas, F.D.M., Viveros, J.A.F., 2020. Genifix: biofertilizante a base de bacterias fijadoras de nitrógeno. Innovación en Biotecnología I 123.

Morínigo Aguayo, J., Achinelli Báez, M., Barrios Leiva, O., 2018. La soja en el Paraguay. Una aproximación a la cuantificación económica. Periodo 1994-2016*. Población y Desarrollo 24 (46), 24–36. https://doi.org/10.18004/pdfce/2076-054x/2018.024(46).024-036.

Richter, M., Rosselló-Móra, R., Oliver Glöckner, F., Peplies, J, 2016. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics. 32 (6), 929–931.

Rissman, A., Mau, B., Biehl, B., Darling, A., Glasner, J., Perna, N., 2009. Reordering contigs of draft genomes using the Mauve Aligner. Bioinformatics. 25 (16), 2071–2073. https://doi.org/10.1093/bioinformatics/btp356.

Rondon, M.R., Ballering, K.S., Thomas, M.G., 2004. Identification and analysis of a siderophore biosynthetic gene cluster from Agrobacterium tumefaciens C58. Microbiology 150 (11), 3857–3866.

Tokgöz, S., Lakshman, D.K., Ghozlan, M.H., Pinar, H., Roberts, D.P., Mitra, A., 2020. Soybean nodule-associated non-rhizobial bacteria inhibit plant pathogens and induce growth promotion in tomatoes. Plants 9 (11), 1494.

Valenzuela-Aragon, B., Parra-Cota, F., Santoyo, G., Arellano-Wattenbarger, G., de los Santos-Villalobos, S., 2018. Plant-assisted selection: a promising alternative for in vivo identification of wheat (*Triticum turgidum* L. subsp. *Durum*) growth-promoting bacteria. Plant Soil. 435, 367–384. https://doi.org/10.1007/s11104-018-03901-1.

Yoon, S., Ha, S., Lim, J., Kwon, S., Chun, J., 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110, 1281–1286.