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GENOME SEQUENCES

Draft Genome Sequence of *Clostridium senegalense* Strain AGRFS4, Isolated from a Dairy Farm in New Zealand

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ABSTRACT We report the draft genome sequence of a new *Clostridium senegalense* strain, AGRFS4, which was isolated from a dairy farm environment in the Manawatu region in New Zealand. The genome is 3.98 Mb, with a GC content of 27%. The genome sequence was found to be 86.6% similar to that of the type strain *Clostridium senegalense* JC122. Until now, no *Clostridium senegalense* strain from New Zealand has been reported.

The genus *Clostridium* consists of more than 180 species of obligate and facultative anaerobic rod-shaped bacilli that are capable of producing highly heat- and chemical-resistant endospores (1, 2). While some species, such as *Clostridium botulinum*, *Clostridium difficile*, *Clostridium tetani*, and *Clostridium perfringens*, are known pathogens (1, 3–6), *Clostridium butyricum* can be used as a probiotic (7, 8).

Clostridium senegalense JC122 is a type strain that was first isolated from a stool sample from a healthy male subject in Dielmo, Senegal; its draft genome sequence was published in 2012 (9). Here, we report the whole-genome sequence of a new *C. senegalense* strain, AGRFS4, which was isolated from a soil sample from a New Zealand dairy farm. The sequences obtained will be used to investigate any pathogenic or beneficial traits of this isolate.

Bacteria were isolated using previously described methods (10). Briefly, 10 g of soil was suspended in 50 ml of phosphate buffer (PB) and centrifuged at 3,466 × *g* for 1 h. The pellet was resuspended in 5 ml of PB and heated at 80°C for 10 min. One milliliter of the heated sample was added to cooked meat-glucose-starch medium (11) and incubated anaerobically at 35°C for 48 h. The growth suspension was serially diluted, plated on Shahidi-Ferguson agar (12), and incubated anaerobically for 24 h to yield pure colonies. Genomic DNA was extracted from the pure cultures grown in tryptic soy broth (Fort Richard Laboratories, New Zealand) by using a phenol-chloroform extraction method (13). The quality and concentration of DNA were determined using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, USA). Initial identification was conducted using 16S rRNA amplicon sequencing with the forward primer pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and the reverse primer pH* (5′-AAGGAGGTGATCCAGCCGCA-3′) (14). The amplification method consisted of 93°C for 3 min; 92°C for 1 min, 55°C for 1 min, and 72°C for 2 min for 30 cycles; and a final extension at 72°C for 3 min.

The whole genome of *C. senegalense* strain AGRFS4 was prepared with the NuGEN Celero PCR workflow with enzymatic fragmentation DNA library preparation kit and sequenced using the Illumina MiSeq version 3 sequencing platform (Massey Genome Services, Palmerston North, New Zealand) to produce 500,848 read pairs of 300 nucleotides and 301,510,496 bp, giving ~75-fold coverage. The reads were quality trimmed, filtered, and assembled via the A5-miseq pipeline version 20160825 with default settings (15). The assembly produced 95 contigs with a total genome size of 3.98 Mb,

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an N_{50} value of 14 kb, and a GC content of 27%. A BUSCO version 3.0.2 (16) test using the bacterial reference produced a completeness score of 98%.

Analysis of 16S rRNA sequencing data showed 98.2% sequence similarity between *C. senegalense* AGRFS4 and *C. senegalense* JC122^T. A comparative genomic analysis was performed with the genome sequences of these organisms using the Type (strain) Genome Server (TYGS) (https://tygs.dsmz.de). *In silico* digital DNA-DNA hybridization (dDDH) was used to calculate genome-to-genome distances (17), which resulted in a dDDH (d_6) value of 86.6%, indicating the same species but with probable differences at the strain level. Further studies are required to investigate these differences.

Data availability. The raw reads have been deposited in the NCBI database under accession number PRJNA605262. This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under accession number JAAGPU000000000.

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