






Draft Genome Sequences of Six Isolates of the *Bacillus cereus* Group Isolated from Pet Reptiles

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ABSTRACT Bacteria of the *Bacillus cereus* group are Gram-positive rods and are widespread in nature, but little information is currently available about their presence in reptiles. Here, we report draft genome sequences of six *Bacillus* isolates belonging to three species, namely, *Bacillus cereus*, *Bacillus paranthracis*, and *Bacillus toyonensis*, isolated from pet reptiles in Poland.

The *Bacillus cereus* group consists of Gram-positive, rod-shaped, aerobic, or facultatively anaerobic bacteria that belong to 21 closely related species (1, 2). One of the most relevant characteristics of the *Bacillus* genus is the ability of the bacteria to form spores which allow them to survive under harsh environmental conditions. The bacilli are widely distributed in the environment, being present in the soil, water, and other ecological niches (3). The presence of *Bacillus* species was also confirmed in the feces of cows as well as in the gastrointestinal tracts of poultry (4). In this study, we present draft genome sequences of six fecal isolates belonging to three species, namely, *Bacillus cereus*, *Bacillus paranthracis*, and *Bacillus toyonensis*, and compare these sequences with reference genomes deposited in GenBank (5).

Fecal samples were incubated in buffered peptone water (BWP) for 18 h at 37°C, and then 10 μL of each culture was streaked onto polymyxin pyruvate-egg yolk-mannitol-bromothymol blue agar (PEMBA) (homemade) and incubated for 24 h at 37°C (6). Single colonies were passaged onto the nutrition agar and incubated overnight at 37°C (7). The pure culture was identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using the extraction method following the manufacturers' guidelines (Bruker Daltonik GmbH). After the incubation onto the nutrition agar at 37°C for 24 h, genomic DNA was extracted with the Maxwell Rapid Sample Concentrator (RSC) cultured cells DNA kit (Promega) according to the manufacturer's instructions. The concentration and quality parameters of the DNA were defined with a spectrophotometer (NanoDrop One) and a Qubit 3 fluorometer. The DNA libraries were prepared using the Nextera XT sample preparation kit following the manufacturer's recommendations. Sequencing was performed using the MiSeq reagent kit on the MiSeq platform (Illumina) with the 2 × 300-bp paired-end protocol, to 100× depth of sequencing. All bioinformatic tools were used with default parameters, except where otherwise noted. Raw paired-end reads were quality filtered using FastQC v0.11.5 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>) (8), and trimming and adapter sequence removal were executed using Trimmomatic 0.36 (ILLUMINACLIP: 2:30:10, LEADING: 3, TRAILING:3, SLIDINGWINDOW:4:15, MINLEN:36) (9). Trimmed reads were then merged using BBMerge from the bbtools software suite (<https://jgi.doe.gov/data-and-tools/software-tools/bbtools/bb-tools-user-guide/bbmerge-guide/>) and assembled using SPAdes v3.9.0 with the “-careful” flag (10). The draft genome sizes varied from 5,135,335 to 5,937,563 bp, and

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TABLE 1 Genome properties of the *Bacillus* strains isolated from reptiles in Poland

Isolate	Species	Host	Genome size (bp)	No. of contigs	Total no. of reads	Coverage	GC content (%)	N_{50} (bp)	MLST type	SRA accession no.	Genome accession no.
PIW23	<i>Bacillus paranthracis</i>	<i>Eumeces schneideri</i>	5,135,275	35	3,949,460	205×	35.3	1,174,839	Unknown (nearest STs: 869, 182, 761)	SRR18728991	JAKQA000000000000
PIW25	<i>Bacillus toyonensis</i>	<i>Testudo hermanni</i>	5,937,563	58	4,533,936	208×	35.0	648,267	ST972	SRR18728986	JAKJPV000000000000
PIW26	<i>Bacillus cereus</i>	<i>Testudo hermanni</i>	5,866,545	103	2,083,574	89×	34.9	334,014	Unknown (nearest STs: 1197, 193)	SRR18728987	JAKJPW000000000000
PIW27	<i>Bacillus toyonensis</i>	<i>Testudo horsfieldii</i>	5,646,249	63	3,602,000	192×	35.0	556,185	ST718	SRR18728988	JAKJPX000000000000
PIW28	<i>Bacillus cereus</i>	<i>Testudo horsfieldii</i>	5,534,170	93	3,694,768	200×	35.0	328,652	ST197	SRR18728989	JAKJPY000000000000
PIW162	<i>Bacillus cereus</i>	<i>Testudo horsfieldii</i>	5,442,449	29	4,761,076	263×	35.1	1,736,072	ST511	SRR18728990	JAKJPZ000000000000

the GC content was approximately 35.1%. Genomic features (number of contigs, N_{50} value, GC content, and total size) were defined with QUAST (11) and included in Table 1. Average nucleotide identity (ANI) was analyzed using the JSpeciesWS online service (12) which revealed that the PIW23 genome sequence had the highest ANI based on MUMer (ANIm) value to *B. paranthracis* (GenBank accession number GCF_001883995.1; 99.23%); PIW25 and PIW27 to *B. toyonensis* (GCA_000496285.1; >99.58%); and PIW26, PIW28, and PIW162 to *B. cereus* (GCA_000007825.1; >96.05%). The multilocus sequence type (MLST) was validated by using MLST 2.0 (<https://cge.cbs.dtu.dk/services/MLST/>) (13). The MLST scheme for *B. cereus* was applied to all isolates. The MLST type was found in PIW25, PIW27, PIW28, and PIW162, and other isolates (PIW23, PIW26) were identified as an unknown sequence type (ST).

Data availability. The draft genome sequences reported here were deposited in GenBank under the BioProject accession number [PRJNA799608](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA799608). The raw sequence read and genome assembly accession numbers are listed in Table 1.

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