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Draft genome sequence of *Bacillus* sp. strain FSQ1, a biological control agent against white mold in common bean (*Phaseolus vulgaris* L.)

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ABSTRACT

Bacillus sp. strain FSQ1 was isolated from the common bean (*Phaseolus vulgaris* L.). The genome of this strain presented 3,598,499 bp; 43.0% G + C content; 925,913 bp N50; 2 L50; 33 contigs; 97 RNAs and 3,908 predicted coding DNA sequences (CDS) distributed in 315 subsystems. Based on genome mining, the biological control activity of strains FSQ1 could be associated with the biosynthesis of rhizocticin A and bacillibactin. Thus, this strain is a promising active ingredient for the formulation of biopesticides.

Common bean (*Phaseolus vulgaris L.*) is an important legume on a global scale, found in tropical highlands (Myers and Kmiecik, 2017). Mexico is the second-largest producer of this crop worldwide with 373, 750 MT per year, whilst the main producer is Brazil with 495,100 MT (US Dry Bean, 2020). The common bean in Mexico, and around the world, is very popular due to its cultural tradition, being a good source of nutrients (22% of protein, 62% of complex carbohydrates, and micronutrients) (Myers and Kmiecik, 2017). However, the production of this crop is highly affected by diseases, i.e. white mold caused by *Sclerotinia sclerotiorum* (Wang et al., 2019; Chen et al., 2020). *Sclerotinia sclerotiorum* is a necrotrophic fungus that infects more than 400 plant species, including several economically important crops, such as canola (*Brassica napus*), chickpea (*Cicer arietinum*), beans (*Phaseolus vulgaris*), soybeans (*Glycine* max), peas (*Pisum sativum*), onion (*Allium cepa*), lentils (*Lens culinaris*) (Allan et al., 2019; Wang et al., 2019). The economic loss caused by this phytopathogenic fungus is reported to be up to USD $1.2 billion per year in Mexico (FAO, 2019); and worldwide annually 40% of the common bean production is lost (Wang et al., 2019). Currently, synthetic fungicides are applied to control *S. sclerotiorum*, such as boscalid, potassium bicarbonate, carbendazim, fluazinam, fluoxastrobin, copper octanate, fludioxonil + ciprodinil, boscalid + pyraclostrobin (Ayala et al., 2015). However, the excessive use of these synthetic fungicides has generated several problems in the environment, due to the production of toxic waste and the phytopathogen resistance (Peláez-Álvarez et al., 2016), also generating economic losses and negative effects on human health (Mondéjar-Jiménez et al., 2016; Peláez-Álvarez et al., 2016). Thus, the development of innovative and sustainable alternatives for controlling plant diseases is crucial to warrant food security, mitigating the economic, social, and environmental impacts of the use of synthetic fungicides in agriculture.

Through time, microorganisms have been studied due to their genetic and functional diversity associated with fundamental roles in agroecosystems (Reali et al., 2017). 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). *Bacillus* is a Gram-positive bacteria with a variable size of 0.5 to 10 μm, aerobic and/or anaerobic growth at a temperature between 30 and 45 °C, and endospores producer has been studied due to its great metabolic diversity to promote plant growth and control of phytopathogens (Villarreal-Delgado et al., 2018). *Bacillus paralicheniformis* (Valenzuela-Ruiz et al., 2019) and *B. cabrialesii* (de los Santos-Villalobos et al., 2019) can inhibit *Bipolaris sorokiniana* (the causal agent of spot blotch in wheat), through the production of secondary metabolites such as biosurfactant lipopeptides (surfactin and fengycin), and dipeptide rhizocticin A (Valenzuela-Ruiz et al., 2019; Villa-Rodríguez et al., 2021); *B. subtilis* inhibit the growth of *Botrytis cinerea* and *Fusarium oxysporum,* through the production of fengycins and iturins (Cawoy et al., 2015; Andrić et al.,

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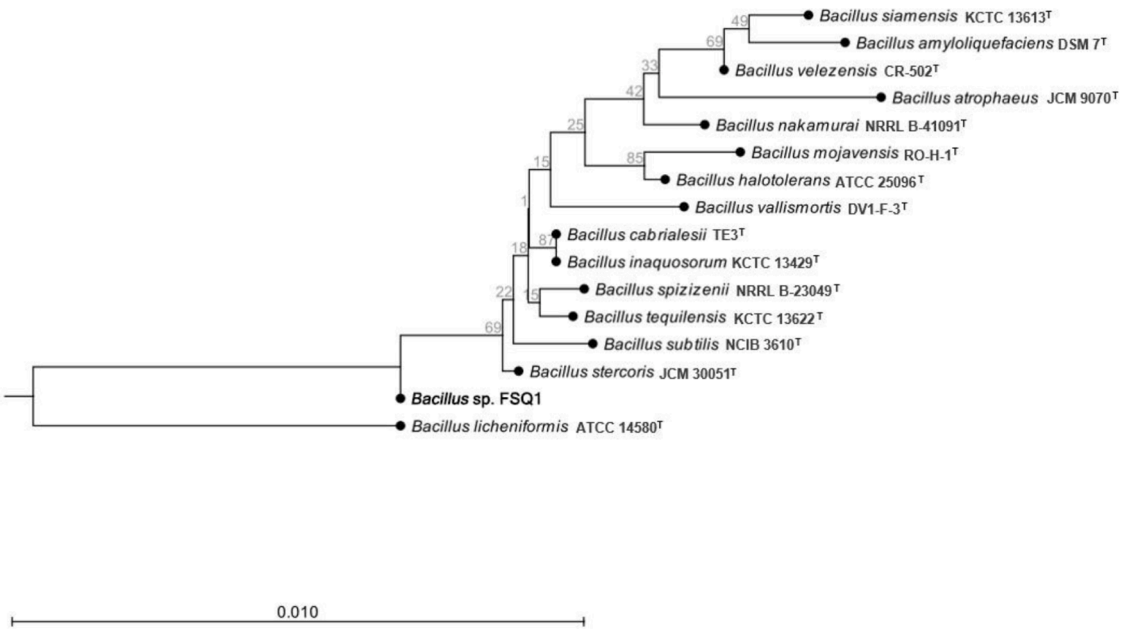

Fig. 1. Phylogenetic relation between *Bacillus* sp. strain FSQ1 and closely related species.

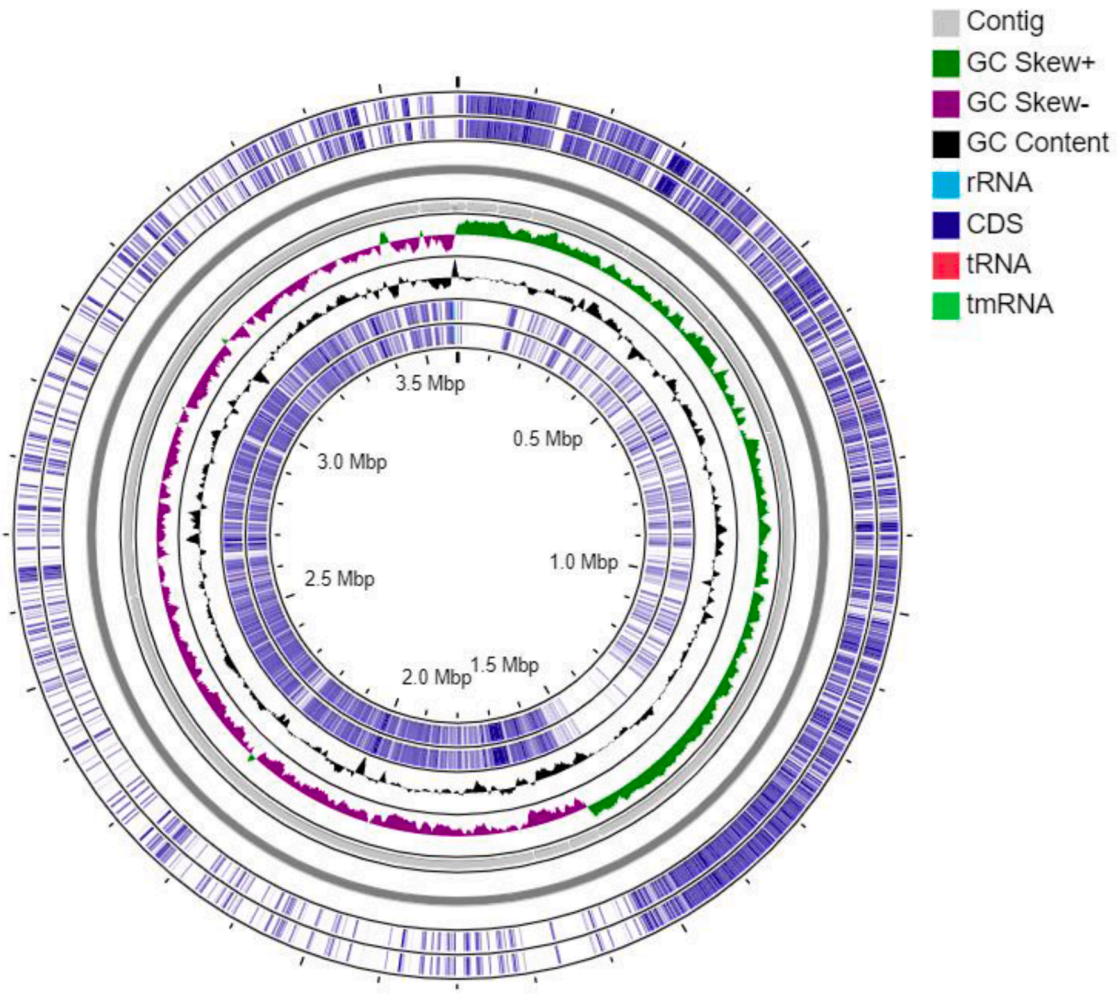

Fig. 2. Circular chromosome map of *Bacillus* sp. strain FSQ1 showing the distribution of coding DNA sequences (CDS), tRNAs, rRNAs, and GC content skew (50% of the total base-pair window), by CGView Server beta online tool.

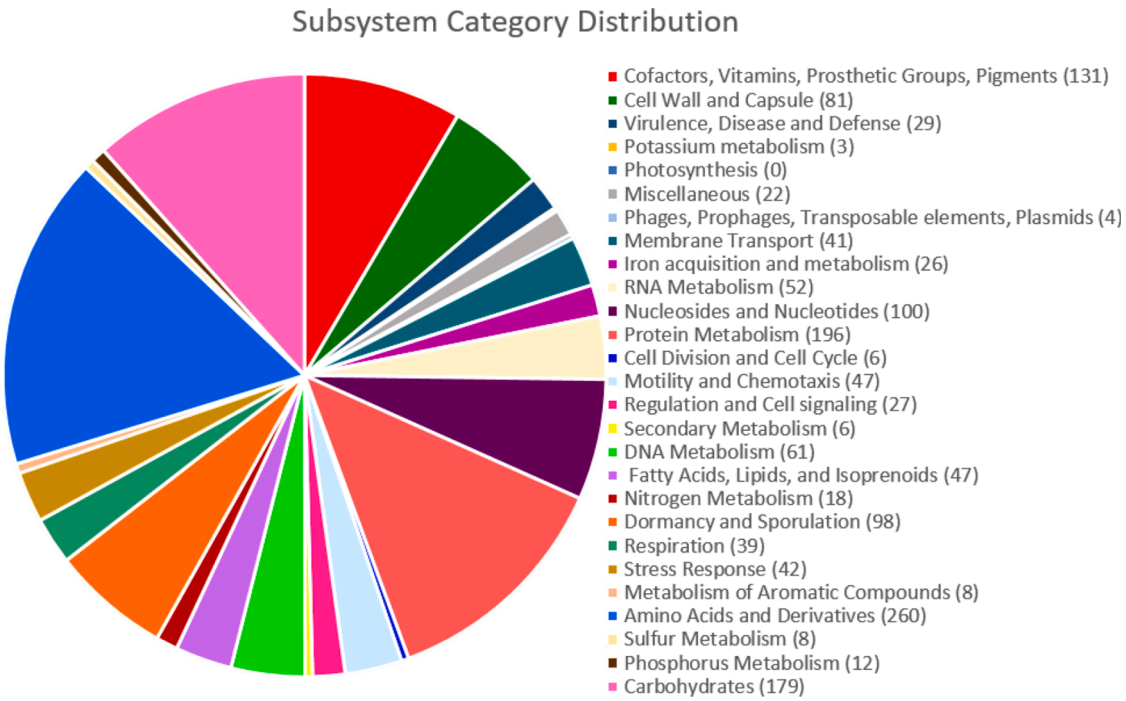

Fig. 3. Genome annotation of *Bacillus* sp. strain FSQ1 by Rapid Annotation Using Subsystem Technology (RAST) server version 2.0 (http://rast.nmpdr.org).

2020; Chen et al., 2020); and *B. velezensis* controls *F. solani, Ralstonia solani, F. oxysporum, S. sclerotiorum,* and *Phytophthora parasitica,* through the production of bacillibactin and bacillomycin D (Li et al., 2015; Zihalirwa et al., 2017; Andrić et al., 2020; Chen et al., 2020).

During the fall-winter agricultural season (2013–2014), our research team isolated strain FSQ1 from the rhizosphere of common bean (*Phaseolus vulgaris* L.) in a commercial field located in Gabriel Leyva Solano Guasave municipality, Sinaloa, Mexico (25.631012, −108.657778). The isolation was carried out by using a serial dilution method on Nutrient Agar (NA) at 28 °C for 2 days (Valenzuela-Aragon et al., 2018). After purification, 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). Then, under *in vitro* confrontation assay, this strain showed a significant growth inhibitory effect (35%) on *Sclerotinia sclerotiorum*, through extracellular diffusible compounds (Villarreal-Delgado et al., 2021). Once the high potential of strain FSQ1 as a biocontrol agent was determined, and given that very little is currently known about its genome, it was sequenced to carry out genome mining to identify promising biosynthetic gene clusters associated with biocontrol.

High-quality extraction of genomic DNA was prepared from an axenic culture of strain FSQ1 grown in nutrient broth [24 h at 30 °C, using an orbital shaker at 121 rpm, obtaining 1×10^6 Colony Forming Units (CFU/mL)] (Valenzuela-Aragon et al., 2018). The quality and quantity of the extracted DNA (OD 260/280 = 1.8–2.0, total amount of DNA ≥ 1 μg, concentration ≥ 20 ng/μL) were determinate by the NanoDrop spectrophotometer (catalog no. ND-2000; Thermo Fisher Scientific), and agarose (2%) electrophoresis. The bacterial DNA was sequenced by the Illumina MiSeq platform, obtaining a total of 3390,725 total reads [2 × 300 base pairs (bp)], and a sequence coverage of 848x. The quality of the obtained reads was obtained by FastQC v 0.11.5 (Andrews, 2010). Trimmomatic v 0.32 (Bolger et al., 2014) was used to remove adapter sequences and low-quality bases, where only 7.28% was dropped. Then, *de novo* assembly was generated by SPAdes v 3.14.1 (Bankevich et al., 2012), using the "–careful" parameter for error correction in reads. The draft genome of strain FSQ1 presented 3598, 499 bp; 43.0% G + C content; 925,913 bp N50; 2 L50; and 33 contigs (> 200 bp). The assembled contigs were ordered by Mauve contig Mover v 2.4.0 (Darling et al., 2004; Rissman et al., 2009), using the reference genome of *Bacillus spizizenii* TU-B-10^T (accession number GCA_000227465.1), based on the highest similarity of the 16S rRNA gene sequence (99.85%) (Fig. 1, Supplementary Table 1), by using EzBiocloud (https://www.ezbiocloud.net/) database (Yoon et al., 2017; Chun et al., 2018). Thus, strain FSQ1 was taxonomically affiliated to the genus *Bacillus*, which also corresponds to its macro and microscopic morphology, bacillary morphology, rod-shaped, strictly aerobic, spore formation, Gram-positive bacterium (Piggot, 2009; Martínez, 2013; Villarreal-Delgado et al., 2018).

In addition, the circular chromosome map of strain FSQ1 was generated by using the CGView Server (Grant and Stothard, 2008), showing 11 rRNAs, 1 tmRNAs, 86 tRNAs, and 3688 CDS (Fig. 2). The genome annotation of *Bacillus* sp. FSQ1 was created through 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 (Fig. 3). This strain consists of a total of 97 RNAs, and 3908 predicted Coding DNA Sequences (CDS) distributed into 315 subsystems. The most abundant subsystem was Amino Acids and Derivatives (260 CDS), followed by Protein Metabolism (197 CDS), Carbohydrates (179 CDS), Cofactors, Vitamins, Prosthetic Groups, Pigments (131 CDS), Nucleosides and Nucleotides (100 CDS), and Dormancy and Sporulation (98 CDS).

Genome mining of strain FSQ1 was carried out by using antiSMASH v6.0 (Blin et al., 2021) predicted the existence of nonribosomal peptide gene clusters, such as rhizocticin A (77%) and bacillibactin (100%). Bacillibactin is used as catechol siderophores, which are secondary metabolites that eliminate iron from the environment, and are delivered to cells through receptors, and provide nutrients to the bacteria (Zhou et al., 2018; Kramer et al., 2020). However, the *Bacillus's* ability to synthesize siderophores leaves other microorganisms without iron in the medium, inhibiting their growth (Villarreal-Delgado et al., 2018). On the other hand, rhizocticin A is a natural phosphonate antibiotic also known as a phosphono-oligopeptide antibiotic, which has antifungal activity since it releases APPA (unusual nonproteinogenic amino acid (Z)-L-2-amino-5-phosphono-3-pentenoic acid), which inhibits the growth of fungi and affects protein synthesis that intervenes in the

threonine biosynthesis and related metabolic pathways (Kugler et al., 1990; Borisova et al., 2010; Petronikolou et al., 2019).

Strain FSQ1 belongs to the genus *Bacillus* and has antifungal activity against *Sclerotinia sclerotiorum*, the causal agent of white mold in the common bean (*Phaseolus vulgaris* L.). In addition, based on genome mining, potential metabolites involved in its biological control activity were identified, such as rhizocticin A and bacillibactin. Thus, strain FSQ1 should be further studied as an active ingredient for the biopesticide formulation due to its biocontrol ability by the potential secretion of anti-fungicidal metabolites.

Data availability

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number JAHAWP000000000. The version described in this paper is JAHAWP010000000 under BioSample accession SAMN19077229 and BioProject number PRJNA728358. Raw data have been deposited in NCBI SRA under accession number SRR14492029 and the Genome submission is under the accession number SUB9604365.

CRediT author statement

Félix-Pablos, Carmen María: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing - review & editing.**Parra-Cota, Fannie I.**: Data curation, Formal analysis, Writing-original draft, Writing - review & editing.**Santoyo, Gustavo**: Visualization, Writing – original draft, Writing - review & editing.**Orozco-Mosqueda, Ma. del Carmen**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review& editing.**de los Santos-Villalobos, Sergio**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

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.The authors declare the following financial interests/personal relationships which may be considered as potential competing interests

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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.2022.100138.

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