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Draft Genome Sequences of *Bacillus velezensis* Strains AF_3B and OS2, *Bacillus amyloliquefaciens* Strain BS9, *Bacillus halotolerans* Strain A1, and *Bacillus* sp. Strain BS3, Producing Biosurfactants with Antimicrobial Potential

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ABSTRACT Five environmental *Bacillus* strains were sequenced, of which three were isolated from the rhizosphere of agricultural soil and one each from Attock Oil Refinery and Khewra Salt Mine in Pakistan. The strains can be used for plant growth promotion and biosurfactant activity brought about by secondary metabolites.

The demand for biosurfactants is rising, and members of the genus *Bacillus* are the pivotal in their production (1). Biosurfactants can lower the critical micelle concentration and surface tension of water (2–4). They can replace synthetic surfactants due to their strong antimicrobial potential against a range of pathogens (5).

Bacilli (6) are well-studied producers of secondary metabolites, including lipopeptides, bacteriocins, terpenes, polyketides, and lanthipeptides, used as biocontrol for plant growth promotion. Almost, 4 to 5% of the genome of *Bacillus* species is responsible for the production of these antagonistic metabolites (6, 7).

Five potential *Bacillus* strains were isolated from various regions of Pakistan. Two strains (AF_3B and A1) were isolated from the soil rhizosphere around the roots of wheat plants, and strain BS9 was isolated from the rhizosphere around the roots of the cotton plants. The other two strains (OS2 and BS3) were isolated from oily sludge at Attock petroleum oil refinery and salty water collected from Khewra Salt Mine in Pakistan, respectively. The soil and oil samples collected were dissolved in sterile distilled water and incubated at 37°C for 24 h until the optical density at 600 nm (OD_{600}) was observed to be above 0.5. The samples were serially diluted; 1 mL of a 10^9 dilution was spread onto LB agar plates and incubated at 37°C for 48 h. Bacterial colonies on agar plates were selected on the basis of apparent *Bacillus*-like colony morphology, circular, rough, opaque, or slightly yellowish with lifted edges. Each selected colony was monitored using the DeltaVision system (Applied Precision, Washington) with an IX71 phase contrast microscope (Olympus, PA, USA) for further confirmation of spore-forming rod-shaped cell morphology.

For genome sequencing, a single colony from the LB plate was cultured in LB broth and incubated at 37°C and 20 rpm for 16 h. Cells were harvested by centrifugation at 10,000 rpm for 20 min at 4°C (Beckman Coulter Avanti centrifuge), and DNA extraction was performed using the manufacturer's protocol with the GenElute bacterial genomic DNA isolation kit (Sigma-Aldrich, Germany). The 16S rRNA genes of the five selected isolates were amplified using 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-ACGGTACCTGTGAC GACTT-3' as the forward and reverse primers, respectively (8), and sequenced, and a BLASTN analysis was performed to confirm that all five isolates belonged to the genus *Bacillus*. The sequences have been submitted to GenBank and accession numbers are given in Table 1.

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The authors declare no conflict of interest.

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TABLE 1 Genomic properties and accession numbers for five *Bacillus* strains

| Bacterial strain | Genome size (bp) | G + C content (%) | No. of coding sequences | No. of reads | N ₅₀ (bp) | No. of contigs | Coverage (x) | No. of RNAs | GenBank accession no. for: | | SRA accession no. |
|---------------------------------------|------------------|-------------------|-------------------------|--------------|----------------------|----------------|--------------|-------------|----------------------------|---------------|-------------------|
| | | | | | | | | | Whole genome | 16S rRNA gene | |
| <i>Bacillus velezensis</i> AF_3B | 3,949,216 | 46.3 | 4,041 | 6,159,113 | 581,547 | 28 | 150 | 65 | JAJBB0000000000 | OL771696.1 | SRX13297035 |
| <i>Bacillus velezensis</i> OS2 | 3,968,045 | 46.3 | 4,054 | 6,183,522 | 418,400 | 32 | 154 | 65 | JAJBCC0000000000 | OL771697.1 | SRX13452167 |
| <i>Bacillus amyloliquefaciens</i> BS9 | 3,949,726 | 46.3 | 4,034 | 6,158,224 | 428,550 | 30 | 152 | 65 | JAJOLX0000000000 | OL757572.1 | SRX13404315 |
| <i>Bacillus halotolerans</i> A1 | 4,058,484 | 43.8 | 4,205 | 6,201,404 | 525,368 | 29 | 156 | 71 | JAJEIN0000000000 | OL757644.1 | SRX13187240 |
| <i>Bacillus</i> sp. BS3 | 4,351,328 | 45.9 | 4,652 | 6,159,214 | 399,602 | 27 | 150 | 69 | JAJNDM0000000000 | OL757643.1 | SRX13347513 |

The genomes of the five screened strains were sequenced using an Illumina HiSeq sequencing system at BGI Tech Solutions (Hong Kong). For each sample, a minimum of 5 million high-quality paired-end raw reads (150 bp) were acquired (Table 1). The quality of the reads was assessed using FastQC version 0.11.9 (9), and contamination by low-quality reads was removed using Trimmomatic version 0.38 (10). The high-quality reads were assembled using Unicycler version 0.4.8 (11) embedded in SPAdes version 3.12.0 (12). All software was used with default parameters. The genome sequences were annotated by NCBI using the Prokaryotic Genome Annotation Pipeline (PGAP) (13) and identified by phylogenetic analysis as *Bacillus* spp. (13).

Data availability. The draft genome sequences of the five selected strains have been submitted to GenBank under the accession numbers listed in Table 1.

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