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GENOME SEQUENCES

Genome Sequence of a *Staphylococcus aureus* Strain Isolated from a Dairy Cow That Was Nonresponsive to Antibiotic Treatment

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ABSTRACT *Staphylococcus aureus* can cause mastitis in dairy cattle. We report the genome sequence of a *Staphylococcus aureus* strain isolated from a dairy cow with a chronic case of mastitis. The infection with this strain of *Staphylococcus aureus* was not cleared from the animal with antibiotic treatment.

Staphylococcus aureus is a pathogen that can cause mastitis in dairy animals. *S. aureus* infections can frequently become chronic and are difficult to effectively treat. There is a large variability in the clinical outcomes of *S. aureus* infection, and evidence suggests that virulence traits could be linked to various strains (1). The identification of specific genes responsible for virulence or the establishment of a chronic infection has to date not been successful. However, genetic differences that are unique to bovine-adapted strains have been found (2). Therefore, greater knowledge about the genomes of *S. aureus* isolates from dairy cows with mastitis may enhance our understanding of the genes responsible for the adaptation to cattle and for the variable severity of the disease.

An *S. aureus* strain was isolated from a multiparous Holstein cow with subclinical mastitis. The infection was treated in accordance with standard dairy procedures with a combination of Pirsue (pirlimycin hydrochloride; once daily; Zoetis) and ToDAY (cephapirin sodium; twice daily; Boehringer Ingelheim) for 5 days. Shortly after cessation of treatment, *S. aureus* was again cultured from the same quarter. A milk sample from the infected quarter was plated on blood agar, and a single colony was isolated and replated. A single colony was grown in brain heart infusion (BHI) broth and glycerol stocks and was frozen at −80°C in 10% glycerol.

The bacterial strain SA1428 was grown in BHI at 37°C overnight in a flask agitated at 180 rpm. DNA was isolated from the SA1428 strain using the Promega Wizard genomic DNA purification kit following the recommendations for Gram-positive bacteria (kit A-1120). DNA was quantified, and the quality was determined with an Implen nanophotometer (MidSci). DNA was sequenced using both Illumina MiSeq and Oxford Nanopore GridION platforms. MiSeq sequencing was performed using the Nextera DNA Flex library prep kit (Illumina) and 500-cycle v2 chemistries (2 × 250-bp paired ends). Nanopore sequencing was performed using a single FLO-MIN106 flow cell on a GridION instrument (Oxford Nanopore Technologies). The sequencing library was prepared with genomic DNA (unsheared) using Oxford Nanopore's ligation sequencing kit (SQK-LSK109). Illumina sequencing generated 369.718 Mb of reads. The raw read quality was evaluated using FastQC (3), with the adapters and low-quality reads (<Q 30) being removed using Cutadapt (4). Nanopore sequencing reads were obtained using Guppy 2.1.3 (Oxford Nanopore Technologies). A total of 12.23 Gb of reads passed with an N_{50} value of 44,231 bp and a mean read quality of 13.4 were generated and split into 132 FASTA files. Software limitations required us to split the Nanopore long sequences so

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that the first 66 files of the Nanopore long sequences were merged and *de novo* assembled with the Illumina short read sequences using Unicycler (5). This assembly resulted in 2 scaffolds. The first circularized scaffold was 2,752,113 bases long (GC content, 32.9%). The second scaffold was 25,797 bases long (GC content, 30.4%), and BLAST analysis using the NCBI nucleotide collection indicated that it was aligned to part of multiple *Staphylococcus aureus* strain plasmid sequences with an E-score of 0 and 88.13% to 98.50% identity. A second assembly was generated using the remaining 66 files of Nanopore long sequences with the Illumina short sequence reads. The second assembly contained two scaffolds, which were exactly the same as the scaffolds in the first assembly based on CLUSTAL W alignment analysis through MEGA7 (6, 7). The NCBI Prokaryotic Genome Annotation Pipeline (8) was used to identify a total of 2,756 genes (2,600 protein-coding genes, 81 RNA genes, and 75 pseudogenes).

Genomic sequencing of *S. aureus* strains with different clinical outcomes could be critical in the identification of genes that allow some strains to cause a chronic disease that is resistant to treatment versus strains that result in less severe disease. Rapid identification of strains for severity could be a tool used to determine the type of treatment and its probability of success in dairy cows.

Data availability. The whole-genome sequence project for the *S. aureus* strain SA1428 has been deposited in NCBI under the accession number CP048431. The associated plasmid has been deposited under the accession number CP048432. The raw data for this project are available at NCBI under the accession number PRJNA609126.

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