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GENOME SEQUENCES

Complete Genome Sequence and Annotation for *Romboutsia* sp. Strain CE17

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ABSTRACT Here, we describe the complete genome sequence of *Romboutsia* sp. strain CE17, which was isolated during a screen for spore-forming anaerobic microorganisms that colonized the surface of chicken eggs.

Romboutsia is a genus of obligately anaerobic bacteria commonly detected in 16S rRNA gene amplicon studies of the gastrointestinal (GI) tract of numerous vertebrate species, including poultry (1–4). Here, we describe the complete genome sequence of *Romboutsia* sp. strain CE17, which was isolated from the surface of a white leghorn chicken eggshell. *Romboutsia* sp. CE17 was isolated during a screen for spore-forming anaerobes on chicken eggshells as part of a 2018 study of bacterial succession in the poultry GI tract (National Animal Disease Center, Ames, IA) (5). Eggs were washed in 1× phosphate-buffered saline to suspend eggshell-associated bacteria. The bacterial suspension was then treated with an equal volume of 70% ethanol for 4 h, to kill non-spore-forming bacteria, prior to plating on brain heart infusion (BHI) broth supplemented with 0.1% whole chicken bile. Resultant colonies were selected for DNA sequencing.

Romboutsia sp. CE17 was grown anaerobically at 42°C on BHI broth supplemented with 0.1% whole chicken bile prior to genomic DNA extraction. After 48 h of growth, total genomic DNA was extracted using the PureLink genomic DNA extraction minikit (Life Technologies, Carlsbad, CA), following the manufacturer's instructions. The quality of extracted DNA was assessed using a Qubit fluorimeter (double-stranded DNA [dsDNA] broad-range [BR] kit; Life Technologies), a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA), and gel electrophoresis. The same DNA preparation was used for both Illumina MiSeq and Nanopore sequencing. A Nextera Flex barcoding kit (Illumina, San Diego, CA) was used to prepare a genomic library for Illumina MiSeq sequencing, according to the manufacturer's instructions, and sequencing was carried out on a MiSeq instrument (Illumina). A genomic library for Nanopore sequencing was prepared using the SQK-RBK004 rapid barcoding kit (Oxford Nanopore Technologies, Oxford, UK), according to the manufacturer's instructions. A FLO-MIN106 R9.4.1 flow cell was used for sequencing on a MinION instrument (Oxford Nanopore), which was run for 48 h.

A total of 493,845 paired-end Illumina reads (2 × 250 bp) were generated. Quality was assessed with FastQC v0.11.9 (6). MinION reads were base called, demultiplexed, and trimmed with Guppy v3.1.5 (7). MinION reads with a quality score of ≥7 were kept for downstream assembly. A total of 183,317 MinION reads, with an average length of 3,288 bp (N_{50}, 5,484 bp), were generated. Illumina (70.8× genomic coverage) and Nanopore (193.4× genomic coverage) reads were assembled into a single circular chromosomal contig with Unicycler v0.4.7 using the "bold" setting without rotation (8).

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This contig was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v4.11 (9).

The genome of *Romboutsia* sp. CE17 is composed of a single contiguous chromosome that is 3,116,687 bp long. The GC content of *Romboutsia* sp. CE17 is 28.2%. *Romboutsia* sp. CE17 has 2,845 protein-coding sequences, including 40 pseudogenes. The *Romboutsia* sp. CE17 genome contains 89 tRNA genes and 16 rRNA operons, similar to other members of the genus *Romboutsia* (1).

Data availability. This genome sequence has been deposited in GenBank under the accession no. CP051144. The Illumina MiSeq reads and Nanopore reads are available in the NCBI Sequence Read Archive (SRA) under accession no. SRR11475325 and SRR11475326, respectively.

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