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GENOME SEQUENCES

Complete Genome Sequences of Sequence Type 71 (ST71) and ST97 *Staphylococcus aureus* Isolates from Bovine Milk

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ABSTRACT This is the announcement of draft genome sequences for *Staphylococcus aureus* strains belonging to sequence type 97 (ST97) and ST71. These sequence types are commonly associated with bovine mastitis, and the strains were isolated in Ireland in 2010 from the milk of cows with clinical mastitis.

Staphylococcus aureus is a common cause of bovine mastitis and is frequently associated with subclinical infections that are refractory to treatment (1). A number of bovine-adapted *S. aureus* lineages are responsible for the majority of cases of bovine mastitis worldwide (2). Livestock-associated *S. aureus* is also a public health concern due to the potential for zoonotic transfer and the ability to act as a reservoir of antimicrobial resistance determinants (3). Sequence type 97 (ST97) is the founder of clonal complex 97 (CC97), a globally distributed bovine-adapted lineage which has increasingly been associated with human infections (4). A subgroup of CC97, founded by ST71, was recently described. Isolates in this subgroup have undergone a genomic rearrangement of over 300 kb in the region of the origin of replication (5) and differ in their expression of some virulence traits (6). Complete genome sequences of ST71 and ST97 will contribute to comparative genomics studies of bovine-adapted *S. aureus* and to clearly defining the breakpoints between ST71 and ST97.

S. aureus strains MOK042 (ST71) and MOK063 (ST97), each isolated from a single colony, were grown overnight in 4 ml of Trypticase soy broth at 37°C. The bacterial pellet was subsequently resuspended in 3.5 ml of Qiagen buffer B2 containing 87.5 μl RNase A (4 mg/ml; Promega); 80 μl lysostaphin (10 mg/ml; Sigma-Aldrich) and 100 μl proteinase K stock solution (Qiagen) were added, followed by incubation at 37°C for 1 h. Genomic DNA was then extracted using the Qiagen Genomic-tip 100/G kit according to the manufacturer's protocol.

Whole-genome sequences were generated by the Earlham Institute (Norwich, UK) using one single-molecule real-time (SMRT) cell of a PacBio RS II sequencer (Pacific Biosciences). *De novo* assembly utilizing Hierarchical Genome Assembly Process 3 (HGAP3) (Pacific Biosciences) yielded two circular contigs for MOK042 and one circular contig for MOK063, with 216- and 259-fold coverage, respectively. The mean subread lengths for MOK042 and MOK063 were 8,682 and 9,209, respectively, and the G+C content of each isolate was 32.8%. The assembled genome sequences of *S. aureus* MOK042 and MOK063 are 2,844,513 bp and 2,808,798 bp long, respectively, while the second contig of MOK042 encoded a plasmid of 31,625 bp displaying 98% sequence identity (over 90% coverage) with plasmid pWBG707 from *S. aureus* WBG7410 (7). The Prokaryotic Genome Annotation Pipeline (PGAP) at NCBI was used to predict 3,006 genes and 2,813 protein coding sequences for *S. aureus* MOK042 and 2,907 genes and 2,722 protein coding sequences for *S. aureus* MOK063 (8). The antibiotic resistance and virulence gene profiles of these isolates have been previously described (5). Three incomplete and two intact (49.3 and 46.5 kb) prophages were identified in the genome of MOK042, and five incomplete and one intact (49.3 kb) prophages were identified in

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the genome of MOK063 using the PHAge Search Tool (PHAST) (9). Strain MOK063 also displayed a 1.72-Mbp genomic inversion relative to MOK042 and RF122 (10).

Data availability. This complete genome project has been deposited in GenBank under the accession numbers CP029627, CP029628, and CP029629.

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