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GENOME SEQUENCES

Complete Genome Sequence of *emm*1 *Streptococcus pyogenes* 10-85, a Strain Isolated from a Patient with Streptococcal Toxic Shock Syndrome in Japan

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ABSTRACT Here, we announce the complete genome sequence of *Streptococcus pyogenes* strain 10-85 (type *emm*1), isolated from a patient with streptococcal toxic shock syndrome (STSS). The strain lacks the genomic regions encoding SalR-SalK, a two-component regulatory system, and the adjacent type I restriction modification system.

Streptococcus pyogenes (group A *Streptococcus* [GAS]) is a Gram-positive bacterium that infects the upper respiratory tract, including the tonsils and pharynx, and it is responsible for postinfection diseases, such as rheumatic fever and glomerulonephritis. *S. pyogenes* also causes severe invasive diseases, including necrotizing fasciitis and streptococcal toxic shock syndrome (STSS) (1–5). The virulence strength is considered to be dependent upon mutations in *covR*, *covS*, or *rgg*, negative regulators in *emm*1 clinical isolates, as reported previously (6–10).

Recently, we reported that the genomic regions encoding SalR-SalK, a two-component regulatory system, and the adjacent type I restriction modification system were deleted in some *emm*1-type isolates from both STSS and non-STSS patients in Japan. *S. pyogenes* strain 10-85 from an STSS patient is one of the isolates with the deletion (11), and it contained no mutations in *covR*, *covS*, and *rgg* (12). The strain 10-85 is resistant to macrolide and has a conjugative prophage Φ1207.3 (formerly Tn*1207.3*), which carries the macrolide resistance genes *mef*(A) and *msr*(D) (13, 14).

The *S. pyogenes* strain 10-85 genome has been previously sequenced, and a total of 27 contigs were obtained (12). To obtain the complete genome sequence, the strain 10-85 genome was resequenced on a PacBio RS II instrument (Pacific Biosciences, Menlo Park, CA) at TaKaRa Bio, Inc. (Shiga, Japan). The strain was cultured at 37°C in brain heart infusion (BHI) broth (E-MC62; Eiken Chemical Co., Tokyo, Japan) supplemented with 0.3% yeast extract (BD, Sparks, MD, USA) broth for 18 h without agitation. The cells collected by centrifugation were incubated at 37°C in 3.3 mg/ml achromopeptidase and 5 mM EDTA. After sodium dodecyl sulfate was added at 1.43% of the final concentration, the cells were further incubated for 10 min at 90°C. The genomic DNA was isolated by three freeze-thaw cycles and phenol-chloroform extraction. The genomic DNA was fragmented prior to PacBio RS II sequencing using the Covaris g-TUBE device (Woburn, MA), in accordance with the manufacturer's instructions. PacBio RS II sequencing runs were performed using the PacBio SMRTbell template prep kit 1.0 and polymerase binding kit P6 after size selection using BluePippin (Sage Science, Beverly, MA) with a cutoff value of 15 kb. The high-quality filtered 105,098 subreads (with subread lengths of ≥500 bases, polymerase read lengths of ≥100 bases, and polymerase read qualities of ≥0.80) were assembled *de novo* using the Hierarchical Genome Assembly Process (HGAP) version 3 in the SMRT Analysis software version 2.3.0 (Pacific Biosciences), and a single contig was obtained. The overlap was removed to

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circularize the contig. Annotation was performed using the DDBJ Fast Annotation and Submission Tool (15).

The *S. pyogenes* strain 10-85 harbored a single circular genome of 1,778,006 bp, with an average G+C content of 38.6%. We observed 1,664 protein-coding regions, 18 rRNA operons, and 67 tRNA genes. The gene content matched the previously reported results (11–14).

Data availability. The whole-genome sequence of *S. pyogenes* strain 10-85 was submitted to DDBJ/ENA/GenBank under the accession number AP019548 and BioProject accession number PRJDB4033. The version described in this paper is the first version, AP019548.1. The accession number for the PacBio data in DDBJ/ENA/NCBI is DRA008374.

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We have no conflicts of interest to declare.

REFERENCES

1. Cone LA, Woodard DR, Schlievert PM, Tomory GS. 1987. Clinical and bacteriologic observations of a toxic shock-like syndrome due to *Streptococcus pyogenes*. N Engl J Med 317:146–149. https://doi.org/10.1056/NEJM198707163170305.
2. Hoge CW, Schwartz B, Talkington DF, Breiman RF, MacNeill EM, Englender SJ. 1993. The changing epidemiology of invasive group A streptococcal infections and the emergence of streptococcal toxic shock-like syndrome. A retrospective population-based study. JAMA 269:384–389. https://doi.org/10.1001/jama.1993.03500030082037.
3. Schwartz B, Facklam RR, Breiman RF. 1990. Changing epidemiology of group A streptococcal infection in the USA. Lancet 336:1167–1171. https://doi.org/10.1016/0140-6736(90)92777-F.
4. Stevens DL. 1994. Invasive group A streptococcal infections: the past, present and future. Pediatr Infect Dis J 13:561–566.
5. Hasegawa T, Hashikawa SN, Nakamura T, Torii K, Ohta M. 2004. Factors determining prognosis in streptococcal toxic shock-like syndrome: results of a nationwide investigation in Japan. Microbes Infect 6:1073–1077. https://doi.org/10.1016/j.micinf.2004.06.001.
6. Sumby P, Whitney AR, Graviss EA, DeLeo FR, Musser JM. 2006. Genome-wide analysis of group A streptococci reveals a mutation that modulates global phenotype and disease specificity. PLoS Pathog 2:e5. https://doi.org/10.1371/journal.ppat.0020005.
7. Engleberg NC, Heath A, Miller A, Rivera C, DiRita VJ. 2001. Spontaneous mutations in the CsrRS two-component regulatory system of *Streptococcus pyogenes* result in enhanced virulence in a murine model of skin and soft tissue infection. J Infect Dis 183:1043–1054. https://doi.org/10.1086/319291.
8. Walker MJ, Hollands A, Sanderson-Smith ML, Cole JN, Kirk JK, Henningham A, McArthur JD, Dinkla K, Aziz RK, Kansal RG, Simpson AJ, Buchanan JT, Chhatwal GS, Kotb M, Nizet V. 2007. DNase Sda1 provides selection pressure for a switch to invasive group A streptococcal infection. Nat Med 13:981–985. https://doi.org/10.1038/nm1612.
9. Tatsuno I, Okada R, Zhang Y, Isaka M, Hasegawa T. 2013. Partial loss of CovS function in *Streptococcus pyogenes* causes severe invasive disease. BMC Res Notes 6:126. https://doi.org/10.1186/1756-0500-6-126.
10. Ikebe T, Ato M, Matsumura T, Hasegawa H, Sata T, Kobayashi K, Watanabe H. 2010. Highly frequent mutations in negative regulators of multiple virulence genes in group A streptococcal toxic shock syndrome isolates. PLoS Pathog 6:e1000832. https://doi.org/10.1371/journal.ppat.1000832.
11. Okada R, Matsumoto M, Zhang Y, Isaka M, Tatsuno I, Hasegawa T. 2014. Emergence of type I restriction modification system-negative *emm*1 type *Streptococcus pyogenes* clinical isolates in Japan. APMIS 122:914–921. https://doi.org/10.1111/apm.12230.
12. Tatsuno I, Okada R, Matsumoto M, Hata N, Matsui H, Zhang Y, Isaka M, Hasegawa T. 2016. Relevance of spontaneous fabT mutations to a streptococcal toxic shock syndrome to non-streptococcal toxic shock syndrome transition in the novel-type Streptococcus pyogenes isolates that lost a salRK. APMIS 124:414–424. https://doi.org/10.1111/apm.12521.
13. Zhang Y, Tatsuno I, Okada R, Hata N, Matsumoto M, Isaka M, Isobe K, Hasegawa T. 2016. Predominant role of *msr*(D) over *mef*(A) in macrolide-resistance in *Streptococcus pyogenes*. Microbiology 162:46–52. https://doi.org/10.1099/mic.0.000206.
14. Tatsuno I, Isaka M, Masuno K, Hata N, Matsumoto M, Hasegawa T. 2018. Functional predominance of *msr*(D), which is more effective as *mef*(A)-associated than *mef*(E)-associated, over *mef*(A)/*mef*(E) in macrolide resistance in *Streptococcus pyogenes*. Microb Drug Resist 24:1089–1097. https://doi.org/10.1089/mdr.2017.0277.
15. Tanizawa Y, Fujisawa T, Nakamura Y. 2018. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. Bioinformatics 34:1037–1039. https://doi.org/10.1093/bioinformatics/btx713.