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GENOME SEQUENCES

# Genomic Alterations of *Staphylococcus aureus* ATCC 25923 after Prolonged Passage in the Laboratory

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**ABSTRACT** *Staphylococcus aureus* reference strain ATCC 25923 has been maintained for more than a decade in our laboratory. Genomic study revealed that the resulting strain AFIPCBER_B_8.4 has lost a 37-kb genomic fragment of the ATCC 25923 parental strain. The missing fragment showed sequence similarity to genes of bacteriophage proteins.

*Staphylococcus aureus* is an important opportunistic human pathogen (1, 2). *S. aureus* strain ATCC 25923 in bacterial collections has been used in many studies (3–6). This strain has been passed for more than a decade in our laboratory and used as a laboratory reference bacterium for molecular assays (7). We conducted a comparative genomic study of the resulting strain, designated AFIPCBER_B_8.4, and the ATCC 25923 strain obtained recently from the American Type Culture Collection (ATCC).

The genomic DNA of *S. aureus* was isolated from overnight tryptic soy agar (TSA) broth cultures inoculated with a single colony picked from agar plates streaked separately with ATCC 25923 or AFIPCBER_B_8.4. The purified DNA was subjected to library preparation using the Nextera XT DNA library prep kit (Illumina). The prepared libraries were sequenced using the Illumina MiSeq platform with 2 × 125-bp paired-end mode. The raw reads of *S. aureus* strains ATCC 25923 and AFIPCBER_B_8.4 were mapped directly to the ATCC 25923 reference genome (GenBank accession number NZ_CP009361) (6) with CLC Genomic Workbench 11.0.1 (Qiagen). The sequencing reads from the new ATCC 25923 strain mapped perfectly on the entire reference genome. The sequencing reads from AFIPCBER_B_8.4 also mapped well on NZ_CP009361, except the 1,518 to 1,555-bp region of the reference genome. Analysis of the 37-kb gene fragment missing in strain AFIPCBER_B_8.4 revealed that 15 out of 19 BLASTn hits shared more than 50% nucleotide similarity with various bacteriophage gene sequences. According to the annotations on the reference sequence NZ_CP009361, the lost genome fragment contained 62 predicted genes; 10 genes were closely associated with bacteriophage proteins, including holin, phage protein, peptidase U35, HNH endonuclease, and transcriptional activator RinB.

The genome was assembled using SPAdes version 3.11.0 (8) with BayesHammer correction (9) and annotated with the built-in RAST tool kit (10) of the PATRIC server (11) with default options. The draft genome of *S. aureus* AFIPCBER_B_8.4 consisted of 60 contigs with 2,687,792 nucleotides ($N_{50}$, 110,114 bp; GC content, 32.72%) and 2,574 predicted genes, of which 2,535 were protein-coding genes and 39 were RNA-related genes. Sixty-two were classified as virulence factor associated by the Virulence Factors Database (VFDB); 18 were predicted to be related to antibiotic resistance by the Comprehensive Antibiotic Resistance Database (CARD) (12).

The plasmid DNA from AFIPCBER_B_8.4 was gel purified with a GeneClean III kit (MB Biomedicals) and sequenced with the Illumina MiSeq platform. Three contigs were assembled with plasmidSPAdes version 3.11.0 with the –careful parameter (13) and

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*k*-mer sizes of 21, 33, 55, 77, 99, and 127. The largest contig consisted of 27,617 bp (GC content, 32.5%), and its circular topology was confirmed with Mauve (14) and Bandage (15) with a 127-bp sequence in both linear ends forming the circular DNA named plasmid pAFIPCBER_B84. The plasmid shared 100% sequence similarity with the ATCC 25923 plasmid pS1945 reported in GenBank accession number NZ_CP009362.

In summary, ATCC strain 25923, which has been passed in our laboratory for years, developed a deletion of a genomic segment that could have originated from the integration of one or more bacteriophages. Here, we report the results of the genomic study of *S. aureus* strain AFIPCBER_B_8.4 maintained in our laboratory.

**Data availability.** The draft genome sequences of the chromosome and plasmid have been deposited in GenBank with the accession number QNQM00000000. The fastq reads for this study were deposited in the Sequence Read Archive (SRA) under BioProject number PRJNA478947.

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