Genome Sequence of *Staphylococcus aureus* Newbould 305, a Strain Associated with Mild Bovine Mastitis

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Staphylococcus aureus is a major etiological agent of mastitis in ruminants. We report here the genome sequence of bovine strain Newbould 305, isolated in the 1950s in a case of bovine mastitis and now used as a model strain able to reproducibly induce chronic mastitis in cows.

Staphylococcus aureus is one of the main pathogens responsible for ruminant mastitis. Staphylococcal mastitis severity is highly variable, ranging from subclinical to gangrenous infection. *S. aureus* strains isolated from bovine or ovine/caprine hosts differ from human isolates, as documented previously (4). However, detailed genomic data regarding ruminant isolates are still scarce (4, 5, 8, 10, 11). There is thus a need for genomic data to better understand mastitis and identify bacterial factors responsible for the severity of the disease.

We previously characterized *S. aureus* bovine strain Newbould 305 (ATCC 29740) (12), which was isolated in 1958 from a cow in Orangeville, Ontario, Canada, with a clinical case of mastitis (4, 12). Newbould 305 reproducibly induces chronic mastitis with mild symptoms in cases of experimental cow mastitis (2, 6). It was previously shown to be clonally related to other bovine strains in ST115, whereas *S. aureus* RF122, another well-documented bovine strain (8) associated with severe mastitis symptoms, clustered in ST151 and was more closely related to ovine and caprine strains yet at a considerable genetic distance (4).

We sequenced the Newbould 305 genome using an Illumina HiSeq 2000 genome analyzer (Fasteris, Geneva, Switzerland). Base calling was performed using the HiSeq Control Soft v. 1.4.8 Pipeline 1.4.0 software. After barcode selection, 12.8 million pair reads of 100 bases in length were obtained. Sequence reads were *de novo* assembled using the Edena assembler (7), version 120430dev. Assembly resulted in 28 contigs (sum, 2.79 Mbp; N_{50}, 503 Kbp; maximum, 729 Kbp; minimum, 219 bp). One of the contigs (3,379 bases) was reported as a potential complete plasmid. A total of 2,755 predicted coding sequences (CDSs) were detected by using Glimmer3 (PubMed identification no. [PMID] 17237039). More than 55% of the genes were assigned to specific subsystem categories by RAST (1). Gene products were subjected to protein location prediction using the software package SurfG+ (3). The Newbould 305 sequence was compared to that of RF122 (ET3-1) using MUMmer (9). The overall Newbould 305 genome shows high similarity to that of RF122. The majority of the genes ($n = 2,518$) were common to both strains (E value, $1E-5$; identity, $>70\%$). A total of 48,371 single nucleotide polymorphisms (SNPs) were found in Newbould 305 compared to RF122. Among the SNPs located in CDSs, 10,470 were nonsynonymous and 1,690 corresponded to insertions or deletions. They were evenly distributed among the contigs and did not correlate with protein

location. Newbould 305 analysis revealed the presence of a putative prophage containing 45 CDSs (contig 5) and of two putative *S. aureus* pathogenicity islands (SaPI) containing 16 genes (contig 2) and 17 genes (contig 6). Most of these genes had homology to SaPI genes in *S. aureus* strains of bovine (RF122), ovine (O46), or human (MW2) origin. The contig that was reported as a potential plasmid was further confirmed by homology and was designated pNewbould305.

Further analysis of the Newbould 305 genome is now under way. It will be further compared to the RF122 genome to identify specific factors that might explain the different phenotypes observed in infection acuteness.

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number AKYW00000000. The version described in this paper is the first version, AKYW01000000.

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REFERENCES

1. **Aziz RK, et al.** 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics **9**:75.
2. **Bannerman DD, et al.** 2004. Escherichia coli and Staphylococcus aureus elicit differential innate immune responses following intramammary infection. Clin. Diagn. Lab Immunol. **11**:463–472.
3. **Barinov A, et al.** 2009. Prediction of surface exposed proteins in Streptococcus pyogenes, with a potential application to other Gram-positive bacteria. Proteomics **9**:61–73.
4. **Ben Zakour NL, et al.** 2008. Genome-wide analysis of ruminant Staph-

ylococcus aureus reveals diversification of the core genome. J. Bacteriol. **190**:6302–6317.

5. **Guinane CM, et al.** 2010. Evolutionary genomics of Staphylococcus aureus reveals insights into the origin and molecular basis of ruminant host adaptation. Genome Biol. Evol. **2**:454–466.

6. **Hensen SM, Pavicic MJ, Lohuis JA, de Hoog JA, Poutrel B.** 2000. Location of Staphylococcus aureus within the experimentally infected bovine udder and the expression of capsular polysaccharide type 5 in situ. J. Dairy Sci. **83**:1966–1975.

7. **Hernandez D, Francois P, Farinelli L, Osteras M, Schrenzel J.** 2008. De novo bacterial genome sequencing: millions of very short reads assembled on a desktop computer. Genome Res. **18**:802–809.

8. **Herron-Olson L, Fitzgerald JR, Musser JM, Kapur V.** 2007. Molecular correlates of host specialization in Staphylococcus aureus. PLoS One **2**:e1120. doi:10.1371/journal.pone.0001120.

9. **Kurtz S, et al.** 2004. Versatile and open software for comparing large genomes. Genome Biol. **5**:R12. doi:10.1186/gb-2004-5-2-r12.

10. **Le Marechal C, et al.** 2011. Genome sequences of two Staphylococcus aureus ovine strains that induce severe (strain O11) and mild (strain O46) mastitis. J. Bacteriol. **193**:2353–2354.

11. **Le Marechal C, et al.** 2011. Molecular basis of virulence in Staphylococcus aureus mastitis. PLoS One **6**:e27354. doi:10.1371/journal.pone.0027354.

12. **Prasad LB, Newbould FH.** 1968. Inoculation of the bovine teat duct with Staph. aureus: the relationship of teat duct length, milk yield and milking rate to development of intramammary infection. Can. Vet. J. **9**:107–115.