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Draft Genome Sequences of Five *Enterococcus* Species Isolated from the Gut of Patients with Suspected *Clostridium difficile* Infection

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ABSTRACT We present draft genome sequences of five *Enterococcus* species from patients suspected of *Clostridium difficile* infection. Genome completeness was confirmed by presence of bacterial orthologs (97%). Gene searches using Hidden-Markov models revealed that the isolates harbor between seven and 11 genes involved in antibiotic resistance to tetracyclines, beta-lactams, and vancomycin.

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Numerous reports link microbial compositional changes (dysbiosis) in the human gut microbiota to diverse disease states ranging from inflammatory illness to psychiatric conditions (1–4). In particular, studies in human and animal models have shown the importance of the gut microbiota's capability of providing colonization resistance against *C. difficile* (5, 6). Consequently, this announcement is part of a larger project aimed at characterizing the microbiota of individuals infected by *C. difficile* in Chile, both in terms of individual isolates and microbiota compositions.

We collected fecal samples from patients suspected of being infected by *C. difficile* that presented aqueous diarrhea associated with antimicrobial drug intake. We plated samples on blood agar medium (Merck; anaerobic conditions) and grew colonies in Brucella broth at 37°C without agitation (7). For DNA extraction, we used the Wizard genomic DNA purification kit (Promega) following the manufacturer's instructions. DNA was quantified in a fluorimeter (Qubit; Invitrogen) and its integrity was checked by agarose gel electrophoresis. We prepared sequencing libraries as in the TruSeq nano DNA LT kit (Illumina) using an average insert size of 450 bp.

We obtained between 1.7 and 2.3 million paired-end reads that we subsequently filtered to allow no undetermined bases and an average quality score per read of $>$ Q20. We also trimmed the 5′ and 3′ ends to remove bases with quality scores of $<$ Q20. The resulting reads were *de novo* assembled using a De Bruijn graph strategy as implemented in SPAdes 3.8 (8). Genome coverage was 48 to 173× (median = 109×). We interrogated the resulting contigs for evidence of contamination using the GenomePeek web server and found no evidence of contaminating DNA (9). We annotated the assembled genome sequences using the NCBI Prokaryotic Genome Annotation Pipeline (released 2013) (10). Information about the Pipeline can be found here: https://www.ncbi.nlm.nih.gov/genome/annotation_prok/. Additionally, genome completeness was confirmed by BUSCO analysis of prokaryotic orthologs, where we found 97% of bacteria-wide orthologs present (11). Current standards suggest that genomes with $>$ 85% orthologs present are considered high-quality genomes (reference *E. faecium* was 93% complete; accession no. NC_017960.1) (12).

TABLE 1 Whole-genome shotgun projects accession numbers

Species	Strain	Source	Genome size (Mb)	No. of coding sequences	Accession no.	Assembly
Enterococcus faecium	97-19_S17	Human feces	2.7	2,915	MRYE00000000	GCA_001990575.1
Enterococcus faecium	97-3_S3	Human feces	2.9	3,165	MRYF00000000	GCA_001990605.1
Enterococcus faecium	97-6_S5	Human feces	2.5	2,698	MRYG00000000	GCA_001990565.1
Enterococcus faecium	97-7_S6	Human feces	3.1	3,390	MRYH00000000	GCA_001990615.1
Enterococcus mundtii	CGB1038-1_S1	Human feces	3.3	3,150	MSTR00000000	GCA_001990645.1

Of the five genome sequences presented here, only two were classified as known multilocus sequence types (97-19_S17 and 97-7_S6 as ST262 and ST822, respectively) (13). However, all strains were found to carry antibiotic resistance genes including *vanR* and *vanS* genes (ARO:3000574; ARO:3000071), *vanX*, and *vanY* genes (ARO:3000011; ARO:3000077), class B beta-lactamase (ARO:3000004), antibiotic efflux pumps (ARO:0010001), and tetracycline resistance genes (ARO:3000186; ARO:3000194; ARO:3000190; ARO:3000192; ARO:3000239; ARO:0000002), among others (14). This report highlights the need for comprehensive open genomic reference databases of human gut members to better address scientific questions regarding epidemiology, virulence and pathogenicity, and drug resistance. All five genomic sequences are compliant with the MIGS package "cultured bacteria/archaea, human-associated; version 4.0" (15).

Accession number(s). The whole-genome shotgun projects have been deposited in GenBank under the accession numbers provided in Table 1. The versions described in this paper are the first versions.

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