



Complete Genome Sequence of Nonhemolytic *Streptococcus agalactiae* Serotype V Strain 1, Isolated from the Buccal Cavity of a Canine

Leeanne K. Harden, Karina M. Morales, Jeffery R. Hughey

Division of Mathematics, Science and Engineering, Hartnell College, Salinas, California, USA

L.K.H. and K.M.M. contributed equally to this work.

The complete genome sequence from a nonhemolytic strain of *Streptococcus agalactiae* from the oral cavity of a canine was assembled. The genome is 2,165,968 bp, contains 2,055 genes, and is classified as group B streptococcus (GBS) serotype V, strain 1. A comparison to other *S. agalactiae* sequences shows high gene synteny with human and bovine strains.

Received 23 November 2015 Accepted 7 December 2015 Published 28 January 2016

Citation Harden LK, Morales KM, Hughey JR. 2016. Complete genome sequence of nonhemolytic *Streptococcus agalactiae* serotype V strain 1, isolated from the buccal cavity of a canine. Genome Announc 4(1):e01612-15. doi:10.1128/genomeA.01612-15.

Copyright © 2016 Harden et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Jeffery R. Hughey, jhughey@hartnell.edu.

S*treptococcus agalactiae* (group B streptococcus [GBS]) is a common Gram-positive colonizer of terrestrial and aquatic vertebrates, including humans, cows, rabbits, cats, dogs, horses, frogs, trout, dolphins, and seals (1, 2). GBS is a leading cause of invasive infections in neonates (1) and is implicated in a number of animal diseases, including bovine mastitis (3). Characterization of the genetic diversity of GBS within the oral microbiota of animals, especially pets that interact closely with humans, is critical to understanding better the source of opportunistic pathogens and to documenting bacterial communities. Ten serotypes are currently described for *S. agalactiae* (4); here, we report the complete genome sequence of *S. agalactiae*, serotype V, strain 1 (ST-1), isolated from the buccal cavity of *Canis lupus familiaris*.

Genomic analysis of *S. agalactiae* ST-1 was performed using Illumina 36-bp paired-end sequencing methods and resulted in 23,645,928 reads. The data were assembled with default *de novo* settings in CLC Genomics Workbench (Qiagen), with an average coverage of 345×. Gaps were closed by mapping in Geneious version 8.1.5 (Biomatters Ltd., San Francisco, CA) against GenBank accession numbers CP010867 and HF952104, and by using standard PCR and Sanger sequencing. The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline 3.0 with GeneMarkS+ (http://www.ncbi.nlm.nih.gov/genome/annotation _prok/).

S. agalactiae ST-1 has a chromosome length of 2,165,968 bp. The genome contains 2,055 protein-coding genes (CDSs), 80 tRNAs, and 21 rRNAs, with 7 copies each of 16S, 23S, and 5S rRNA genes. It also includes 3 prophage-like elements and 1 clustered regularly interspaced short palindromic repeat. The genome is most closely related to *S. agalactiae* strains isolated from the blood of a human, *S. agalactiae* SS1 (1), and from bovine milk from a cow with mastitis, *S. agalactiae* 09mas018883 (3). These two strains differ in genetic distance from the canine *S. agalactiae* ST-1 by 0.016 and 0.027, respectively. Alignment of the sequences against *Streptococcus dysgalactiae* (accession no. AP011114) using MAUVE version 2.3.1 (5) showed high gene synteny for the three genomes of *S. agalactiae*, supporting the pangenome proposal of Tettelin et al. (6). The alignment identified 129 locally collinear blocks (LCBs), of which three LCBs were unique to *S. agalactiae* ST-1. The LCBs were 9,662 bp (containing 9 genes), 12,794 bp (17 genes), and 50,260 bp (58 genes) in length. The LCB genes were predominantly hypothetical proteins. A single large LCB of 46,595 bp in length (47 genes) is present in *S. agalactiae* 09mas018883 but was not found in *S. agalactiae* ST-1 or *S. agalactiae* SS1. Analysis of the *S. agalactiae* ST-1 genome using IslandViewer 3 (7) found 14 genomic islands; however, no virulence, resistance, or pathogenassociated genes were predicted.

Although *S. agalactiae* is reported in canines (8), several extensive genetic analyses of the canine oral microbiome identifying >350 taxa failed to identify *S. agalactiae* (9–11). These data represent the first record of *S. agalactiae* isolated from the oral cavity of a dog and suggest that further work is needed to fully characterize the canine oral microbiome.

Nucleotide sequence accession number. This whole-genome shotgun project has been deposited in GenBank under the accession number CP013202. The version described in this paper is the first version.

ACKNOWLEDGMENTS

This work was funded through the Title V grant PO31C110168 to Hartnell College and a private family trust from Paul W. Gabrielson. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

We thank Joni Black for laboratory assistance and for the swab of *S. agalactiae* from her pet Foxy.

FUNDING INFORMATION

U.S. Department of Education (DoED) provided funding to Jeffery Ryan Hughey under grant number PO31C110168.

REFERENCES

 Brochet M, Couvé E, Zouine M, Vallaeys T, Rusniok C, Lamy M, Buchrieser C, Trieu-Cuot P, Kunst F, Poyart C, Glaser P. 2006. Genomic diversity and evolution within the species *Streptococcus agalac*- *tiae*. Microbes Infect 8:1227–1243. http://dx.doi.org/10.1016/ j.micinf.2005.11.010.

- Delannoy CM, Crumlish M, Fontaine MC, Pollock J, Foster G, Dagleish MP, Turnbull JF, Zadoks RN. 2013. Human Streptococcus agalactiae strains in aquatic mammals and fish. BMC Microbiol 13:41. http:// dx.doi.org/10.1186/1471-2180-13-41.
- Zubair S, de Villiers EP, Fuxelius HH, Andersson G, Johansson K-E, Bishop RP, Bongcam-Rudloff E. 2013. Genome sequence of *Streptococcus* agalactiae Strain 09mas018883, isolated from a Swedish cow. Genome Announc 1(4):e00456-13. http://dx.doi.org/10.1128/genomeA.00456-13.
- 4. Flores AR, Galloway-Peña J, Sahasrabhojane P, Saldaña M, Yao H, Su X, Ajami NJ, Holder ME, Petrosino JF, Thompson E, Margarit y Ros I, Rosini R, Grandi G, Horstmann N, Teatero S, McGeer A, Fittipaldi N, Rappuoli R, Baker CJ, Shelburne SA. 2015. Sequence type 1 group B *Streptococcus*, an emerging cause of invasive disease in adults, evolves by small genetic changes. Proc Natl Acad Sci USA 112:6431–6436. http://dx.doi.org/10.1073/pnas.1504725112.
- 5. Darling ACE, Mau B, Blatter FR, Perna NT. 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 14:1394–1403. http://dx.doi.org/10.1101/gr.2289704.
- 6. Tettelin H, Masignani V, Cieslewicz MJ, Donati C, Medini D, Ward NL, Angiuoli SV, Crabtree J, Jones AL, Durkin AS, Deboy RT, Davidsen TM, Mora M, Scarselli M, Margarit y Ros I, Peterson JD, Hauser CR, Sundaram JP, Nelson WC, Madupu R, Brinkac LM, Dodson RJ, Rosovitz MJ, Sullivan SA, Daugherty SC, Haft DH, Selengut J, Gwinn ML, Zhou L, Zafar N, Khouri H, Radune D, Dimitrov G, Watkins K,

O'Connor KJ, Smith S, Utterback TR, White O, Rubens CE, Grandi G, Madoff LC, Kasper DL, Telford JL, Wessels MR, Rappuoli R, Fraser CM. 2005. Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: implications for the microbial "pan-genome." Proc Natl Acad Sci USA 102:13950–13955. http://dx.doi.org/10.1073/pnas.0506758102.

- Langille MGI, Brinkman FSL. 2009. IslandViewer: an integrated interface for computational identification and visualization of genomic islands. Bioinformatics 25:664–665. http://dx.doi.org/10.1093/bioinformatics/ btp030.
- Yildirim AÃ, Lämmler C, Weiss R, Kopp P. 2002. Pheno- and genotypic properties of streptococci of serological group B of canine and feline origin. FEMS Microbiol Lett 212:187–192. http://dx.doi.org/10.1111/j.1574 -6968.2002.tb11265.x.
- Dewhirst FE, Klein EA, Thompson EC, Blanton JM, Chen T, Milella L, Buckley CMF, Davis IJ, Bennett M, Marshall-Jones ZV. 2012. The canine oral microbiome. PLoS One 7:e36067. http://dx.doi.org/10.1371/ journal.pone.0036067.
- Sturgeon A, Stull JW, Costa MC, Weese JS. 2013. Metagenomic analysis of the canine oral cavity as revealed by high-throughput pyrosequencing of the 16S rRNA gene. Vet Microbiol 162:891–898. http://dx.doi.org/ 10.1016/j.vetmic.2012.11.018.
- Lamm CG, Ferguson AC, Lehenbauer TW, Love BC. 2010. Streptococcal infection in dogs: a retrospective study of 393 cases. Vet Pathol 47: 387–395. http://dx.doi.org/10.1177/0300985809359601.