Draft Genome Sequences of the Bap-Producing Strain *Staphylococcus aureus* V329 and Its Derived Phage-Resistant Mutant BIM-1

Lucía Fernández,[a,b] Ana Catarina Duarte,[a,b] Beatriz Martínez,[a,b] Ana Rodríguez,[a,b] Pilar García[a,b]

aInstituto de Productos Lácteos de Asturias (IPLA-CSIC), Villaviciosa, Asturias, Spain
bDairySafe Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain

ABSTRACT   This study reports the draft genome sequences of *Staphylococcus aureus* V329, a Bap-producing strain isolated from a case of subclinical bovine mastitis in Spain, and a derived mutant (BIM-1) resistant to phage phiIPLA-RODI. Comparison of the two genomes revealed that the mutant strain has a point mutation in the gene *tagO*.

Although *Staphylococcus aureus* is best known for being an opportunistic pathogen in humans, it can also cause infections in a wide range of animal hosts (1). For instance, this microbe is one of the etiological agents of mastitis in dairy cows, leading to economic losses in this sector and posing a potential risk to human health due to milk contamination. Such infections often exhibit a persistent nature and cannot be successfully cleared with antibiotic treatment.

It is widely accepted that biofilms play a role in chronic, recalcitrant bacterial infections. For most *S. aureus* strains, the main component of the extracellular matrix is the polysaccharide PIA/PNAG (2). However, some bovine mastitis isolates form strong biofilms rich in the Bap protein (3), a characteristic that has also been observed in other staphylococcal species (4). The first strain identified to produce such biofilms was *S. aureus* V329, isolated in Spain from a mastitic cow (3). More recently, Duarte et al. (5) isolated a V329-derived mutant, BIM-1, exhibiting resistance to phage phiIPLA-RODI and decreased susceptibility to the lytic protein CHAPSH3b. This study aimed to sequence the genome of V329, kindly provided by A. Toledo-Arana (Instituto de Agrobiotecnología, CSIC-Universidad Pública de Navarra, Spain), and compare it to the mutant BIM-1, recently derived in our laboratory, in order to identify the potential mutation(s) responsible for its phenotype.

Both strains were grown on plates containing TSB (tryptic soy broth; Scharlau Microbiology, Barcelona, Spain) supplemented with 2% agar (Roko, S.A., Llanera, Spain) at 37°C. A single colony was then streaked onto a second plate and incubated overnight under the same conditions. All cells grown on this plate were harvested and stored in Microbank cryovials, which contain beads and a cryopreservative (Pro-Lab Diagnostics UK, UK), until genomic DNA (gDNA) isolation. Then, the beads were washed with extraction buffer containing lysostaphin and RNase A and incubated for 25 min at 37°C. Afterwards, proteinase K and RNase A were added, and the samples were further incubated for 5 min at 65°C. gDNA purification was performed using solid-phase reversible immobilization (SPRI) beads (Beckman Coulter, Brea, CA), and the genomic libraries were prepared using a Nextera XT library prep kit (Illumina, San Diego, CA) according to the manufacturer's protocol. The resulting libraries were subsequently sequenced on an Illumina HiSeq platform using a 250-bp paired-end protocol. Genome sequencing was provided by MicrobesNG. Default parameters were used for all software utilized for the genome analysis unless otherwise noted. The quality of the reads was assessed using FASTQC v. 0.11.3 (6). Trimming was carried out using Trimmomatic v. 0.39 with a sliding window quality cutoff of Q15 (7). The genomes were assembled *de novo* using SPAdes v. 3.14.1 (8), and the quality of the assembly was assessed using QUAST v. 5.0.2 (9). The genome

TABLE 1 Genome assembly statistics and annotation features

Feature	Data for strain:	
	V329	BIM-1
No. of reads	610,303	572,792
Assembly statistics		
No. of contigs	50	53
No. of contigs >500 bp	20	19
Largest contig size (bp)	636,777	636,777
Genome size (bp)	2,719,662	2,718,812
G+C content (%)	32.8	32.8
N_{50} (bp)	356,410	356,410
No. of Ns per 100 kbp	0	0
Genome completeness (%)	100	100
Annotation features		
No. of genes (coding)	2,539	2,537
No. of tRNAs	53	53
No. of rRNAs	12	11

completeness was assessed using BUSCO v. 5 (10) and the ortholog set "Bacteria" on the gVolante Web server (11). The contigs were reordered using Mauve v. 20150226 (12). Then, sequences shorter than 500 bp were removed prior to genome annotation using the NCBI Prokaryotic Genome Annotation Pipeline v. 5.1 (13). Details regarding the genome assembly and annotation for both strains are shown in Table 1. A comparison of the two genomes using Breseq v. 0.35.5 (14) subsequently revealed that strain BIM-1 had a point mutation in the gene *tagO* (G210E), involved in the biosynthesis of teichoic acids (15). *tagO* deletion is known to result in phage resistance in *S. aureus* (16). Further work will be necessary to elucidate whether this mutation is responsible for the BIM-1 phenotype.

Data availability. The whole-genome shotgun projects corresponding to *S. aureus* V329 and BIM-1 have been deposited at DDBJ/ENA/GenBank under accession numbers JAGTJH000000000 and JAGTJI000000000, respectively. The version described in this article is the first version. The corresponding files containing the raw reads have been deposited at NCBI under BioProject accession number PRJNA723827.

REFERENCES

1. Haag AF, Fitzgerald JR, Penadés JR. 2019. *Staphylococcus aureus* in animals. Microbiol Spectr 7:7.3.11. https://doi.org/10.1128/microbiolspec.GPP3-0060-2019.
2. Cramton SE, Gerke C, Schnell NF, Nichols WW, Götz F. 1999. The intercellular adhesion (*ica*) locus is present in *Staphylococcus aureus* and is required for biofilm formation. Infect Immun 67:5427–5433. https://doi.org/10.1128/IAI.67.10.5427-5433.1999.
3. Cucarella C, Solano C, Valle J, Amorena B, Lasa I, Penadés JR. 2001. Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. J Bacteriol 183:2888–2896. https://doi.org/10.1128/JB.183.9.2888-2896.2001.
4. Valle J, Fang X, Lasa I. 2020. Revisiting Bap multidomain protein: more than sticking bacteria together. Front Microbiol 11:613581. https://doi.org/10.3389/fmicb.2020.613581.
5. Duarte AC, Fernández L, De Maesschalck V, Gutiérrez D, Campelo AB, Briers Y, Lavigne R, Rodríguez A, García P. 2021. Synergistic action of phage phiIPLA-RODI and lytic protein CHAPSH3b: a combination strategy to target *Staphylococcus aureus* biofilms. NPJ Biofilms Microbiomes 7:39. https://doi.org/10.1038/s41522-021-00208-5.
6. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.
7. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
8. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
9. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.

10. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31:3210–3212. https://doi.org/10.1093/bioinformatics/btv351.

11. Nishimura O, Hara Y, Kuraku S. 2017. gVolante for standardizing completeness assessment of genome and transcriptome assemblies. Bioinformatics 33:3635–3637. https://doi.org/10.1093/bioinformatics/btx445.

12. Rissman AI, Mau B, Biehl BS, Darling AE, Glasner JD, Perna NT. 2009. Reordering contigs of draft genomes using the Mauve aligner. Bioinformatics 25:2071–2073. https://doi.org/10.1093/bioinformatics/btp356.

13. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.

14. Deatherage DE, Barrick JE. 2014. Identification of mutations in laboratory-evolved microbes from next-generation sequencing data using breseq. Methods Mol Biol 1151:165–188. https://doi.org/10.1007/978-1-4939-0554-6_12.

15. Soldo B, Lazarevic V, Karamata D. 2002. tagO is involved in the synthesis of all anionic cell-wall polymers in Bacillus subtilis 168. Microbiology (Reading) 148:2079–2087. https://doi.org/10.1099/00221287-148-7-2079.

16. Azam AH, Hoshiga F, Takeuchi I, Miyanaga K, Tanji Y. 2018. Analysis of phage resistance in Staphylococcus aureus SA003 reveals different binding mechanisms for the closely related Twort-like phages φSA012 and φSA039. Appl Microbiol Biotechnol 102:8963–8977. https://doi.org/10.1007/s00253-018-9269-x.