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Short Communication

Draft genome sequences of *Enterococcus durans* EDD2 strain associated with honeybees

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Abstract: We report the annotated draft genome sequence of *Enterococcus durans* EDD2 strain isolated from freshly collected pollen granules derived from three different beehives. This isolate showed strong inhibitory activity *in vitro* against *Paenibacillus larvae* ATCC 9545; thus the genome sequence presented here will contribute to a better understanding of its protective potential against bacterial pathogen invasion, as well as to the assessment of its beneficial potential for the bee colonies.

Keywords: draft genome sequence; American foulbrood disease (AFB); *Paenibacillus larvae*; *Enterococcus durans*

Apis mellifera is among the most prominent natural pollinators of agricultural crops worldwide. In addition, its economic importance in apiculture is also quite significant. Honey bees can be affected by various types of diseases including the American foulbrood (AFB) that is the most severe bacterial condition in this group. It is caused by the spore-forming bacteria *Paenibacillus larvae* ssp. [1]. This infection is not stress related, and can affect even the strongest colony in an apiary with devastating effects and considerable financial losses. Given both the economic value of *Apis mellifera* species, and its prominence in nature, it is important to develop novel efficient approaches to control the AFB disease. Furthermore, the use of antibiotics cannot be considered as an option, not only due to the rapid emergence of resistant bacteria but also because of the strict EU regulations, requiring zero tolerance for the presence of such compounds in honey and bee products. In light of the above factors, the application

of bacterial species that are part of the normal honeybee associated microbiota, and can produce antimicrobial compounds active against *P. larvae,* raises as an important alternative to control the highly infectious AFB disease.

Enterococci are Gram-positive lactic acid bacteria (LAB) with ubiquitous presence in the environment, various types of fermented food and the gastrointestinal tracts of diverse animals [2]. Some *Enterococcus* strains have already been isolated from the gut of the bees *Apis mellifera* indicating the presence of these microbes in beehives [3]. Here, we report the draft genome sequences of *Enterococcus durans* EDD2 strain, which showed strong inhibitory activity *in vitro* against *P. larvae* ATCC 9545, determined by the well diffusion method. The protein nature of the inhibitory agent was confirmed by proteinase K treatment which led to a loss of the inhibitory activity. To our knowledge till now this is the first beehive proliferating *E. durans* strain which was subjected to whole-genome sequencing in order to characterize it by a genomic analysis.

The *E. durans* EDD2 strain was isolated from freshly collected pollen granules derived from three different beehives in a small apiary located in Dushantzi village (Pirdop region, Bulgaria). Overnight culture was grown in BHI broth at 30°C, and total genomic DNA was extracted using the "Gram Plus & Yeast Genomic DNA Purification Kit" (EURx) according to the manufacturer's instructions. The 2 × 150 bp Illumina HiSeq paired-end genome sequencing of *E. durans* EDD2 was carried out by the BGI Genomics Company (BGI, China). It resulted in total of 4,370,827 raw read pairs that were uploaded to the Galaxy online platform [4]. These read pairs where then inspected with FastQC [5], and finally Trimmomatic [6] was used to remove adapters and discard sequences with a low Phred score.

Initial *de novo* assembly of the processed reads was performed using the Shovill pipeline with default settings [7]. Additionally, prior to assembly, the reads were randomly subsampled down to approximately 100× coverage. The assembly consisted of 139 contigs with sizes larger than 500 bp (largest contig: 155,300 bp). Analysis with QUAST [8] revealed its metrics which indicated that the final draft genome comprised of 2,961,262 bp with a mean GC content of 37.71% and N_{50} value of 57,760 bp. Next, raw assembled contigs were re-ordered against the genome of *E. durans* KLDS6.0930 (GCA_001267865.1) using Mauve [9]. *E. durans* KLDS6.0930 was selected as a reference due to its probiotic properties, and the accessible complete genome sequence of this strain [10]. Aligning both genomes (using the progressive Mauve algorithm) resulted in one large block of correspondence, indicating an overall relatedness between these two sequences. Additional analysis with MiGA [11] revealed that the selected *E. durans* KLDS6.0930 genome is the most related representative one in the database of this tool. Both genomes have an average nucleotide identity (ANI) of 99.71%, and share 91.6% of their proteins with average amino acid identity (AAI) of 99.76%. Annotation of the *E. durans EDD2* draft genome was carried out with NCBI's Prokaryotic Genome Annotation Pipeline (PGAP) [12], which predicted 2,648 protein-coding genes, 3 complete rRNA gene clusters (5S, 16S, 23S) and 62 tRNA genes. In addition, in two contigs PlasmidFinder tool [13] identified plasmid replication related sequences. Having in mind that enterococci are considered as opportunistic human pathogens [14], both the resistome and the virulome of *E. durans* EDD2 were assessed using ResFinder 3.2 and VirulenceFinder 2.0 respectively [15]. The presence of genetic determinants of bacteriocins was monitored through the BAGEL4 mining platform [16] which revealed two putative clusters for production of Enterocin L50A and Enterocin P variants.

To the best of our knowledge, *E. durans* EDD2 is the first beehive-associated isolate of this species that produces bacteriocins active against *P. larvae.* Previous study reports the isolation of one *E. durans*

strain from adult worker bee intestines, but further investigations demonstrate absence of inhibitory activity *in vitro* against *P. larvae* [17,18]. In contrast, the strain EDD2 can play a role in the defense against bacterial pathogen invasion, especially having in mind that our analysis found no virulence factors or resistance determinants for clinically important antibiotics in its genome. Indeed, one strain should also possess other important qualities in order to be considered probiotic, such as being non-pathogenic, genetically stable, and viable in high populations. Moreover, it should be able to survive the acid and bile environment, and proliferate in the intestine. Other important traits required include adhesion and colonization of intestinal cells, as well as present therapeutic benefits. The availability of the draft genome sequence described here will allow further analyses of these properties on genome scale, which would help us understand better the biological role of enterococci in honey bees and their colonies.

Data availability

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAAILD000000000. The version described in this paper is version JAAILD010000000.

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Conflict of interest

The authors declare there is no conflict of interest.

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