

R FOR DIOGY gen@meAnnouncements™

PROKARYOTES



Complete Genome Sequence of Serotype III *Streptococcus agalactiae* Sequence Type 17 Strain 874391

Matthew J. Sullivan,^a Brian M. Forde,^b Darren W. Prince,^a Deepak S. Ipe,^a Nouri L. Ben Zakour,^{b*} Mark R. Davies,^{c*} Gordon Dougan,^c Scott A. Beatson,^b Glen C. Ulett^a

School of Medical Science, and Menzies Health Institute Queensland, Griffith University, Gold Coast, Australia^a; Australian Infectious Diseases Research Centre and School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, Australia^b; The Wellcome Trust Sanger Institute and The Department of Medicine, University of Cambridge, Cambridge, United Kingdom^c

ABSTRACT Here we report the complete genome sequence of *Streptococcus agalactiae* strain 874391. This serotype III isolate is a member of the hypervirulent sequence type 17 (ST-17) lineage that causes a disproportionate number of cases of invasive disease in humans and mammals. A brief historical context of the strain is discussed.

S(1), is a commensal of the human gastrointestinal and urogenital tracts of up to 30% of healthy adults (2). *S. agalactiae* is an opportunistic pathogen that causes sepsis, meningitis, pneumonia, and soft tissue infections, including urinary tract infection. The changing epidemiology of invasive disease due to *S. agalactiae* has highlighted an increasing incidence of infection in immunocompromised and elderly individuals (3). *S. agalactiae* strain 874391 (former strain number 24) (4, 5) is a human vaginal isolate previously studied in the context of pathogenomics (6–8); surface antigen structure (9); adhesion to, invasion, and killing of macrophages and epithelial cells (10–14); and urogenital tract colonization (15–17). *S. agalactiae* 874391 is of the hypervirulent sequence type 17 (ST-17) lineage that comprises homogenous serotype III clones that are associated with a disproportionately high number of cases of invasive neonatal disease, particularly meningitis (18–20). It is likely that the ST-17 *S. agalactiae* lineage originated from a bovine source (21).

DNA extraction and whole-genome sequencing were performed as follows. For Illumina sequencing, *S. agalactiae* 874391 genomic DNA was isolated using methods previously described (22). The DNA was used to generate 100-bp paired-end reads using the Illumina HiSeq 2000 platform at the Wellcome Trust Sanger Institute, United Kingdom. For Pacific Biosciences (PacBio) sequencing, DNA was isolated using the UltraClean microbial DNA isolation kit (Mo Bio Laboratories). Single-molecule real-time (SMRT) sequencing was performed on an RS-II machine (Pacific Biosciences, CA, USA) using P6-C4 chemistry at The University of Melbourne, Australia. The sequencing provided $477 \times$ coverage (1.17-Gb sequence, 68,825 reads, 17,033-bp mean read length).

For sequence analysis, PacBio sequence read data were assembled *de novo* using Canu version 1.3 (23). Following assembly, the genome was polished using Illumina sequencing data to resolve single nucleotide insertion and deletion errors associated with homopolymer tracts, generating a complete circular genome of 2,153,937 bp with a GC content of 35.5%. Detection of methylation signatures was carried out using the SMRT analysis package version 2.3.0. Annotation of the 2.15-Mb genome was performed using Prokka version 1.12 (24) and the NCBI Prokaryotic Genome Annotation

Volume 5 Issue 42 e01107-17

Received 6 September 2017 Accepted 15 September 2017 Published 19 October 2017 Citation Sullivan MJ, Forde BM, Prince DW, Ipe DS, Ben Zakour NL, Davies MR, Dougan G, Beatson SA, Ulett GC. 2017. Complete genome sequence of serotype III *Streptococcus agalactiae* sequence type 17 strain 874391. Genome Announc 5:e01107-17. https://doi .org/10.1128/genomeA.01107-17.

Copyright © 2017 Sullivan et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Glen C. Ulett, g.ulett@griffith.edu.au.

* Present address: Nouri L. Ben Zakour, Westmead Institute for Medical Research, Westmead, Sydney, NSW, Australia; Mark R. Davies, The Doherty Institute for Infection & Immunity, University of Melbourne, Melbourne, VIC, Australia. Pipeline. Annotated features include 2,157 genes with 2,023 coding sequences (CDS), 21 ribosomal RNAs (rRNAs), 80 transfer RNAs (tRNAs), 3 noncoding RNAs (ncRNAs), 32 pseudogenes, and 1 clustered regularly interspaced short palindromic repeat (CRISPR) array. Annotation using the Rapid Annotation Subsystem Technology (RAST) server (25) showed that of the 2,023 CDS, 53% of the genes covered subsystem features. Of these, 68 were associated with virulence and 10 were associated with phages and prophages. Additionally, gene networks were linked to carbohydrate metabolism (n = 231), protein metabolism (n = 261), cell wall and capsule (n = 139), and resistance to antibiotics and toxic compounds (n = 34), including β -lactams (n = 1), fluoroquinolones (n = 4),

Accession number(s). The genome has been deposited in GenBank under accession no. CP022537 (PacBio BioProject no. PRJNA395243, BioSample no. SAMN07374522), BioSample no. SAMEA1324071 (Illumina BioProject no. PRJEB2837), and the European Nucleotide Archive (accession no. ERS086616 and ERR126909).

tetracyclines (n = 2), vancomycin (n = 5), and multidrug efflux (n = 3).

ACKNOWLEDGMENTS

This study was supported in part by Griffith University and the Wellcome Trust, United Kingdom.

We do not have a commercial or other association that might pose a conflict of interest.

REFERENCES

- Anthony BF, Okada DM. 1977. The emergence of group B streptococci in infections of the newborn infant. Annu Rev Med 28:355–369. https://doi .org/10.1146/annurev.me.28.020177.002035.
- Hickman ME, Rench MA, Ferrieri P, Baker CJ. 1999. Changing epidemiology of group B streptococcal colonization. Pediatrics 104:203–209.
- Skoff TH, Farley MM, Petit S, Craig AS, Schaffner W, Gershman K, Harrison LH, Lynfield R, Mohle-Boetani J, Zansky S, Albanese BA, Stefonek K, Zell ER, Jackson D, Thompson T, Schrag SJ. 2009. Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990–2007. Clin Infect Dis 49:85–92. https://doi.org/10.1086/599369.
- Takahashi S, Nagano Y, Nagano N, Fujita K, Taguchi F, Okuwaki Y. 1993. Opsonisation of group B streptococci and restriction endonuclease digestion patterns of their chromosomal DNA. J Med Microbiol 38: 191–196. https://doi.org/10.1099/00222615-38-3-191.
- Takahashi S, Nagano Y, Nagano N, Hayashi O, Taguchi F, Okuwaki Y. 1995. Role of C5a-ase in group B streptococcal resistance to opsonophagocytic killing. Infect Immun 63:4764–4769.
- Brochet M, Couvé E, Zouine M, Vallaeys T, Rusniok C, Lamy MC, Buchrieser C, Trieu-Cuot P, Kunst F, Poyart C, Glaser P. 2006. Genomic diversity and evolution within the species *Streptococcus agalactiae*. Microbes Infect 8:1227–1243. https://doi.org/10.1016/j.micinf.2005.11.010.
- Fleming KE, Bohnsack JF, Palacios GC, Takahashi S, Adderson EE. 2004. Equivalence of high-virulence clonotypes of serotype III group B *Strep-tococcus agalactiae* (GBS). J Med Microbiol 53:505–508. https://doi.org/ 10.1099/jmm.0.05443-0.
- Bohnsack JF, Whiting AA, Bradford RD, Van Frank BK, Takahashi S, Adderson EE. 2002. Long-range mapping of the *Streptococcus agalactiae* phylogenetic lineage restriction digest pattern type III-3 reveals clustering of virulence genes. Infect Immun 70:134–139. https://doi.org/10 .1128/IAI.70.1.134-139.2002.
- Seifert KN, Adderson EE, Whiting AA, Bohnsack JF, Crowley PJ, Brady LJ. 2006. A unique serine-rich repeat protein (Srr-2) and novel surface antigen (epsilon) associated with a virulent lineage of serotype III Streptococcus agalactiae. Microbiology 152:1029–1040. https://doi.org/10 .1099/mic.0.28516-0.
- Adderson EE, Takahashi S, Wang Y, Armstrong J, Miller DV, Bohnsack JF. 2003. Subtractive hybridization identifies a novel predicted protein mediating epithelial cell invasion by virulent serotype III group B Streptococcus agalactiae. Infect Immun 71:6857–6863. https://doi.org/10.1128/ IAI.71.12.6857-6863.2003.
- Chattopadhyay D, Carey AJ, Caliot E, Webb RI, Layton JR, Wang Y, Bohnsack JF, Adderson EE, Ulett GC. 2011. Phylogenetic lineage and pilus protein Spb1/SAN1518 affect opsonin-independent phagocytosis

gen@meAnnouncements™

and intracellular survival of group B streptococcus. Microbes Infect 13:369–382. https://doi.org/10.1016/j.micinf.2010.12.009.

- Ulett GC, Bohnsack JF, Armstrong J, Adderson EE. 2003. Beta-hemolysinindependent induction of apoptosis of macrophages infected with serotype III group B streptococcus. J Infect Dis 188:1049–1053. https://doi .org/10.1086/378202.
- Ulett GC, Adderson EE. 2005. Nitric oxide is a key determinant of group B streptococcus-induced murine macrophage apoptosis. J Infect Dis 191:1761–1770. https://doi.org/10.1086/429693.
- Ulett GC, Maclean KH, Nekkalapu S, Cleveland JL, Adderson EE. 2005. Mechanisms of group B streptococcal-induced apoptosis of murine macrophages. J Immunol 175:2555–2562. https://doi.org/10.4049/jimmunol.175.4 .2555.
- Carey AJ, Tan CK, Mirza S, Irving-Rodgers H, Webb RI, Lam A, Ulett GC. 2014. Infection and cellular defense dynamics in a novel 17betaestradiol murine model of chronic human group B streptococcus genital tract colonization reveal a role for hemolysin in persistence and neutrophil accumulation. J Immunol 192:1718–1731. https://doi.org/10.4049/ jimmunol.1202811.
- Sullivan MJ, Leclercq SY, Ipe DS, Carey AJ, Smith JP, Voller N, Cripps AW, Ulett GC. 2017. Effect of the *Streptococcus agalactiae* virulence regulator CovR on the pathogenesis of urinary tract infection. J Infect Dis 215: 475–483. https://doi.org/10.1093/infdis/jiw589.
- Carey AJ, Weinberg JB, Dawid SR, Venturini C, Lam AK, Nizet V, Caparon MG, Walker MJ, Watson ME, Ulett GC. 2016. Interleukin-17A contributes to the control of *Streptococcus pyogenes* colonization and inflammation of the female genital tract. Sci Rep 6:26836. https://doi.org/10.1038/ srep26836.
- Musser JM, Mattingly SJ, Quentin R, Goudeau A, Selander RK. 1989. Identification of a high-virulence clone of type III *Streptococcus agalactiae* (group B Streptococcus) causing invasive neonatal disease. Proc Natl Acad Sci U S A 86:4731–4735. https://doi.org/10.1073/pnas.86.12.4731.
- Jones N, Bohnsack JF, Takahashi S, Oliver KA, Chan MS, Kunst F, Glaser P, Rusniok C, Crook DW, Harding RM, Bisharat N, Spratt BG. 2003. Multilocus sequence typing system for group B streptococcus. J Clin Microbiol 41:2530–2536. https://doi.org/10.1128/JCM.41.6.2530-2536 .2003.
- Luan SL, Granlund M, Sellin M, Lagergård T, Spratt BG, Norgren M. 2005. Multilocus sequence typing of Swedish invasive group B streptococcus isolates indicates a neonatally associated genetic lineage and capsule switching. J Clin Microbiol 43:3727–3733. https://doi.org/10.1128/JCM .43.8.3727-3733.2005.
- 21. Bisharat N, Crook DW, Leigh J, Harding RM, Ward PN, Coffey TJ, Maiden

MC, Peto T, Jones N. 2004. Hyperinvasive neonatal group B streptococcus has arisen from a bovine ancestor. J Clin Microbiol 42:2161–2167. https://doi.org/10.1128/JCM.42.5.2161-2167.2004.

- 22. Ipe DS, Ben Zakour NL, Sullivan MJ, Beatson SA, Ulett KB, Benjamin WHJ, Davies MR, Dando SJ, King NP, Cripps AW, Schembri MA, Dougan G, Ulett GC. 2015. Discovery and characterization of human-urine utilization by asymptomatic-bacteriuria-causing *Streptococcus agalactiae*. Infect Immun 84:307–319. https://doi.org/10.1128/IAI.00938-15.
- 23. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer

weighting and repeat separation. Genome Res 27:722–736. https://doi .org/10.1101/gr.215087.116.

- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST Server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.