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Draft Genome Sequences of a *Bacillus subtilis* Strain, a *Bacillus velezensis* Strain, a *Paenibacillus* Strain, and an *Acinetobacter baumannii* Strain, All Isolated from the Phyllosphere of *Lactuca sativa* or *Solanum lycopersicum*

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Draft Genome Sequences of a *Bacillus subtilis* Strain, a *Bacillus velezensis* Strain, a *Paenibacillus* Strain, and an *Acinetobacter baumannii* Strain, All Isolated from the Phyllosphere of *Lactuca sativa* or *Solanum lycopersicum*

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ABSTRACT Four strains isolated from tomato and lettuce phyllosphere were sequenced in order to investigate the presence of novel antimicrobial gene clusters and to get a better understanding of plant microbe interactions. These strains comprise two *Bacillus* strains, one *Paenibacillus* strain, and one *Acinetobacter* strain.

Crop production is facing several challenges, such as a higher demand due to an increased population, climate change, and yield loss caused by pathogens. The extensive use of chemical pesticides in agricultural production has caused negative effects including contamination of soils and water and toxicity in animals, including humans (1). Beneficial microbes in the plant microbiome have shown good potential for application as pathogen control and plant growth promoters in order to increase crop yields. Therefore, we can regard them as environmentally friendly alternatives to chemical products in agricultural practices (2).

Bacillus species are Gram-positive, spore-forming bacteria widely distributed in the environment; they are well known as producers of a wide array of antimicrobials, having between 5 and 8% of the total genome devoted to the biosynthesis of secondary metabolites. (3). *Paenibacillus* is another Gram-positive bacterium that has shown major positive effects in sustainable agriculture, medicine, and biotechnology (4); thus, *Bacillus* and *Paenibacillus* have become attractive biological control agents. Finally, during the last 2 decades, Gram-negative *Acinetobacter* bacteria have been reported for the first time as new possible biocontrol agents. However, some species, such as *Acinetobacter baumannii*, are highly pathogenic for humans (5).

Four possible biocontrol strains were isolated from the phyllosphere of healthy tomato and lettuce crops in Groningen, the Netherlands. Briefly, 2 g of leaves from tomato and lettuce were macerated to a homogenous liquid state using 5 ml of 10 mM MgSO₄ buffer. Serial dilutions were prepared, and 1 ml of each dilution was exposed to heat treatment of 80°C for 15 min. After the heat treatment, the dilutions were spread onto separate LB agar plates and incubated at 28°C for the next 72 h. Colony growth was monitored every day, and each colony was isolated and cultured on a separate plate. The isolated colonies were finally grown overnight in liquid LB medium at 28°C with shaking at 220 rpm, and stocks were created by using glycerol at 80% solutions with 720 μl of culture and 750 μl of glycerol and stored at –80°C.

For genome sequencing, strains from the –80°C glycerol stocks were streaked onto LB agar plates, and a single colony of each strain was grown in 3 ml of LB medium at 28°C with shaking at 220 rpm. Overnight cultures of the 4 strains in LB medium were collected by centrifugation.

Genomic DNA was isolated with a GenElute bacterial genomic DNA isolation kit (Sigma-Aldrich) according to the manufacturer's protocol. The genomes were sequenced

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TABLE 1 Genome features and GenBank accession numbers of the strains

Strain	Genome size (bp)	G+C content (%)	No. of coding sequences	N_{50} (bp)	No. of contigs	Coverage (×)	No. of RNAs	GenBank accession no.	SRA accession no.
<i>Bacillus subtilis</i> STRP31	4,362,346	43.9	4,987	1,049,484	931	>150	97	JABBYF000000000	SRX9245527
<i>Bacillus velezensis</i> SPL51	4,390,708	45.5	4,522	229,106	57	>150	70	JABBYI000000000	SRX9245528
<i>Paenibacillus</i> sp. strain PL91	8,014,288	47.3	7,583	409,462	80	>150	65	JABBYG000000000	SRX9234476
<i>Acinetobacter baumannii</i> PL81	4,001,457	38.9	3,575	221,958	40	>150	66	JABBYH000000000	SRX9234475

at BGI Tech Solutions (Hong Kong), with an Illumina HiSeq sequencing system. On average, 5 million paired-end raw reads (150 bp) were generated per sample. FastQC version 0.11.9 (6) was used to examine the quality of the reads, and low-quality reads were removed with Trimmomatic version 0.38 (7). The reads were assembled *de novo* using SPAdes version 3.11.1 with default parameters (8). The genome sequences were annotated by the NCBI using the Prokaryotic Genome Annotation Pipeline (PGAP) and are publicly available (9).

Within the manuscript, the draft genome sequences were annotated with the Rapid Annotations using Subsystems Technology (RAST) server and identified as *Bacillus*, *Paenibacillus*, and *Acinetobacter* by phylogenetic analysis with available whole-genome sequences (10). Genome mining was conducted with antiSMASH, showing potential novel nonribosomal peptides (NRPs) and polyketides (PKs), which are under investigation (11).

Data availability. The draft genome sequences of the 4 strains have been deposited in GenBank under the accession numbers listed in Table 1. The raw reads have been registered and submitted to the Sequence Read Archive (SRA) under the accession numbers listed in Table 1.

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