Complete Genome Sequences of 11 Staphylococcus sp. Strains Isolated from Buffalo Milk and Milkers’ Hands

Lucas J. L. Pizauro,a Camila C. de Almeida,b Iman M. Gohari,c Janet I. MacInnes,c Luiz Francisco Zafalon,d Andrew M. Kropinski,c Alessandro M. Varania

a Department of Technology, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil
b Department of Microbiology, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil
c Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada
d Brazilian Agricultural Research Corporation (EMBRAPA), Embrapa Southeast Livestock, Sao Carlos, Sao Paulo, Brazil

ABSTRACT Here, we present data on the complete genome sequences of 11 Staphylococcus sp. isolates (three S. chromogenes isolates and one isolate each of S. saprophyticus, S. xylosus, S. hominis, S. agnetis, S. caprae, S. aureus, and S. warneri), obtained as part of a mastitis study of buffalo milk (from healthy animals and from those with subclinical mastitis) and milkers’ hands.

Like dairy cattle, dairy buffaloes with clinical or subclinical mastitis may have increased somatic cell counts (SCC) and decreased milk production, factors which both have important economic impacts (1). S. aureus is arguably the most important agent of mastitis, although other Staphylococcus spp. have also been implicated (2). For example, some coagulase-negative Staphylococcus strains are now known to affect the udder of cows and other dairy animals (3). The growing number of genome sequences of Staphylococcus species isolated from ruminants with and from those without mastitis is a valuable resource for a better understanding of this important disease (4).

Here, we report the complete genome sequences of 11 Staphylococcus sp. strains obtained from buffaloes in Sao Paulo State, Brazil, and from hand swabs of consenting milkers (Table 1). Milk samples were collected after mammary gland physical examination (5), strip cup test, and California mastitis test (CMT) (6) and then submitted for SCC analysis and microbiological culture according to National Mastitis Council guidelines (7). As recommended, a cutoff of 200,000 cells/ml was used to identify subclinical mastitis (8). Milk and hand swab samples were streaked on sheep blood agar and MacConkey agar. Isolates were characterized by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) analysis at the Animal Health Laboratory, University of Guelph (Guelph, Ontario, Canada), and the identity was confirmed by cydB quantitative PCR testing (9, 10). Antibiotic resistance testing (Kirby Bauer) was performed according to the manual of the Clinical and Laboratory Standards Institute (CLSI) (11), and the disk inhibition zones were interpreted according to CLSI guidelines.

Total cellular DNA was extracted using a bacterial DNA extraction protocol (Qiagen, Limburg, Netherlands) with an additional lysostaphin digestion step (12). The quality of the genomic DNA (gDNA) was evaluated by agarose gel electrophoresis and Qubit fluorometric spectrophotometry quantitation. SMRTbell libraries were prepared from gDNA using the PacBio SMRTbell template prep kit 1.0. SMRTbell libraries were size fractionated using a SageELF device (Sage Sciences, Beverly, MA). Genome sequencing was done using PacBio RS II technology at the Génome Québec Innovation Centre (McGill University, Quebec, Canada) with one single-molecule real-time (SMRT) cell per
sample. On average, 130,000 reads were generated for each genome (read \( N_{50} \), 12 kbp), and quality control was performed using FASTQC v0.11.8 software (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). The reads were trimmed and assembled using SMRT Analysis v2.3.0 software, and then assembled contigs were circularized using the minimus2 tool in the AMOS package (13). Trimmed reads were mapped against the assembled and circularized genomes; single-nucleotide polymorphism (SNP) corrections were done with variant-caller software v4.2 in the SMRT package (14); antibiotic resistance genes were predicted using the quiver algorithm. The genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (14); antibiotic resistance genes were predicted with ResFinder v3.1.0 (15). All software was run using default parameters. To our knowledge, this is the first report of complete genome sequences of \( S. \) hominis, \( S. \) caprae, \( S. \) pasteuri, \( S. \) chromogenes, \( S. \) saprophyticus, \( S. \) agnetis, \( S. \) warneri, and \( S. \) xylosus.

### Data availability
Sequence and annotation data of the strains were deposited in the GenBank database under BioProject accession number PRJNA482667 and the BioSample accession numbers SAMN09714551 (\( S. \) chromogenes 34B), SAMN09714428 (\( S. \) chromogenes 17A), SAMN09714506 (\( S. \) chromogenes 20B), SAMN09710868 (\( S. \) saprophyticus 1A), SAMN09714559 (\( S. \) xylosus 2), SAMN09714635 (\( S. \) pasteuri 3C), SAMN09714578 (\( S. \) hominis 19A), SAMN09714665 (\( S. \) agnetis 12B), SAMN09714418 (\( S. \) caprae 26D), SAMN09714411 (\( S. \) aureus 13), and SAMN09714427 (\( S. \) warneri 16A). Raw sequence data were deposited to the Sequence Read Archive (SRA) and linked to BioProject PRJNA482667.
ACKNOWLEDGMENT

This work was conducted during a scholarship supported by the International Cooperation Program CAPES/COFECUB at the University of Guelph, which was financed by CAPES–Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Ministry of Education of Brazil.

REFERENCES


