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GENOME SEQUENCES

Draft Genome Sequence of *Clostridium cochlearium* Strain AGROS13, Isolated from a Sheep Dairy Farm in New Zealand

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ABSTRACT We report the draft genome sequence of a new *Clostridium cochlearium* strain, AGROS13, which was isolated from a sheep dairy farm environment in New Zealand. The genome is 2.7 Mbp, with a GC content of 28.2%. The genome sequence was found to be closely related to that of *Clostridium cochlearium* ATCC 17787. The new strain harbors a biosynthetic gene cluster coding for an unknown sactipeptide.

Clostridium species are obligate or facultative anaerobic bacteria, producing endospores that are highly resistant to heat and other environmental factors (1, 2). Some *Clostridium* species are well-known pathogens (1, 3–6), whereas some are detrimental to milk and dairy product quality (7, 8). Of all the important *Clostridium* species known, not much has been stated about *Clostridium cochlearium*, a species that has been found to spoil dairy products and also has been isolated from infant formula milk (9, 10). Here, we report the whole-genome sequence of a new *Clostridium cochlearium* strain, AGROS13, which was isolated from a New Zealand sheep dairy farm silage sample.

Bacteria were isolated using a previous methodology, with slight modifications (11). Briefly, 20 g of silage was weighed in a stomacher bag, suspended in 50 ml of phosphate buffer to blend the sample, and centrifuged at 3,466 × *g* for 1 h. The pellet was resuspended in 10 ml of phosphate buffer and heated at 80°C for 10 min. One milliliter of the heated sample was added to cooked meat-glucose starch medium (12) and incubated anaerobically at 35°C for 48 h. The growth suspension was serially diluted, plated on Shahidi-Ferguson agar, and incubated anaerobically for 24 h (13). Proteolytic activity was preliminarily investigated by visualizing a clear zone around the bacterial growth on a skimmed milk agar plate (14). The presumptive *C. cochlearium* strain AGROS13 was found to be proteolytic, indicating potentially a dairy spoilage bacterium. Genomic DNA was extracted from pure cultures grown in tryptic soy broth (Fort Richard, New Zealand) by using the phenol-chloroform extraction method (15). The quality and concentration of DNA were determined using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, USA).

The whole genome of *Clostridium* strain AGROS13 was prepared with the NuGEN Celero enzymatic fragmentation DNA library kit and sequenced using the Illumina MiSeq sequencing platform version 3 (Massey Genome Services, Palmerston North, New Zealand), producing 484,293 read pairs of 300 nucleotides and 291,544,386 bp and giving a coverage of ~109-fold. The reads were quality trimmed, filtered, and assembled via the A5-miseq pipeline version 20160825 with default settings (16). The assembly produced 75 contigs, with a total genome size of 2.7 Mb, an N_{50} value of 78 kb, and a GC content of 28.2%. A BUSCO version 3.0.2 (17) test using the bacterial reference produced a completeness score of 93.9%.

A two-way average nucleotide identity test (http://enve-omics.ce.gatech.edu/ani) of

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the new *Clostridium* strain AGROS13 produced a 98.96% match with *Clostridium cochlearium* NCTC 13027 (GenBank accession number NZ_LT906477.1) (18). A comparative genomic analysis of these two genomes using *in silico* digital DNA-DNA hybridization (dDDH) via the Type (strain) Genome Server (TYGS) (https://tygs.dsmz.de) (19) resulted in a dDDH (d_6) value of 80%, indicating the same species but with probable differences at the strain level. We investigated the presence of biosynthetic gene clusters (BGCs) in strain AGROS13 using antiSMASH version 5.1.2 (https://antismash.secondarymetabolites.org) (20). The software predicted the presence of a BGC encoding an unknown sactipeptide, a ribosomally synthesized and posttranslationally modified peptide (RiPP) (21). RiPPs have been recognized as a predominant group of natural antimicrobial compounds, of which sactipeptides and lanthipeptides are the dominant ones identified in some *Clostridium* species (22, 23). Further studies are required to identify the sactipeptide and to investigate its properties. As part of the submission process, NCBI annotated the genomic scaffolds with PGAP version 4.11 (24), resulting in 2,692 genes being annotated in total.

Data availability. The raw reads have been deposited in the NCBI SRA under the accession number SRX8326676. This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number JABFIF000000000. The version described in this paper is version JABFIF010000000.

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