



# Draft Genome Sequence of an Invasive *Streptococcus agalactiae* Isolate Lacking Pigmentation

## Pallavi Singh,<sup>a</sup> David M. Aronoff,<sup>b</sup> H. Dele Davies,<sup>c</sup> Shannon D. Manning<sup>a</sup>

Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan, USA<sup>a</sup>; Department of Medicine, Vanderbilt University, Nashville, Tennessee, USA<sup>b</sup>; University of Nebraska Medical Center, Omaha, Nebraska, USA<sup>c</sup>

This report provides the whole-genome sequence of *Streptococcus agalactiae* isolate GB00037 isolated from a newborn in Calgary, Canada. This serotype V isolate is unique because it lacks pigment production previously shown to be critical for *S. agalactiae* virulence.

Received 5 January 2016 Accepted 6 January 2016 Published 25 February 2016

**Citation** Singh P, Aronoff DM, Davies HD, Manning SD. 2016. Draft genome sequence of an invasive *Streptococcus agalactiae* isolate lacking pigmentation. Genome Announc 4(1):e00015-16. doi:10.1128/genomeA.00015-16.

Copyright © 2016 Singh et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Shannon D. Manning, mannin71@msu.edu.

*treptococcus agalactiae*, also known as group B Streptococcus (GBS), is a leading cause of sepsis and meningitis in neonates worldwide. GBS typically has hemolytic activity and produces a yellow-orange pigment in culture, which are phenotypes encoded by genes within the *cyl* operon (1, 2). Pigment production is important for diagnostics and has been shown to be critical for virulence (3-6). However, not all invasive GBS strains are hemolytic and a small proportion lack pigment (7); hence, the mechanism of pathogenesis in these strains is likely due to other virulence factors. Strain GB00037 was recovered from the blood of a septic neonate with early onset disease in Calgary, Canada in 2000 (8). GB00037 is a serotype V, nonpigmented and nonhemolytic strain that was classified as multilocus sequence type (ST)-1. Genome analysis revealed an intact cyl operon, and therefore, additional phenotypic and sequencing analyses are warranted to identify the genes and pathways required for pathogenesis. Because GB00037 represents an atypical invasive strain, the genome is an important addition to GenBank.

For sequencing, genomic DNA was extracted and purified using the UltraClean microbial DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA), and sequencing was performed using an Illumina MiSeq (Illumina Inc., San Diego, CA) with a 500 cycle, paired-end 250 post library preparation using the Illumina Nextera XT kit. Post ambiguous sequences and adapters were trimmed with Trimmomatic (9) followed by quality checking using FastQC (http://www.bioinformatics.babraham.ac.uk/projects /fastqc/) and assembly with Velvet 1/2/07 (10) resulting in 34× coverage.

Annotation of the 2,045,700 bp draft genome was performed using the Prokaryotic Genomes Annotation Pipeline (http://www .ncbi.nlm.nih.gov/genome/annotation\_prok/). Annotated features include 2,159 genes with 2,117 coding sequences (CDS), 3 rRNAs, 16 tRNAs, and 1 noncoding RNA (ncRNA). Functional annotation using with the Rapid Annotation using Subsystem Technology (RAST) Server (11) identified 1,988 coding sequences with 19 RNAs. Furthermore, 56% of the genes covered subsystem features and 67 of these genes were associated with virulence, while 16 genes were phage-associated. Many genes (n = 259) were linked to carbohydrates and carbohydrate metabolism, protein metabolism (n = 177), and cell wall and capsule (n = 141). The Resistance Gene Identifier (RGI) in the Comprehensive Antibiotic Resistance Database (12) identified 10 genes conferring resistance to fluoroquinolones (n = 2),  $\beta$ -lactams (n = 5), peptides (n = 1), a tetracycline derivative (n = 1), and multidrug resistance to macrolides and lincosamides (n = 1).

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. LGAH000000000. The version described in this paper is version LGAH01000000.

#### ACKNOWLEDGMENTS

This study was supported in part by the Global Alliance to Prevent Prematurity and Stillbirth (GAPPS) in collaboration with the Bill and Melinda Gates Foundation (project N015615). We thank the National Center for Streptococcus (Alberta, Canada) for their role in serotyping the strain.

#### FUNDING INFORMATION

Global Alliance to Prevent Prematurity and Stillbirth provided funding to David M. Aronoff and Shannon D. Manning under grant number N015615.

### REFERENCES

- 1. Spellerberg B, Pohl B, Haase G, Martin S, Weber-Heynemann J, Lütticken R. 1999. Identification of genetic determinants for the hemolytic activity of *Streptococcus agalactiae* by ISS1 transposition. J Bacteriol 181: 3212–3219.
- Spellerberg B, Martin S, Brandt C, Lütticken R. 2000. The cyl genes of Streptococcus agalactiae are involved in the production of pigment. FEMS Microbiol Lett 188:125–128. http://dx.doi.org/10.1111/j.1574 -6968.2000.tb09182.x.
- Fallon RJ. 1974. The rapid recognition of Lancefield group B haemolytic streptococci. J Clin Pathol 27:902–905. http://dx.doi.org/10.1136/ jcp.27.11.902.
- 4. Randis TM, Gelber SE, Hooven TA, Abellar RG, Akabas LH, Lewis EL, Walker LB, Byland LM, Nizet V, Ratner AJ. 2014. Group B *Streptococcus* beta-hemolysin/cytolysin breaches maternal-fetal barriers to cause pre-

term birth and intrauterine fetal demise in vivo. J Infect Dis 210:265–273. http://dx.doi.org/10.1093/infdis/jiu067.

- 5. Whidbey C, Harrell MI, Burnside K, Ngo L, Becraft AK, Iyer LM, Aravind L, Hitti J, Waldorf KM, Rajagopal L. 2013. A hemolytic pigment of group B *Streptococcus* allows bacterial penetration of human placenta. J Exp Med 210:1265–1281. http://dx.doi.org/10.1084/jem.20122753.
- Whidbey C, Vornhagen J, Gendrin C, Boldenow E, Samson JM, Doering K, Ngo L, Ezekwe EA, Jr, Gundlach JH, Elovitz MA, Liggitt D, Duncan JA, Adams Waldorf KM, Rajagopal L. 2015. A streptococcal lipid toxin induces membrane permeabilization and pyroptosis leading to fetal injury. EMBO Mol Med 7:488–505. http://dx.doi.org/10.15252/emmm.201404883.
- Six A, Firon A, Plainvert C, Caplain C, Touak G, Dmytruk N, Longo M, Letourneur F, Fouet A, Trieu-Cuot P, Poyart C. 2016. Molecular characterization of nonhemolytic and nonpigmented group B streptococci responsible for human invasive infections. J Clin Microbiol 54:75–82. http://dx.doi.org/10.1128/JCM.02177-15.
- Davies HD, Adair C, McGeer A, Ma D, Robertson S, Mucenski M, Kowalsky L, Tyrell G, Baker CJ. 2001. Antibodies to capsular polysaccharides of group B *Streptococcus* in pregnant Canadian women: relationship to colonization status and infection in the neonate. J Infect Dis 184: 285–291. http://dx.doi.org/10.1086/322029.

- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. http:// dx.doi.org/10.1093/bioinformatics/btu170.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18:821–829. http:// dx.doi.org/10.1101/gr.074492.107.
- 11. 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. http://dx.doi.org/10.1186/ 1471-2164-9-75.
- 12. McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, Bhullar K, Canova MJ, De Pascale G, Ejim L, Kalan L, King AM, Koteva K, Morar M, Mulvey MR, O'Brien JS, Pawlowski AC, Piddock LJ, Spanogiannopoulos P, Sutherland AD, Tang I, Taylor PL, Thaker M, Wang W, Yan M, Yu T, Wright GD. 2013. The comprehensive antibiotic resistance database. Antimicrob Agents Chemother 57:3348–3357. http:// dx.doi.org/10.1128/AAC.00419-13.