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Complete genome sequencing of *Bacillus cabrialesii* TE3^T: A plant growth-promoting and biological control agent isolated from wheat (*Triticum turgidum* subsp. *durum*) in the Yaqui Valley

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ABSTRACT

Bacillus cabrialesii TE3^T is a strictly aerobic and Gram-stain-positive plant growth-promoting bacterium, motile and catalase-positive. In addition, strain TE3^T was also recently described as a biological control agent. Here, we present the complete circularized genome of this type strain, as well as a whole genome analysis identifying genes of agricultural interest. Thus, a hybrid assembly method was performed using short-read sequencing through the Illumina MiSeq platform, and long-read sequencing through the MinION sequencing technology by Oxford Nanopore Technology (ONT). This assembly method showed a closed circular chromosome of 4,125,766 bp and 44.2% G + C content. The strain TE3^T genome annotation, based on the RAST platform, presented 4,282 Coding DNA sequences (CDS) distributed in 335 subsystems, from which 4 CDS are related to the promotion of plant growth and 28 CDS to biological control. Also, Prokka (Rapid Prokaryotic Genome Annotation) predicted a total of 119 RNAs composed of 87 tRNAs, 31 rRNA, and 1 tmRNA; and the PGAP (Prokaryotic Genome Annotation Pipeline) predicted a total of 4,212 genes (3,991 CDS). Additionally, seven putative biosynthetic gene clusters were identified by antiSMASH, such as Fengycin, Bacilysin, Subtilosin A, Bacillibactin, Bacillaene, Surfactin, and Rizocticin A, which are related to antimicrobial and antifungal properties, whose gene presence was further supported by the Prokaryotic Genome Annotation Pipeline (PGAP) annotation. Thus, the complete genome of *Bacillus cabrialesii* TE3^T showed promising bioactivities for the use of this type strain to bioformulate bacterial inoculants for sustainable agriculture.

The growing population and severe agricultural affectations caused by climate change have threatened global food security. Sustainable food production has become a worldwide issue to address. In this sense, to contribute to this issue many have opted for traditional strategies using chemical fertilizers, pesticides, and insecticides due to their rapid action (Ferrusquía-Jiménez et al., 2022). However, excessive use of chemical fertilizers causes problems such as eutrophication, root weakening, and soil acidification (Kumar et al., 2018). On the other hand, the excessive use of synthetic pesticides leads to soil and aquifer contamination with recalcitrant wastes, soil, chemical, and microbial degradation, and biodiversity loss (Córdova-Albores et al., 2021). Therefore, sustainable alternatives for food production are continuously being researched, where beneficial microorganisms are of great interest due to their genetic and functional diversity associated with key roles in soil and plant health (Villareal-Delgado et al., 2018). Thus, several microbial genera interact with crops, regulating their growth and productivity by increasing tolerance to abiotic and biotic stress, plant nutrition, and antagonism against phytopathogenic agents (Díaz-Rodríguez et al., 2021), being *Bacillus* species one of the most studied plant growth-promoting bacteria (PGPB) and biological control agents (BCA) (Córdova-Albores et al., 2021; Villarreal-Delgado et al., 2018).

Bacillus cabrialesii TE3^T was isolated as an endophytic bacterial strain

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from wheat (*Triticum turgidum* L. subsp. *durum*) in the Yaqui Valley, and preserved in the Colección de Microorganismos Edáficos y Endófitos Nativos (COLMENA) (de los Santos-Villalobos et al., 2018, 2021). This type strain was first described by de los Santos Villalobos et al. (2019) as a strictly aerobic and Gram-stain-positive PGPB, motile and catalase-positive. *Bacillus cabrialesii* TE3^T plant growth-promoting traits were previously described, including its ability to produce indole acetic acid -an important phytohormone for plant physiological development- up to 8.21 ± 1.35 μg mL^{-1} (Rojas Padilla et al., 2020), phosphate solubilization -the second most critical macronutrient after nitrogen required for metabolism, growth, and development of plants; which is mainly found in unavailable forms for plant uptake in soil (Rawat et al., 2021)- of up 1.43 ± 0.04 IS (Rojas Padilla et al., 2020), as well as thermal (43.5 °C), hydric [Polyethylene glycol (PEG), 10%, −0.84 mPa)], saline (NaCl, 5%, 6.8 dS m-1) and chemical (chlorothalonil) stress tolerance (Valenzuela-Aragón et al., 2018; Díaz-Rodríguez et al., 2019). Valenzuela-Aragon et al. (2018), demonstrated the ability of TE3^T to significantly promote wheat root length, dry root weight, and aerial dry weight. Similarly, TE3^T plant growth-promoting bacteria (PGPB) abilities have been analyzed in different consortiums by Rojas Padilla et al. (2020) and Robles Montoya et al. (2020), where positive outcomes were obtained including a significant increase of wheat aerial and root dry weight and biovolume index by 25%, 44%, and 18%, respectively.

In addition, strain TE3^T has also been reported as a promising BCA against *Bipolaris sorokiniana,* showing an inhibition zone of 6.7 ± 0.2 mm *in-vitro* assays, and in wheat plant assays, where the control of spot blotch (caused by *B. sorokiniana*) was reduced [scale 1 (minimum) to 9 (maximum)] to 3–5, and the number of lesions/cm^2 to 3.06 ± 0.6, compared to plants inoculated only with *B. sorokiniana* (disease infection of 8–9, and 6.46 ± 1.46 lesions/cm^2) (Villa-Rodríguez et al., 2019). Also, spot blotch inhibition was observed in wheat leaves, which suggests that the strain TE3^T can colonize the wheat phyllosphere and protect its host against phytopathogens (Villa-Rodríguez et al., 2019). Through an integrated omic approach this biological control activity was mainly attributed to the production of biosurfactant lipopeptides, such as surfactin and fengycin; where the application of that lipopeptide complex to *B. sorokiniana* TPQ3 lead to cellular damage, including swollen coarse and irregular hyphae, as well as its cytoplasmic membranous vesicles were expulsed (Villa-Rodríguez et al., 2021). The presented evidence shows that *Bacillus cabrialesii* TE3^T has promising plant growth-promoting and biological control traits, including *in vivo* biological control activity against *B. sorokiniana*, non-cytotoxic activity, phosphate solubilization, indole acetic acid production and the ability to grow in synthetic minimal medium and under stressful conditions.

Here, we present the complete circularized genome of this type strain, as well as, an extensive *in silico* analysis identifying genes of agrobiotechnological interest. Thus, high-quality genomic DNA (1000 ng μL^{-1}, A260/230 and A260/280 were ~ 1.9) was extracted from a fresh culture of this strain grown in nutrient broth (NB) [24 h at 32 °C, using an orbital shaker at 121 rpm, obtaining 1 × 10^6 CFU mL^{-1}], using a phenol-chloroform method following the protocol described by Valenzuela-Aragon et al. (2018). Then, the bacterial DNA was sequenced by the Illumina MiSeq platform, generating 1739,230 paired-end reads (2 × 300 bp), and the MinION sequencing technology by Oxford Nanopore Technology (ONT), obtaining 14,736 reads. The *de novo* assembly was performed using Unicycler version 0.4.8 (Wick et al., 2017), using short reads as a base and long reads for bridging, therefore making an assembly graph with SPAdes version 3.13.1 (Bankevich et al., 2012), under default parameters, using normal mode. Unicycler polishes its final assembly with Illumina reads and Pilon to reduce the rate of small base-level errors, having a total of five polishing rounds. Thus, this assembly resulted in a closed circular chromosome of 4125,766 bp and 44.2% G + C content. Also, PlasmidFinder 2.0 (Carattoli et al., 2014) was used to identify plasmids; however, no plasmids were found. The assembly quality was analyzed in the KBase online platform (Arkin et al., 2018), through Quast v.4.4 (Mikheenko et al., 2016) and CheckM v.1.0.18 (Parks et al., 2015). As a result, no contamination was found.

The *Bacillus cabrialesii* TE3^T genome annotation was carried out by the Rapid Annotation Using Subsystem Technology (RAST) server version 2.0 (Overbeek et al., 2014), using the RASTtk pipeline based on The PathoSystems Resource Integration Center (PATRIC) under default parameters (Davis et al., 2020). RASTtk predicted 116 RNAs, a total of 4282 DNA coding sequences (CDS) distributed in 335 subsystems (supplementary Table 1). The subsystems with the highest presence of CDS were i) amino acids and their derivatives (310 CDS); ii) carbohydrates (273 CDS); iii) protein metabolism (218 CDS); iv) cofactors, vitamins and prosthetic groups (146 CDS); and v) nucleosides and nucleotides (99 CDS) (Fig. 1). In addition, the genome of this strain presented CDS related to the promotion of plant growth, such as i) virulence, disease, and defense (36 CDS), *i.e.* resistance to antibiotics and toxic compounds (17 CDS), invasion and intracellular resistance (12 CDS), and ribosomally synthesized bacteriocins and antibacterial peptides (7 CDS); ii) iron acquisition and metabolism (30 CDS), including siderophores, related to the production of bacillibactin (10 CDS) and anthrachelin (5 CDS); and iii) secondary metabolism (6 CDS), including auxin biosynthesis (4 CDS). Furthermore, subsystems related to bacterial resiliency ideal for prospecting in agricultural bioproducts were identified including; stress response (43 CDS), composed of osmotic stress (15 CDS), oxidative stress (13 CDS), and detoxification (4 CDS), among others.

In addition, a second annotation platform Proksee (https://proksee.ca/) (Grant and Stothard, 2008) was used, using the Rapid Prokaryotic Genome Annotation (Prokka) version 1.0.0 (Seeman, 2014) (supplementary figure 2). This platform generated a circular chromosome map of *Bacillus cabrialesii* TE3^T (Fig. 1), including the identified biosynthetic clusters obtained *via* antiSMASH 6.0 (Blin et al., 2021). Furthermore, complementing the results obtained by RASTtk, the Prokka annotation predicted a total of 4164 CDS. In contrast to the 116 RNAs predicted by RASTtk, Prokka predicted a total of 119 RNAs composed of 87 tRNAs, 31 rRNA, and 1 tmRNA (Fig. 2).

Additionally, the complete genome of *Bacillus cabrialesii* TE3^T was analyzed using antiSMASH version 7.0 under default parameters (Blin et al., 2021), to identify biosynthetic gene clusters (BGCs) related to biocontrol activity (supplementary Table 2). This resulted in the identification of seven putative BGCs: i) Fengycin (100%), a cyclic lipopeptide, with broad antibacterial activity and antagonistic activity against filamentous fungi, low hemolysis, and safe degradation, with a vast potential in agriculture (Villa-Rodríguez et al., 2021; Lu et al., 2022); ii) Bacilysin (100%), a dipeptide antibiotic compound and a signaling molecule either directly or indirectly affecting various cellular functions, as including spore quality and has a wide range of antagonistic activity against fungi and bacteria (Islam et al., 2022); iii) Subtilosin A (100%), a ribosomally produced bacteriocin with antimicrobial bioactivity (Venkatasamy et al., 2021); iv) Bacillibactin (100%), an archetypal triscatetolate siderophore known for its high affinity for iron (Fe^{3+}) compared to other siderophores, playing a key role in iron chelation and overall plant immunity towards various pathogens (Lalitha and Nithyapriya, 2021); v) Bacillaene (100%), a polypeptide with antimicrobial and inhibition effects on biofilm formation (Erega et al., 2021); vi) Surfactin (86%), a cyclic lipopeptide and one of the most effective biosurfactants due to a large number of biological activities, like antifungal, antitumor, and insecticidal (Rodrigues et al., 2021); and vii) Rizocticin A (93%), an antibiotic that penetrates the fungal cell through the oligopeptide transport system, inhibiting protein synthesis (Sidorova et al., 2018) (Fig. 2 and Fig. 3).

Finally, PGAP version 6.1 was also used under the automatic NCBI annotation method (Tatusova et al., 2016) obtaining a total of 4212 genes (supplementary Table 1). As well as, 121 RNAs being the server with the most identified RNAs out of the three servers used to annotate. Also, PGAP annotation elucidated genes related to fengycin biosynthesis including the genes reported by Lu et al. (2022), *i.e. accA* and *gapA*;

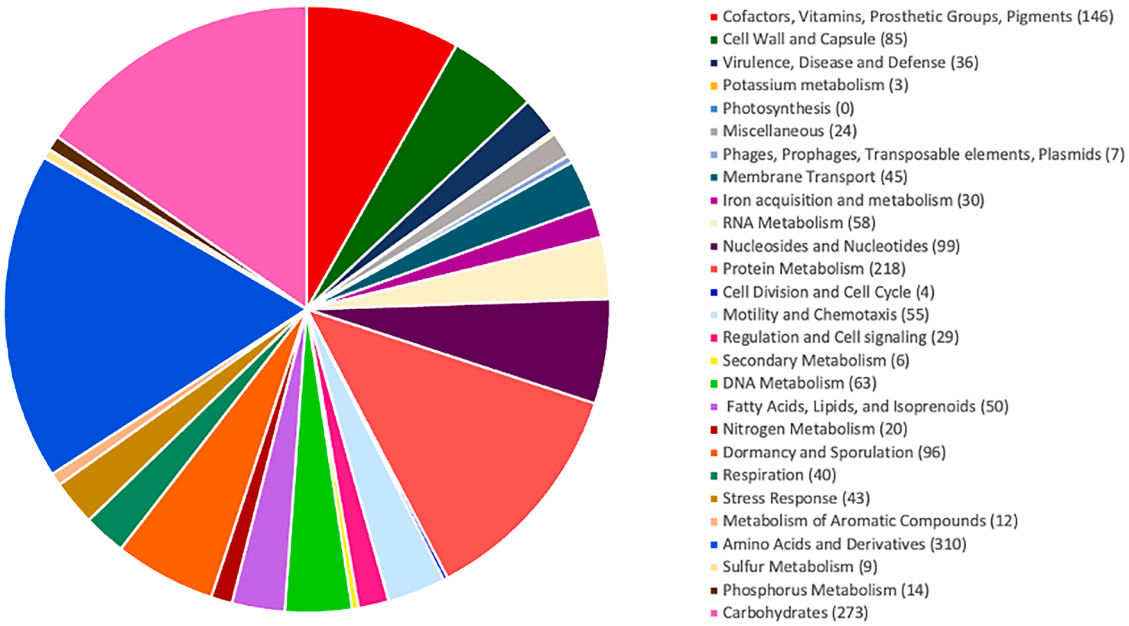

Fig. 1. Distribution of subsystem categories found in the complete genome of *Bacillus cabrialesii* TE3^T, obtained through the genome annotation on RASTtk v 2.0 (http://rast.nmpdr.org).

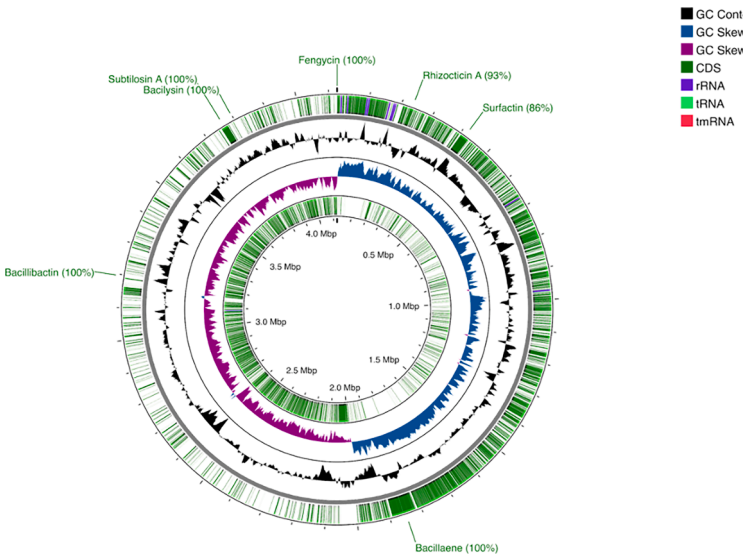

Fig. 2. Circular chromosome map of the complete genome of *Bacillus cabrialesii* TE3^T generated through Proksee (https://proksee.ca), including whole genome annotation from Prokka v 1.0.0 and biosynthetic gene clusters related to biocontrol from antiSMASH v 6.0.

bacilysin biosynthesis (*BacE* and *LutR*); subtilosin production (*AlbA, AlbB, AlbD*, and *sboA*); bacillibactin production (*bcbE* and *besA*); and genes related to surfactin production (*srfAA, srfAB, srfAC, srfAD, srfP*).

Thus, the presence of biological control and plant growth promotion-related genes in the circularized genome of *Bacillus cabrialesii* TE3^T along with the evidence under *in vitro* assays prevails its potential as a powerful active component for the generation of microbial inoculants and biopesticides, for the use in sustainable agriculture.

Author contributions

All authors have read and agreed to the published version of the manuscript.

CRediT authorship contribution statement

Valeria Valenzuela Ruiz: Conceptualization, Writing – original draft, Visualization, Writing – review & editing. **Gustavo Santoyo:** Writing – original draft, Visualization, Writing – review & editing. **Lorena Jacqueline Gómez Godínez:** Writing – original draft, Visualization, Writing – review & editing. **Luis A. Cira Chávez:** Writing – original draft, Visualization, Writing – review & editing. **Fannie I. Parra Cota:** Writing – original draft, Visualization, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Sergio de los Santos Villalobos:** Conceptualization, Writing – original draft, Visualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

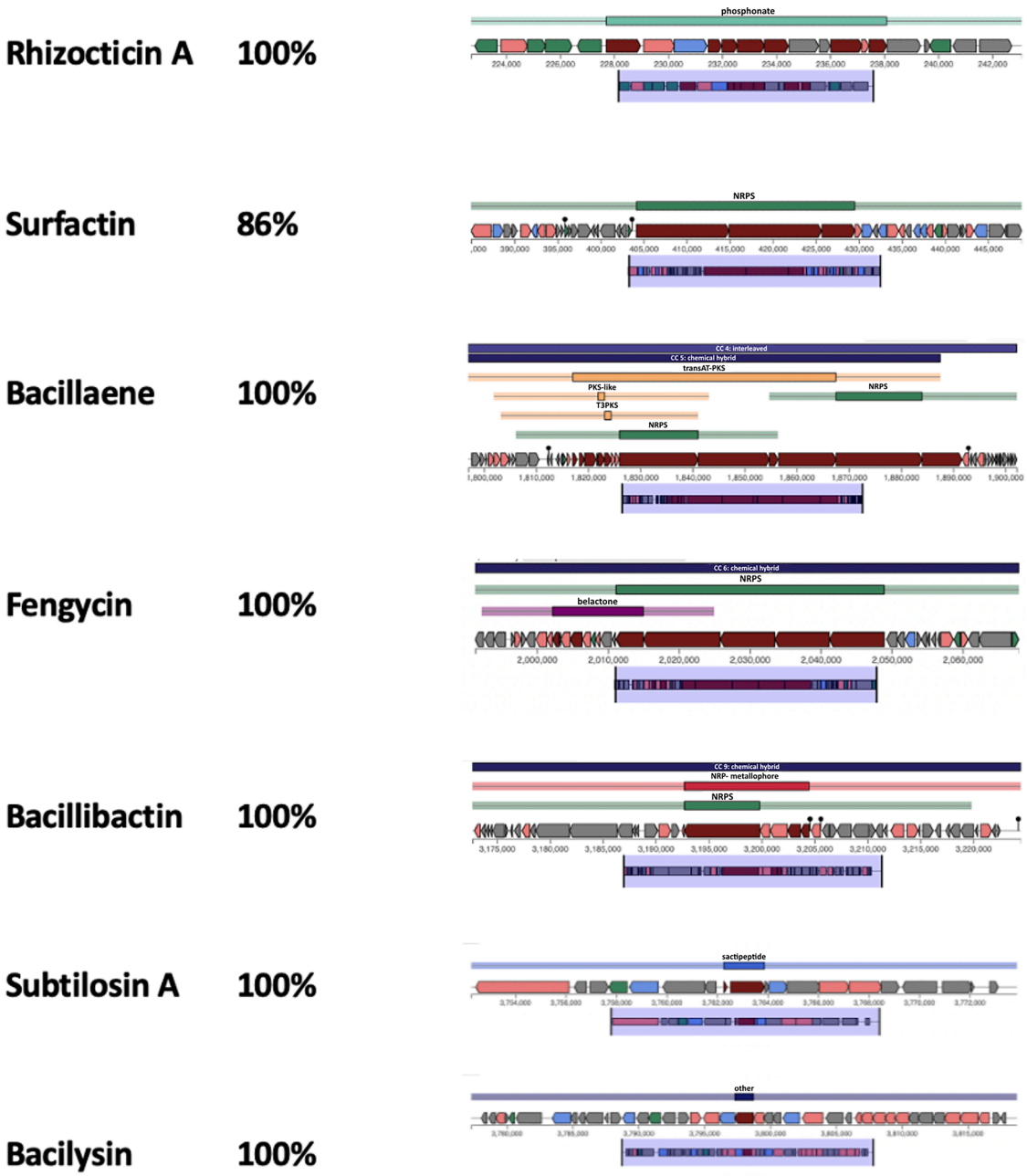

Fig. 3. antiSMASH v. 7.0 *in silico* biosynthetic gene cluster identification related to biological control.

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

The complete genome sequence has been deposited in DDBJ/ENA/GenBank under accession number CP096889.1, under BioProject number PRJNA504313, and BioSample number SAMN10390288. Raw data is available under accession number PRJNA504313, under the following link https://www.ncbi.nlm.nih.gov/sra/PRJNA504313.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2023.100193.

References

Arkin, A., Cottingham, R., Henry, C., Harris, N., Stevens, R., Maslov, S., et al., 2018. KBase: the United States department of energy systems biology knowledgebase. Nat. Biotechnol. 36, 566. https://doi.org/10.1038/nbt.4163.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A., Dvorkin, M., Kulikov, A., Lesin, V., Nikolenko, S., Pham, S., Prjibelski, A., Pyshkin, A., Sirotkin, A., Vyahhi, N., Tesler, G., Alekseyev, M., Pevzner, P., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477. https://doi.org/10.1089/cmb.2012.0021.

Blin, K., Shaw, S., Kloosterman, A., Charlop-Powers, Z., vanWezel, G., Medema, M., Weber, T., 2021. antiSMASH 6.0: improving cluster detection and comparison capabilities. Nucleic Acids Res. 49 (w1), w39–w45. https://doi.org/10.1093/nar/gkab335.

Carattoli, A., Zankari, E., Garcia-Fernandez, A., Voldby Larsen, M., Lund, O., Villa, L., Aarestrup, F., Hasman, H., 2014. PlasmidFinder and pMLST: in silico detection and typing of plasmids. Antimicrob. Agents Chemother. 58 (7), 3895–3903. https://doi.org/10.1128/AAC.02412-14.

Córdova-Albores, L., Zelaya-Molina, L., Ávila-Alistac, N., Valenzuela-Ruíz, V., Cortés-Martínez, N., Parra-Cota, F., Burgos-Canul, Y., Chávez-Díaz, I., Fajardo-Franco, M., de los Santos-Villalobos, S., 2021. Omics sciences potential on bioprospecting of biological control microbial agents: the case of the Mexican agro-biotechnology. Mexican J. Phytopathol. 39 (1), 147–184. https://doi.org/10.18781/R.MEX.FIT.2009-3.

Davis, J., Wattam, A., Aziz, R., Brettin, T., Butler, R., Butler, R., et al., 2020. The PATRIc bioinformatics resource center: expanding data and analysis capabilities. Nucleic Acids Res. 48, D606–D612. https://doi.org/10.1093/nar/gkz943.

de los Santos Villalobos, S., Robles, R., Parra Cota, F., Larsen, J., Lozano, P., Tiedje, J., 2019. *Bacillus cabrialesii* sp. nov., an endophytic plant growth promoting bacterium isolated from wheat (*Triticum turgidum* subsp. *durum*) in the Yaqui Valley, Mexico. Int. J. Syst. Evol. Microbiol. 69, 3939–3945.

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, 1–13. https://doi.org/10.3390/d13080337.

de los Santos-Villalobos, S., Parra-Cota, F., Herrea-Sepulveda, A., Valenzuela-Aragon, B., Estrada-Mora, J., 2018. Colmena: colección de microorganismos edáficos Y endófitos nativos,para contribuir a la seguridad alimentaria nacional. Revista Mexicana de Ciencias Agrícolas 9 (1), 191–202. https://doi.org/10.29312/remexca.v9i1.858.

Díaz Rodríguez, A., Parra Cota, F., Santoyo, G., de los Santos Villalobos, S., 2019. Chlorothalonil tolerance of indole producing bacteria associated to wheat (*Triticum turgidum* L.) rhizosphere in the Yaqui Valley, Mexico. Ecotoxicology. https://doi.org/10.1007/s10646-019-02053-x.

Erega, A., Stefanic, P., Dogsa, I., Danevčič, T., Simunovic, K., Klančnik, A., Smole Možina, S., Mandic Mulec, I., 2021. Bacillaene mediates the inhibitory effect of *Bacillus subtilis* on *campylobacter jejuni* biofilms. Appl. Environ. Microbiol. 87 (12), e0295520 https://doi.org/10.1128/AEM.02955-20.

Ferrusquía-Jiménez, N., González-Arias, B., Rosales, A., Esquivel, K., Escamilla-Silva, E., Ortega-Torres, A., Guevara-González, R., 2022. Elicitation of *bacillus cereus*-amazcala (B.c-A) with SiO2 nanoparticles improves its role as a plant growth-promoting bacteria (PGPB) in Chili pepper plants. Plants 11 (24), 3445. https://doi.org/10.3390/plants11243445.

Grant, J., Stothard, P., 2008. The CGView server: a comparative genomics tool for circular genomes. Nucleic Acids Res. 36, 181–184. https://doi.org/10.1093/nar/gkn179.

Islam, T., Rabbee, M., Choi, J., Baek, K.-.H., 2022. Biosynthesis, molecular regulation, and application of bacilysin produced by *Bacillus* species. Metabolites 12, 397. https://doi.org/10.3390/metabo12050397.

Kumar, M., Reddy, G., Phogat, M., Korav, S., 2018. Role of bio-fertilizers towards sustainable agricultural development: a review. J. Pharmacogn. Phytochem. 7, 1915–1921.

Lalitha, S., Nithyapriya, S., 2021. Production of bacillibactin siderophore from soil bacteria, *Bacillus subtilis*: a bioinoculant enhances plant growth in *Arachis hypogaea* L. through elevated uptake of nutrients. Adv. Biol. Sci. Res. 13, 71–82. https://doi.org/10.2991/absr.k.210609.013.

Lu, H., Xu, H., Yang, P., Bilal, M., Zhu, S., Zhong, M., Zhao, L., Gu, C., Liu, S., Zhao, Y., Geng, C., 2022. Transcriptome analysis of *Bacillus amyloliquefaciens* reveals fructose addition effects on fengycin synthesis. Genes (Basel) 13 (6), 984. https://doi.org/10.3390/genes13060984.

Mikheenko, A., Valin, G., Prjibelski, A., Saveliev, V., Gurevich, A., 2016. Icarus: visualizer for de novo assembly evaluation. Bioinformatics 32 (21), 3321–3323. https://doi.org/10.1093/bioinformatics/btw379.

Overbeek, R., Olson, R., Pusch, G., Olsen, G., Davis, J., Disz, T., Edwards, R., Gerdes, S., Parrello, B., Shukla, M., Vonstein, V., Wattam, A., Xia, F., Stevens, R., 2014. The SEED and the rapid annotation of microbial genomes using sub-systems technology (RAST). Nucleic Acids Res. 42 (D1), 206–214. https://doi.org/10.1093/nar/gkt1226.

Parks, D., Imelfort, M., Skennerton, C., Hugenholtz, P., Tyson, G., 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 25 (7), 1043–1055. https://doi.org/10.1101/gr.186072.114.

Rawat, P., Das, S., Shankhdhar, D., Shankhdhar, S., 2021. Phosphate-solubilizing microorganisms: mechanism and their role in phosphate solubilization and uptake. J. Soil Sci. Plant Nutr. 21, 49–68. https://doi.org/10.1007/s42729-020-00342-7.

Robles Montoya, R., Chaparro Encinas, L., Parra Cota, F., De los Santos Villalobos, S., 2020. Improving biometric traits of wheat seedlings with the inoculation of a consortium native of *Bacillus*. Revista Mexicana Ciencias Agrícolas 11 (1), 229–235. https://doi.org/10.29312/remexca.v11i1.2162.

Rodrigues, A., Gudiña, E., Teixeira, J., Rodrigues, L., 2021. Biosurfactants as biocontrol agents against mycotoxigenic fungi. Biosurfactants for a Sustain. Future 465–490. https://doi.org/10.1002/9781119671022.ch21.

Rojas Padilla, J., Chaparro Encinas, L., Robles Montoya, R., de los Santos Villalobos, S., 2020. Growth promotion on wheat (*Triticum turgidum* L. subsp. *durum*) by coinoculation of native *Bacillus* strains isolated from the Yaqui Valley, Mexico. Nova Scientia 24 (12), 1–27. https://doi.org/10.21640/ns.v12i24.2136.

Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30, 2068–2069. https://doi.org/10.1093/bioinformatics/btu153.

Sidorova, T., Asaturova, A., Homyak, A., 2018. Biologically active metabolites of *Bacillus subtilis* and their role in the control of phytopathogenic microorganisms (review). Agricultural Biol. 53 (1), 29–37. https://doi.org/10.15389/agrobiology.2018.1.29eng.

Tatusova, T., DiCuccio, M., Badretdin, A., Chetvernin, V., Nawrocki, E., Zaslavsky, L., Lomsadze, A., Pruitt, K., Borodovsky, M., Ostell, J., 2016. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res. 44, 6614–6624. https://doi.org/10.1093/nar/gkw569.

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. In Plant and Soil 435, 367–384. https://doi.org/10.1007/s11104-018-03901-1.

Venkatasamy, V., Durairaj, R., Karuppaiah, P., et al., 2021. An in silico evaluation of molecular interaction between antimicrobial peptide subtilosin A of *Bacillus subtilis* with virulent proteins of *aeromonas hydrophila*. Int. J. Pept Res. Ther. 27, 1709–1718. https://doi.org/10.1007/s10989-021-10203-1.

Villa-Rodriguez, E., Moreno-Ulloa, A., Castro-Longoria, E., Parra-Cota, F., de los Santos-Villalobos, S., 2021. Integrated omics approaches for deciphering antifungal metabolites produced by a novel *Bacillus* species, *B. cabrialesii* TE3^{T}, against the spot blotch disease of wheat (*Triticum turgidum* L. subsp. *durum*). Microbiol. Res. 251, 126826 https://doi.org/10.1016/j.micres.2021.126826.

Villa-Rodríguez, E., Parra-Cota, F., Castro-Longoria, E., López-Cervantes, J., de los Santos-Villalobos, S., 2019. *Bacillus subtilis* TE3: a promising biological control agent against *Bipolaris sorokiniana*, the causal agent of spot blotch in wheat (*Triticum turgidum* L. subsp. *durum*). Biol. Control 132, 135–143. https://doi.org/10.1016/j.biocontrol.

Villarreal-Delgado, M., Villa-Rodríguez, E., Cira-Chávez, L., Estrada-Alvarado, M., Parra-Cota, F., De los Santos-Villalobos, S., 2018. The genus *Bacillus* as a biological control agent and its implications in the agricultural biosecurity. Revista Mexicana de Fitopatología 36 (1), 95–130. https://doi.org/10.18781/R.MEX.FIT.1706-5.

Wick, R., Judd, L., Gorrie, C., Holt, K., 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput. Biol. 13, e1005595 https://doi.org/10.1371/journal.pcbi.1005595.