Microbiol. Biotechnol. Lett. (2023), 51(4), 555–558
http://dx.doi.org/10.48022/mbl.2309.09004
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

Genome Reports

Complete Genome Sequence of *Staphylococcus aureus* strain 21SAU_AGRO3 Isolated from Korean Agricultural Products

Sojin Ahn[1,2], Eunbyeol Ahn[3], So Yun Jhang[1,2], Misun Jeong[1], Sangryeol Ryu[3], and Seoae Cho[1]*

[1]*eGnome Inc., Seoul 05836, Republic of Korea*
[2]*Interdisciplinary Program in Bioinformatics, Seoul National University, Seoul 08826, Republic of Korea*
[3]*Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea*

Received: September 19, 2023 / Revised: November 30, 2023 / Accepted: December 4, 2023

Staphylococcus aureus **is a prominent multidrug-resistant pathogen known for its resistance to a variety of antibiotics. To combat this, a wide range of antibiotics, including quinolones, is utilized. While the efficacy of quinolones against *S. aureus* has been established, the rise in quinolone-resistant strains, particularly in methicillin-resistant *S. aureus* (MRSA), has necessitated a shift in their usage patterns. Genomic sequencing plays a crucial role as it offers insights into the genetic mechanisms of resistance. Thus, we report the complete genome sequence of an oxolinic acid-resistant strain of *S. aureus* isolated from sweet potato leaves, a crop commonly cultivated in Korea.**

Keywords: ***Staphylococcus aureus*****, antimicrobial resistance, fluoroquinolones, complete genome**

Staphylococcus aureus is notable for its resistance to various antibiotics, including methicillin-resistant *S. aureus* (MRSA). Historically, quinolone antibiotics have been used against *S. aureus* infections, but the emergence of fluoroquinolone-resistant strains has compromised this efficacy [1]. The widespread use of quinolones in the agricultural sector, including agents like oxolinic acid that target DNA gyrases, has further intensified the challenge of antibiotic resistance [2]. Resistance to quinolones often involves mutations within the quinolone resistance-determining region of the *gyr* and *par* genes [3]. Additionally, multidrug resistance efflux pumps are believed to play a role in mediating fluoroquinolone resistance, although the exact mechanisms are not fully understood [4]. Given these issues, monitoring quinolone-resistant strains is necessary. In this context, we report the complete genome sequence of *S. aureus* strain 21SAU_AGRO3, which possesses antimicrobial resistance genes that includes those specific to fluoroquinolones.

The *S. aureus* strain 21SAU_AGRO3, obtained from the leaves of sweet potato grown in Busan, South Korea, was subjected to antimicrobial susceptibility testing using the disk diffusion assay. For oxolonic acid (2 μg), we observed a zone of inhibition measuring 12 mm. In the absence of established guidelines for oxolonic acid, we followed the CLSI standards for quinolone resistance in *S. aureus* to evaluate the antibiotic susceptibility of 21SAU_AGRO3 [5]. The PureHelix Genomic DNA Prep Kit (Solution Type)-Bacteria was used for DNA extraction in anticipation of next generation sequencing. The quantity and quality of the isolated genomic DNA were assessed using gel electrophoresis at 260/230 nm

***Corresponding author**
Phone: +82-2-876-8820, Fax: +82-2-876-8827
E-mail: seoae@egnome.co.kr

and 260/280 nm absorbance ratio, and the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen). The sequencing library was constructed using the Oxford Nanopore Technology ligation sequencing kit (SQK_LSK112) and the native barcoding expansion kit (EX_NBD114) according to the manufacturer's instructions. Subsequently, the library was loaded onto a MinION Flowcell (MIN112, R10.4) for sequencing, utilizing a MinION MK1b and MinKNOW software (22.03.6, 22.05.5). We then performed *de novo* assembly of long reads using Flye v2.9.1 and validated the genome completeness using BUSCO v5.2.2 [6, 7]. As a result, a high-quality complete genome consisting of a single circular contig was produced with a BUSCO v5.2.2 completeness score of 100%. The overall genome length is 2,869,088 bp with a GC content of 32.82%. The annotated genome composed of 2,755 coding sequences (CDS), 60 tRNA genes, 19 rRNA genes, and a single transfer-messenger RNA gene. Comparison of our data with previously published complete *S. aureus* genomes using Average Nucleotide Identity (ANI) via FastANI identified the *S. aureus* FDAARGOS_412 strain (GCF_002386245.1) as the most closely related strain to ours, exhibiting an ANI value of 99.0453% [8].

Functional category annotation was performed using the Clusters of Orthologous Groups (COG) approach, resulting in the annotation of 2,344 CDS [9]. With the exclusion of Class S, which consists of 633 CDS of unknown function, the most abundant categories were Class E (Amino acid transport and metabolism) with 230 CDS, Class P (Inorganic ion transport and metabolism) with 186 CDS, Class K (Transcription) with 172 CDS, and Class J (Translation, ribosomal structure and biogenesis) with 169 CDS. Subsequently, antibiotic resistance genes in the strict and perfect categories of the 21SAU_ARGO3 strain were annotated using the Comprehensive Antibiotic Resistance Database (CARD) and the Resistance Gene Identifier (RGI) v5.2.1[10]. This led to the annotation of 12 resistance genes, of which 7 were found to be associated with the fluoroquinolone antibiotic drug class (see Table 1). The complete genome sequence of *S. aureus* strain 21SAU_ARGO3 were deposited in GenBank under the accession CP134047.

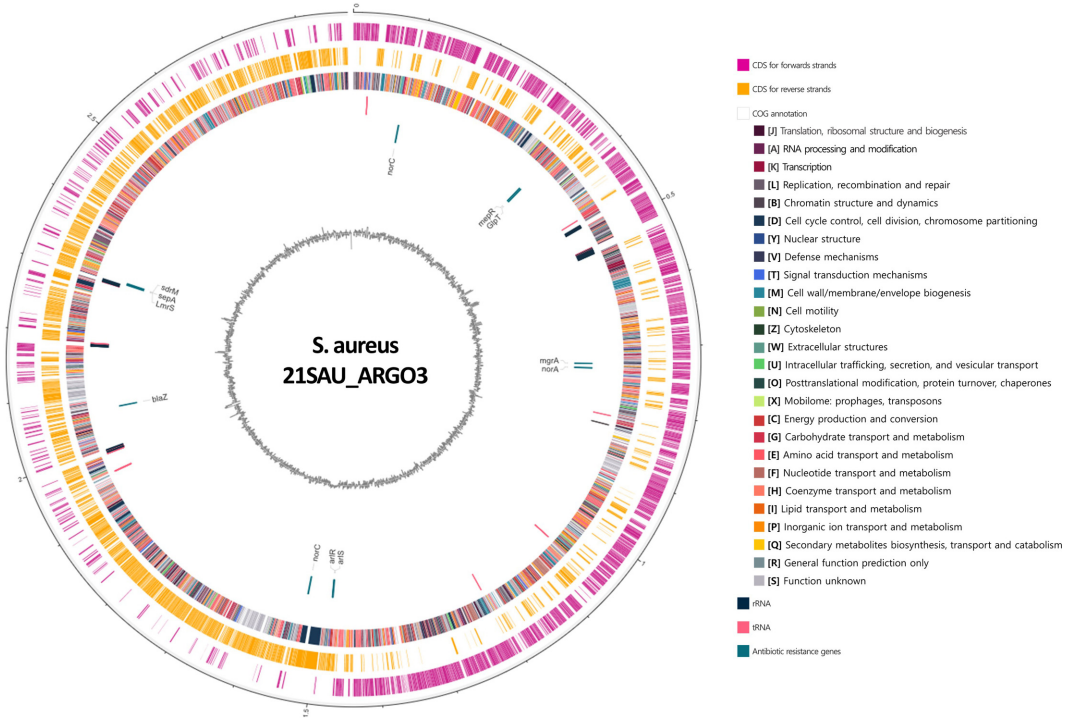

Fig. 1. Circular genome map of *S. aureus* strain 21SAU_AGRO3. Each circle indicates CDS in the leading strand, CDS in the lagging strand, COG distribution, RNA, antibiotic resistance genes, and the GC contents from outer to inner. Antibiotic resistance genes are labeled.

http://dx.doi.org/10.48022/mbl.2309.09004

Table 1. Putative antimicrobial resistance gene in genome of 21SAU_ARGO3 strain based on the CARD and RGI tools.

Locus_tag	Nucleotide Start position	Nucleotide End position	ARO term (CARD Accession)	RGI (percentage identity)	Drug Class	AMR gene family
GLJKLCAL_00081	86094	87482	*norC* (ARO: 3007010)	Strict (99.57)	fluoroquinolone antibiotic; disinfecting agents and antiseptics	MFS antibiotic efflux pump
GLJKLCAL_00306	351859	352278	*mepR* (ARO: 3000746)	Perfect (100.0)	glycylcycline; tetracycline antibiotic	MATE transporter
GLJKLCAL_00309	355725	354367	*glpT* (ARO: 3003901)	Strict (99.78)	fosfomycin	antibiotic-resistant GlpT
GLJKLCAL_00667	726927	726484	*mgrA* (ARO: 3000815)	Perfect (100.0)	fluoroquinolone antibiotic; cephalosporin; penam; tetracycline antibiotic; peptide antibiotic; disinfecting agents and antiseptics	MFS antibiotic efflux pump; ABC antibiotic efflux pump
GLJKLCAL_00676	734578	735744	*norA* (ARO: 3004667)	Perfect (100.0)	fluoroquinolone antibiotic	MFS antibiotic efflux pump
GLJKLCAL_01404	1478968	1477613	*arlS* (ARO: 3000839)	Perfect (100.0)	fluoroquinolone antibiotic; disinfecting agents and antiseptics	MFS antibiotic efflux pump
GLJKLCAL_01405	1479624	1478965	*arlR* (ARO: 3000838)	Strict (99.54)	fluoroquinolone antibiotic; disinfecting agents and antiseptics	MFS antibiotic efflux pump
GLJKLCAL_01985	2067950	2068795	*blaZ* (ARO: 3000621)	Strict (94.31)	penam	BlaZ beta-lactamase
GLJKLCAL_02230	2304179	2302737	*lmrS* (ARO: 3004572)	Strict (99.17)	macrolide antibiotic; aminoglycoside antibiotic; oxazolidinone antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic	MFS antibiotic efflux pump
GLJKLCAL_02231	2304975	2304502	*sepA* (ARO: 3007012)	Strict (96.82)	disinfecting agents and antiseptics	SMR antibiotic efflux pump
GLJKLCAL_02232	2306417	2305074	*sdrM* (ARO: 3007013)	Strict (99.78)	fluoroquinolone antibiotic; disinfecting agents and antiseptics	MFS antibiotic efflux pump

* ARO: Antibiotic Resistance Ontology; MFS: major facilitator superfamily; MATE: multidrug and toxic compound extrusion, ATP-binding cassette; ABC: ATP-binding cassette; SMR: small multidrug resistance

Acknowledgments

This study was carried out with the support of "Cooperative Research Program for Agricultural Science and Technology Development (Project No. PJ01612001)", Rural Development Administration, Republic of Korea

Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Woodford N, Livermore DM. 2009. Infections caused by Gram-positive bacteria: a review of the global challenge. *J. Infect.* **59**: S4-S16.
2. Miller SA, Ferreira JP, LeJeune JT. 2022. Antimicrobial use and resistance in plant agriculture: A one health perspective. *Agriculture* **12**: 289.
3. Redgrave LS, Sutton SB, Webber MA, Piddock LJ. 2014. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends Microbiol.* **22**: 438-445.
4. Truong-Bolduc QC, Strahilevitz J, Hooper DC. 2006. NorC, a new efflux pump regulated by MgrA of *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **50**: 1104-1107.
5. CLSI. 2020. *Performance standards for antimicrobial susceptibility testing*, pp. 30st ed Ed. Clinical and Laboratory Standards Institute.
6. Bzikadze AV, Pevzner PA. 2020. Automated assembly of centromeres from ultra-long error-prone reads. *Nat. Biotechnol.* **38**: 1309-1316.

7. Manni M, Berkeley MR, Seppey M, Simao FA, Zdobnov EM. 2021. BUSCO update: Novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *Mol. Biol. Evol.* **38**: 4647-4654.
8. Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. 2018. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat. Commun.* **9**: 5114.
9. Cantalapiedra CP, Hernandez-Plaza A, Letunic I, Bork P, Huerta-Cepas J. 2021. eggNOG-mapper v2: Functional annotation, orthology assignments, and domain prediction at the metagenomic scale. *Mol. Biol. Evol.* **38**: 5825-5829.
10. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, *et al.* 2020. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res.* **48**: D517-D525.

http://dx.doi.org/10.48022/mbl.2309.09004