



Genome Sequence of *Enterococcus faecalis* NVIT04, Isolated from *Nasonia vitripennis*

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ABSTRACT *Enterococcus faecalis* is a Gram-positive, lactic acid-producing coccus which can be found as a member of the gut microbiome in many animal species and is a potential pathogen in humans. Here, we describe the genome sequence of an *E. faecalis* strain isolated from the gut microbiome of the hymenopteran model *Nasonia vitripennis*.

The *Enterococcus* genus is a consistent member of the mammalian, avian, reptilian, and insect gut microbiome and has been implicated as both a potential pathogen (1, 2) and a beneficial microorganism (3, 4). The species *Enterococcus faecalis* is typically classified as an opportunistic pathogen, especially in immunocompromised patients. While many strains have been reported to possess natural antibiotic resistance, there has been an increasing number of multidrug-resistant strains of the bacterium emerging since the 1970s. This surge of multidrug-resistant strains drives the need for further understanding of the genetic diversity and natural reservoirs of the genus (1).

E. faecalis has been detected in relatively low abundance in the gut microbiome of the parasitic wasp genus *Nasonia* (~2%) (5), a well-established model organism for the host-symbiont interaction (5, 6). However, the potential role of *Enterococcus* species in the health and development of the wasp host is currently unknown. We sought to assess the potential function of *E. faecalis* within the microbiome of *N. vitripennis* strain AsymCx. After surface sterilizing whole *Nasonia* animals with 10% bleach for 2 min and then homogenizing them in sterile phosphate-buffered saline (PBS), we then plated the 1× aliquots of the homogenate onto several bacterial agar medium types. Specifically, we isolated *E. faecalis* using bile esculin agar (catalog no. 48300; Sigma-Aldrich) as a selective medium and confirmed the taxonomic identity of a resulting colony by Sanger sequencing of the 16S rRNA gene before performing whole-genome sequencing.

Bacterial genomic DNA was isolated from *E. faecalis* NVIT04 cells cultured in 100 ml of nutrition broth (catalog no. 1.05443.0500; Sigma-Aldrich) medium at 30°C and 250 rpm for 18 h in a flask. The culture was centrifuged at 6,000 rpm for 5 min, and the resulting cell pellet was used for genome extraction using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany). DNA was quantified using the double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit on the Qubit 2.0 fluorometer (Life Technologies, Waltham, MA, USA).

Library preparation and genome sequencing were performed at MicrobesNG (Birmingham, United Kingdom). The genomic DNA library was prepared using the Nextera XT library prep kit (Illumina), following the manufacturer's protocol, with a Microlab STAR automated liquid-handling system (Hamilton). The library was sequenced on the Illumina HiSeq platform using a 250-bp paired-end protocol.

Sequencing resulted in 727,126 reads that were trimmed using Trimmomatic 0.36 (7), and the quality was assessed using in-house scripts (MicrobesNG, Birmingham, United Kingdom) combined with SAMtools (8), BedTools (9), and the Burrows-Wheeler Aligner MEM algorithm (BWA-MEM) (10). Contig assembly was carried out with SPAdes

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(version 3.8) (11) via MicrobesNG. This resulted in a draft genome consisting of 27 contigs, with an N_{50} value of 1,478,411 bp and a total of 2,773,768 nucleotides, resulting in approximately 110-fold coverage.

The genome of *E. faecalis* NVIT04 shared 99% nucleotide identity with available reference genomes of *E. faecalis* strains sorialis (GenBank accession no. [CP015883](#)), V583 (GenBank accession no. [AE016830](#)) (12), and DD14 (GenBank accession no. [CP021161](#)). The genome of *E. faecalis* NVIT04 consists presumably of a single chromosome (2,765,246 bp) and exhibits a G+C content of 37.46%. No plasmids were detected based on analysis with the PlasmidFinder (version 1.3) (13). Annotation was performed using Prokka 1.12 (14). The genome encodes 2,561 predicted protein-encoding genes with known functions and 794 (31.00%) coding for hypothetical proteins. The draft genome encodes 5 rRNAs and 54 tRNAs identified using RNAmmer (15) and tRNAscan (16), respectively. All the software programs mentioned above were used with default settings.

Four putative antibiotic resistance genes were identified using the CosmosID package (version August 2018; CosmosID, Inc., Rockville, MD, USA), namely, a multidrug resistance efflux pump gene (*emeA*), an efflux pump gene (*isaA*), a dihydrofolate reductase gene (*dfrE*), and a macrolide resistance gene (*mphD*). Likewise, 18 virulence factors were identified using the CosmosID package, namely, a collagen adhesion gene (*ace*), pheromoneprecursor lipoprotein genes (*cad*, *camE*, *cCF10*, and *cOB1*), endocarditis- and biofilm-associated pilus genes (*ebpA* and *ebpC*), an endocarditis antigen A gene (*efaAfs*), a gene encoding enterococcal leucine-rich internalin-like protein A (*elrA*), a gelatinase gene (*gelE*), a hyaluronidase gene (*hylA*), a sortase A gene (*srtA*), a thiol peroxidase gene (*tpx*), FsrABDC signal transduction system genes (*fsrA*, *fsrB*, and *fsrC*), a gene encoding the regulation of malate uptake (*malR*), and a serine protease (*sprE*). The large number of antibiotic resistance genes and virulence factors identified in the *E. faecalis* NVIT04 genome analysis indicates the potential of isolates to be pathogenic and supports the diverse *E. faecalis*-host relationships across animal species.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. [QUAG00000000](#). The version described in this paper is the first version, QUAG01000000, and the GenInfo identifier (GI) no. is 1467817911 2. The BioProject accession no. is [PRJNA484798](#) for the SRA data.

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