

Complete Genome Sequence of a Variant of the Methicillin-Resistant Staphylococcus aureus ST239 Lineage, Strain BMB9393, Displaying Superior Ability To Accumulate *ica*-Independent Biofilm

Maiana Oliveira Cerqueira Costa,^a Cristiana Ossaille Beltrame,^b Fabienne Antunes Ferreira,^b Ana Maria Nunes Botelho,^b Nicholas Costa Barroso Lima,^a Rangel Celso Souza,^a Luiz Gonzaga Paula de Almeida,^a Ana Tereza Ribeiro Vasconcelos,^a Marisa Fabiana Nicolás,^a Agnes Marie Sá Figueiredo^b

Laboratório Nacional de Computação Científica, Laboratório de Bioinformática, Petrópolis, Rio de Janeiro, Brazila; Universidade Federal do Rio de Janeiro, Instituto de Microbiologia Professor Paulo de Góes, Departamento de Microbiologia Médica, Rio de Janeiro, Brazilb

Biofilm is considered an important virulence factor in nosocomial infections. Herein, we report the complete genome sequence of a variant of methicillin-resistant *Staphylococcus aureus*, strain BMB9393, which is highly disseminated in Brazil. This strain belongs to the lineage ST239 and displays increased ability to accumulate *ica*-independent biofilm and to invade human epithelial cells.

Received 26 June 2013 Accepted 8 July 2013 Published 8 August 2013

Citation Costa MOC, Beltrame CO, Ferreira FA, Botelho AMN, Lima NCB, Souza RC, de Almeida LGP, Vasconcelos ATR, Nicolás MF, Figueiredo AMS. 2013. Complete genome sequence of a variant of the methicillin-resistant Staphylococcus aureus ST239 lineage, strain BMB9393, displaying superior ability to accumulate ica-independent biofilm. Genome Announc. 1(4):e00576-13. doi:10.1128/genomeA.00576-13.

Copyright © 2013 Costa et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Agnes Marie Sá Figueiredo, agnes@micro.ufrj.br, or Marisa Fabiana Nicolás, marisa@lncc.br.

Staphylococcus aureus subsp. aureus strains are highly adaptive and versatile Gram-positive bacterial isolates (1, 2). Methicillin-resistant *S. aureus* (MRSA) bacteria displaying highlevel multiresistance are of great concern worldwide (3). In Brazil, a multiresistant *S. aureus* clone (Brazilian epidemic clone [BEC]) of the lineage ST239-SCCmecIII is widely disseminated in hospitals. ST239 isolates have also spread to other South American countries and countries in Europe, Asia, and Oceania (4–9). Here, we report the complete genome sequence of an ST239 variant, strain BMB9393, which displays superior ability to accumulate *ica*-independent biofilm and to adhere to and invade human airway cells. This variant was isolated in 1993 from a case of nosocomial bloodstream infection in Rio de Janeiro (4).

The genome sequencing was performed using a 454 GS FLX titanium (3-kb paired-end library) approach (Roche Diagnostics Corporation). The assembly, based on 285,317 reads corresponding to 102,585,816 bp (29-fold coverage), was carried out using Newbler v 2.6 (Roche) and Celera genome assembly v 6.1 (JCV Institute). Gaps within scaffolds resulting from repetitive sequences were resolved by *in silico* gap filling.

The genome of BMB9393 consists of one circular chromosome with 2,980,548 bp (GC content of 32.92%) and one circular plasmid of 2,908 bp (pBMB9393). Using the Sabia pipeline (10), we performed functional annotation of 2,678 protein-coding sequences (CDS), among which 2,244 were assigned to known functions and 434 were of unknown categories. The genome harbors 5 rRNA operons (5 copies of 16S rRNA, 5 of 23S rRNA, and 6 of 5S rRNA) and 60 tRNA genes, which were identified with RNAmmer and tRNAscan, respectively. Additionally, 75.46% of the CDS were assigned to at least one COG group.

The comparative analyses were performed using the MaGe MicroScope platform (11). The comparison (50% amino acid

identity, 80% alignment coverage) with three other published genomes of ST239 S. aureus revealed that BMB9393 shares 2,579 CDS with strain JKD6008, 2,555 with strain TW20, and 2,541 with strain T0131. BMB9393 has 142 unique open reading frames (ORFs) compared with the other three genomes, from which 14 were associated with virulence and antimicrobial resistance. pBMB9393 is similar to pC194, carries the cat gene encoding chloramphenicol resistance, and is not found in the other three genomes. Approximately 14% of the BMB9393 chromosome corresponds to regions of genomic plasticity (RGPs). Three RGPs are phage related, including phage φNM3, which carries the virulence genes sak, scn, and chp, encoding staphylokinase, staphylococcal complement inhibitor, and chemotaxis-inhibitor protein, respectively. BMB9393 also harbors the genomic island $vSA\alpha$, which carries lipoproteins and superantigen gene clusters, and $\nu SA\beta$ (absent in JKD6008), which harbors genes associated with antibiotic and toxin production, as well as a cluster of genes encoding serine proteases. Finally, BMB9393 carries a staphylococcal pathogenicity island (SAPI) that is a structural mosaic between SAPI1 and SAPI2. BMB9393 has a number of RGPs associated with insertion elements (IS) and transposons (Tn), including 15 copies of IS256, four of Tn554, and two of Tn552.

Nucleotide sequence accession numbers. The complete genome data of BMB9393 have been deposited in GenBank with the accession numbers CP005288 for the chromosome and CP005289 for the plasmid.

ACKNOWLEDGMENTS

This work was supported in part by grants from the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tec-

nológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

There are no conflicts of interest.

REFERENCES

- 1. McCarthy AJ, Lindsay JA. 2010. Genetic variation in *Staphylococcus aureus* surface and immune evasion genes is lineage associated: implications for vaccine design and host-pathogen interactions. BMC Microbiol. 10: 173. doi:10.1186/1471-2180-10-173.
- 2. Gill SR, Fouts DE, Archer GL, Mongodin EF, DeBoy RT, Ravel J, Paulsen IT, Kolonay JF, Brinkac L, Beanan M, Dodson RJ, Daugherty SC, Madupu R, Angiuoli SV, Durkin AS, Haft DH, Vamathevan J, Khouri H, Utterback T, Lee C, Dimitrov G, Jiang L, Qin H, Weidman J, Tran K, Kang K, Hance IR, Nelson KE, Fraser CM. 2005. Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. J. Bacteriol. 187:2426–2438.
- Moellering RC, Jr. 2012. MRSA: the first half century. J. Antimicrob. Chemother. 67:4–11.
- 4. Amaral MM, Coelho LR, Flores RP, Souza RR, Silva-Carvalho MC, Teixeira LA, Ferreira-Carvalho BT, Figueiredo AM. 2005. The predominant variant of the Brazilian epidemic clonal complex of methicillinresistant Staphylococcus aureus has an enhanced ability to produce biofilm and to adhere to and invade airway epithelial cells. J. Infect. Dis. 192: 801–810.
- Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, Stobberingh EE. 2007. The molecular evolution of methicillin-resistant Staphylococcus aureus. Clin. Microbiol. Infect. 13:222–235.

- Harris SR, Feil EJ, Holden MT, Quail MA, Nickerson EK, Chantratita N, Gardete S, Tavares A, Day N, Lindsay JA, Edgeworth JD, de Lencastre H, Parkhill J, Peacock SJ, Bentley SD. 2010. Evolution of MRSA during hospital transmission and intercontinental spread. Science 327: 469-474.
- 7. Feil EJ, Nickerson EK, Chantratita N, Wuthiekanun V, Srisomang P, Cousins R, Pan W, Zhang G, Xu B, Day NP, Peacock SJ. 2008. Rapid detection of the pandemic methicillin-resistant *Staphylococcus aureus* clone ST 239, a dominant strain in Asian hospitals. J. Clin. Microbiol. 46:1520–1522.
- Aires De Sousa M, Miragaia M, Sanches IS, Avila S, Adamson I, Casagrande ST, Brandileone, Palacio R, Dell'Acqua L, Hortal M, Camou T, Rossi A, Velazquez-Meza ME, Echaniz-Aviles G, Solorzano-Santos F, Heitmann I, de Lencastre H. 2001. Three-year assessment of methicillin-resistant *Staphylococcus aureus* clones in Latin America from 1996 to 1998. J. Clin. Microbiol. 39:2197–2205.
- Teixeira LA, Resende CA, Ormonde LR, Rosenbaum R, Figueiredo AM, de Lencastre H, Tomasz A. 1995. Geographic spread of epidemic multiresistant *Staphylococcus aureus* Clone in Brazil. J. Clin. Microbiol. 33: 2400–2404.
- Almeida LG, Paixão R, Souza RC, Costa GC, Barrientos FJ, Santos MT, Almeida DF, Vasconcelos AT. 2004. A system for automated bacterial (genome) integrated annotation—SABIA. Bioinformatics 20:2832–2833.
- 11. Vallenet D, Belda E, Calteau A, Cruveiller S, Engelen S, Lajus A, Le Fèvre F, Longin C, Mornico D, Roche D, Rouy Z, Salvignol G, Scarpelli C, Thil Smith AA, Weiman M, Médigue C. 2013. MicroScope—an integrated microbial resource for the curation and comparative analysis of genomic and metabolic data. Nucleic Acids Res. 41(Database issue): D636–D647. doi:10.1093/nar/gks1194.