



# Assembly of Complete Genome Sequences of Negative-Control and Experimental Strain Variants of *Staphylococcus aureus* ATCC BAA-39 Selected under the Effect of the Drug FS-1, Which Induces Antibiotic Resistance Reversion

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**ABSTRACT** *Staphylococcus aureus* ATCC BAA-39 is the reference organism for a multidrug-resistant *Staphylococcus aureus* (MRSA) strain that was used to study drug-induced resistance reversion by an iodine-containing nanomolecular complex, FS-1. PacBio sequencing was performed on both the experimental and control strains, followed by genome assembly, variant calling, and DNA modification profiling.

The iodine-containing nanomolecular complex FS-1 induces a reversion of drug-resistant bacteria into sensitive phenotypes (1). FS-1 was designed to supplement antibiotic treatment therapy against drug-resistant tuberculosis (2) and has been shown to be successful against multidrug-resistant *Staphylococcus aureus* (MRSA) (3). To investigate the molecular mechanisms of this phenomenon, *Staphylococcus aureus* ATCC BAA-39 was used as a model organism. The strain was cultivated with a sublethal concentration of FS-1 (12  $\mu\text{g/ml}$ ) in 10 daily passages. In parallel, the culture was cultivated in the same medium without FS-1 to serve as a negative control (NC). Afterwards, chromosomal DNA samples were prepared in 3 repeats. DNA samples were extracted from bacterial cells using the PureLink genomic DNA kit (Thermo Fisher). The samples were sequenced by MacroGen (South Korea) on single-molecule real-time (SMRT) 8Pac V3 cells following the SMRTbell 20-kb protocol. The average  $N_{50}$  value and the average length of the subreads were 15 kb and 10 kb, respectively.

*S. aureus* ATCC BAA-39 (4) was isolated in 2010 from a nasal clinical sample (BioProject accession number [PRJNA50533](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA50533)). The whole-genome assembly comprising 83 contigs was published in 2010 ([GCA\\_000146385](https://www.ncbi.nlm.nih.gov/genbank/GCA_000146385)). The PacBio reads generated in this research have provided genome coverages of 966 $\times$  and 906 $\times$  for the FS and NC variants, respectively. PacBio subreads were published at the NCBI under BioProject accession number [PRJNA480363](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA480363). The complete genome assembly of the PacBio reads was performed using SMRT Link v5.0.1 pipelines (<https://www.pacb.com/support/software-downloads/>) with default parameters. The *de novo* assembly pipeline makes use of Hierarchical Genome Assembly Process (HGAP v4), with a default minimum seed length of 6 kb. Two genome variants, NC and FS, were obtained as single-contig sequences without gaps and ambiguities. The completeness of the final assemblies was evaluated using the Benchmarking Universal Single-Copy Orthologs (BUSCO v3.0.2) software (5) with parameters set to default. The lengths of the NC and FS genomes were 2,791,218 bp and 2,792,888 bp, respectively, with an average GC content of 32.9%. Genomes were annotated using the Rapid Annotations using Subsystems Technology (RAST) server (6). A methicillin resistance staphylococcal cassette chromosome *mec* element (SCC*mec*) containing the gene *mecA*, which encodes the penicillin-binding

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protein 2a (7, 8), was found in both genomes. Epigenetic modifications of nucleotides were detected in the genomes by aligning PacBio reads against the complete genome sequences, followed by base call kinetic analysis using the SMRT Link v5.0.1 software package. Identified *N*<sup>6</sup>-adenosine methylation motifs were published at the NCBI together with the complete genome sequences. The obtained genomes will be used in upcoming experiments to study the mechanisms of antibiotic resistance reversion induced by FS-1 in *S. aureus*. A working hypothesis is that FS-1 may cause inactivation of the drug resistance determinants in *SCCmec* by an increased rate of mutations, gene regulation alteration, or epigenetic modifications.

**Data availability.** The genome sequences are available from the NCBI under accession numbers [CP033505](#) and [CP033506](#) (BioProject accession number [PRJNA480363](#)) for the NC and FS variants, respectively. The SRA accession numbers are [SRR9077059](#) for NC and [SRR9067444](#) for FS.

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