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GENOME SEQUENCES

Complete Genome Sequence of *Paenibacillus* sp. Strain E222, a Bacterial Symbiont of an *Epichloë* Fungal Endophyte of Ryegrass

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ABSTRACT We report on the whole-genome sequence of *Paenibacillus* sp. strain E222, a bacterium isolated from a fresh culture of *Epichloë festucae* var. *lolii*, a mutualistic fungal endophyte of perennial ryegrass. The genome has a size of 7.8 Mb and a G+C content of 46% and encodes 6,796 putative protein-coding genes.

Many bacteria form symbiotic associations with plant-associated fungi and can be located either within the body or cells of the fungus (termed endosymbionts) or on the body surface of the fungus (termed ectosymbionts). Although these types of bacteria are associated mainly with fungi, they can also directly affect plant fitness by modulating plant performance and/or by regulating the performance of their plant-associated fungi (1). Bacterial symbionts of fungi have been isolated from many fungal species that form intimate associations with plants (e.g., references 2 and 3). Here, we report the draft genome sequence of *Paenibacillus* sp. strain E222, a bacterium isolated from the cultured mycelia of *Epichloë festucae* var. *lolii*. This fungal strain was isolated from a perennial ryegrass plant according to the method published by Latch and Christensen (1985), with the exception that the agar medium did not contain antibiotics (4). *Epichloë festucae* var. *lolii* has coevolved with perennial ryegrass of the family Poaceae, subfamily Pooideae, with which they form long-lived, mutualistic associations (5). The bacterium was isolated by grinding *Epichloë* fungal mycelia with sterilized glass beads immersed in nutrient broth. After grinding and centrifugation, an aliquot of the supernatant was transferred onto nutrient agar and incubated at 28°C for 48 h in the dark. No other microbial growth was observed.

For sequencing purposes, cells of *Paenibacillus* sp. strain E222 were obtained from a single bacterial colony, transferred to 25 ml of Luria-Bertani broth (pH 8), and incubated at 28°C for 48 h at 300 rpm. The genomic bacterial DNA was extracted using the Qiagen blood and cell culture DNA kit (Bio-Strategy Ltd.) following the manufacturer's instructions. After extraction, the DNA was precipitated with phenol-chloroform (6), and the pellet was purified using the Zymo Clean and Concentrator-25 kit (Ngaio Diagnostics Ltd.). The genomic DNA was sequenced on a Pacific Biosciences (PacBio) Sequel instrument using a library constructed with the SMRTbell express kit and SMRTbell barcoded overhang adapter kit (PacBio, Inc.) as part of a multiplexed experiment. The run produced 160,303 reads with an average length of 9 kb, an N_{50} value of 43.5 kb, and a total output of 1.46 Gb, attaining a coverage of 208-fold. The reads were assembled using Canu version 1.6 with default parameters and an estimated genome size of 5 Mb (7). The assembly process produced one single contig of 7.5 Mb. A BUSCO test was run with this genome assembly using the bacterial database odb9, producing a completeness score of 99.3% (147 complete sets, 0 duplicated, 0 missing, 1 fragmented) (8). A CheckM test produced a completeness score of 99.85 and a contamination score of 0.14 (9).

The whole genome of *Paenibacillus* sp. strain E222 has a size of 7.5 Mb with a G+C content of 46%. The genome annotation was carried out using GAMOLA2 (10). The

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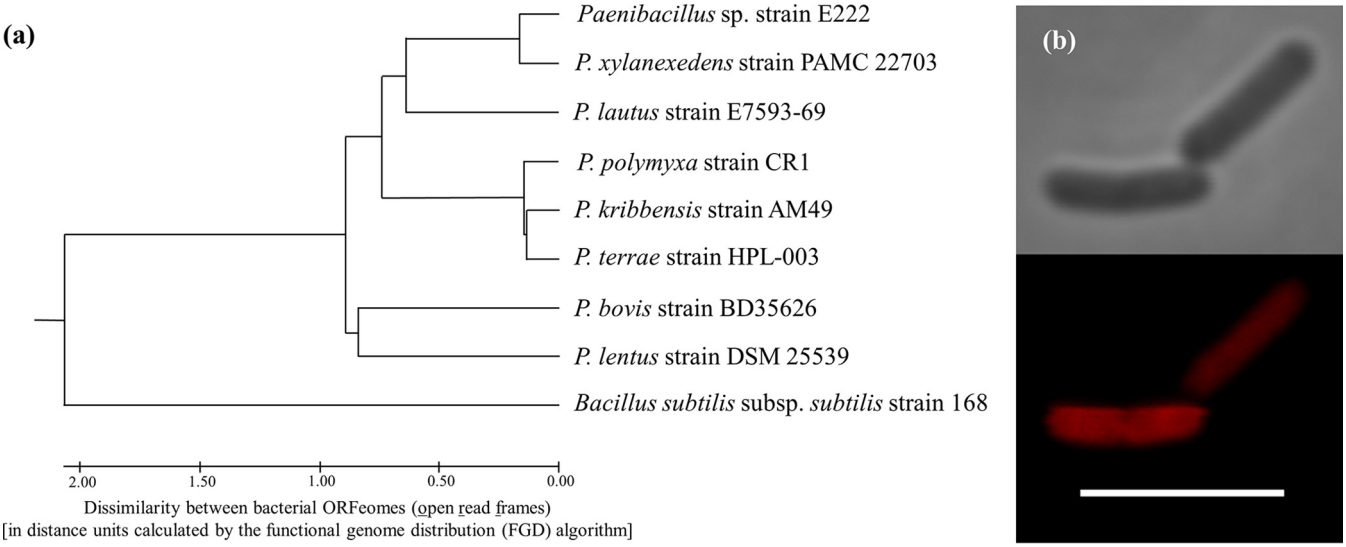

FIG 1 (a) Genome dissimilarity tree between *Paenibacillus* sp. genomes. The genome differences were determined after conducting a functional genome distribution (FGD) analysis (15). This analysis calculates the similitudes between amino acid sequences predicted from bacterial open read frames (ORFeomes). Complete genomes were downloaded from the NCBI genome database and annotated using GAMOLA2 (10) (NCBI accession numbers GCA_001908275.1, GCA_003590055.1, GCA_000507205.2, GCA_002240415.1, GCA_000235585.1, GCA_001421015.2, GCF_003931855.1, and GCA_000009045.1). The tree was inferred using the unweighted pair group method with arithmetic mean (UPGMA) algorithm and drawn to scale in MEGA7 (16), with branch lengths in the same units as those of the distances used to infer the tree. The *Bacillus subtilis* genome was used as an outgroup. The tree scale bar indicates the dissimilarity between bacterial ORFeomes, and approximated branch lengths are depicted by distance units (dus), which reflect the numeric dissimilarity value calculated by the FGD algorithm (see more details about du calculations in reference 15). (b) Micrographs of *Paenibacillus* sp. E222. Bacterial cells were hybridized with the EUB338 oligonucleotide probe labeled with the Cy3 fluorophore. This probe specifically hybridizes with bacterial 16S rRNA genes (17). The top micrograph shows *Paenibacillus* sp. cells under phase-contrast illumination, whereas the bottom image displays the same cells (and field) hybridized with the EUB338 probe. The micrograph scale bar represents 5 μm.

annotated genome contained 6,932 genes, with 6,796 total coding sequences, 110 tRNA genes, 86 rRNA genes, 113 noncoding RNA genes, and 1 CRISPR/CAS system. The whole *Paenibacillus* sp. E222 genome sequence was most similar to that of *Paenibacillus xylanexedens* PAMC 22703 (Fig. 1a). *Paenibacillus* sp. E222 has a rod-shaped morphotype like others within this clade and is a facultative symbiont of *E. festucae* var. *lolii* (Fig. 1b). Preliminary microscopic examination of *E. festucae* var. *lolii* mycelia enriched with cells of *Paenibacillus* sp. E222 suggested that this bacterial strain was not an endosymbiont and that it established an ectosymbiotic relationship with this fungal species. In order to predict the potential of *Paenibacillus* sp. E222 to produce secondary metabolites, the genome of this strain was analyzed with the antiSMASH software (version 5.1.0) (11). This analysis predicted that the genome contained nine gene clusters coding for enzymes involved in the biosynthesis of lanthipeptides, type III polyketides, nonribosomal peptides, bacteriocins, lasso peptides, siderophores, and terpenes. Some of these compounds have been identified and isolated from *Paenibacillus* species (12–14).

Data availability. The genome assembly and the raw sequence data have been deposited in the NCBI nucleotide and SRA databases under accession numbers CP058552 and PRJNA641937, respectively. Data were also deposited under SRA accession number SRR12094900.

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