

Draft Genome Sequence of a Nonhemolytic Fish-Pathogenic Streptococcus agalactiae Strain

Christian M. J. Delannoy,^{a,b} Ruth N. Zadoks,^b Frederick A. Lainson,^b Hugh W. Ferguson,^c Margaret Crumlish,^a James F. Turnbull,^a and Michael C. Fontaine^b

Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling, United Kingdom^a; Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, United Kingdom^b; and School of Veterinary Medicine, St. George's University, St. George's, Grenada, West Indies^c

Streptococcus agalactiae is a significant Gram-positive bacterial pathogen of terrestrial and aquatic animals. A subpopulation of nonhemolytic strains which appear to be pathogenic only for poikilotherms exists. We report here the first draft genome sequence of a nonhemolytic *S. agalactiae* isolate recovered from a diseased fish.

S*treptococcus agalactiae* is a Gram-positive bacterium that can cause a variety of diseases in a wide range of host species, including humans, cattle, and fish (5, 7, 8). To date, 3 complete genome sequences and 7 draft genome sequences of *S. agalactiae* have been made publicly available (3, 10, 12, 13); these include 8 isolates of human origin and 2 of bovine origin. Significantly, comparative analysis of these sequences has permitted the identification of virulence determinants and genes involved in host adaptation (1, 10).

The S. agalactiae STIR-CD-17 genome is the first submission to NCBI of a nonhemolytic strain isolated from fish. The strain was isolated from the heart of a moribund fish during a disease outbreak affecting farmed tilapia (Oreochromis sp.) in Honduras in 2008 and was selected for further analysis based on the outcome of epidemiological, phenotypic, and genotypic characterization (C. M. J. Delannoy, M. Crumlish, M. C. Fontaine, J. Pollock, G. Foster, M. Dagleish, J. F. Turnbull, and R. N. Zadoks, submitted for publication). The strain is nonhemolytic and belongs to serotype Ib. Based on multilocus sequence typing (MLST) (4), it belongs to the sequence type (ST) 260 and clonal complex (CC) 552, corresponding to a cluster of nonhemolytic strains that have been associated exclusively with disease in aquatic poikilotherms (2). Moreover, based on a standardized 3-set genotyping analysis (6), STIR-CD-17 is negative for all surface protein genes and mobile genetic elements screened, further supporting that it is not closely related to other described S. agalactiae strains of human or bovine origin. Experimental intraperitoneal infection of tilapia also revealed that STIR-CD-17 is highly pathogenic for fish (our unpublished data).

Genome sequencing was performed with an Illumina Solexa Genome Analyzer at the GenePool sequencing core facility (University of Edinburgh). *De novo* assembly of Solexa reads was achieved using Velvet 0.6 (14), and the resulting 96 contigs were annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) (9).

The draft genome contains 1,805,303 bp, with an average G+C content of 35%. Altogether, a total of 1,697 protein-encoding genes were predicted, with 505 (29.8%) being annotated as hypothetical proteins. In addition, 102 pseudogenes, in which frameshift and nonsense mutations introduce multiple stop codons throughout the gene, were identified. The predicted genes were sorted on the basis of clusters of orthologous groups (COG) classification (11). A total of 352 (20.7%) genes were associated with

information storage and processing, 268 (15.8%) were associated with cellular processes and signaling, and 548 (32.3%) were associated with metabolism. Finally, 529 (31.2%) residual genes, which were not able to be categorized into COG classes, have poorly characterized functions and features. We anticipate that the comparison of the STIR-CD-17 genome with other published *S. agalactiae* genomes from strains of bovine and human origin will provide further insights into the molecular basis of the host adaptation and pathogenicity of this important bacterial pathogen.

Nucleotide sequence accession number. The draft genome sequence of *S. agalactiae* STIR-CD-17 has been deposited in GenBank under the accession number ALXB00000000.

ACKNOWLEDGMENTS

This work was supported by a joint Ph.D. grant from the Moredun Foundation and the University of Stirling.

We thank Raja Yaga for his contribution to the sequencing project.

REFERENCES

- Brochet M, et al. 2006. Genomic diversity and evolution within the species *Streptococcus agalactiae*. Microbes Infect. 8:1227–1243.
- 2. Evans JJ, et al. 2008. Phylogenetic relationships among *Streptococcus agalactiae* isolated from piscine, dolphin, bovine and human sources: a dolphin and piscine lineage associated with a fish epidemic in Kuwait is also associated with human neonatal infections in Japan. J. Med. Microbiol. 57:1369–1376.
- 3. Glaser P, et al. 2002. Genome sequence of *Streptococcus agalactiae*, a pathogen causing invasive neonatal disease. Mol. Microbiol. **45**:1499–1513.
- 4. Jones N, et al. 2003. Multilocus sequence typing system for group B streptococcus. J. Clin. Microbiol. 41:2530–2536.
- Keefe GP. 1997. Streptococcus agalactiae mastitis: a review. Can. Vet. J. 38:429-437.
- Kong F, Martin D, James G, Gilbert GL. 2003. Towards a genotyping system for *Streptococcus agalactiae* (group B streptococcus): use of mobile genetic elements in Australasian invasive isolates. J. Med. Microbiol. 52: 337–344.
- Mian GF, et al. 2009. Aspects of the natural history and virulence of *S. agalactiae* infection in Nile tilapia. Vet. Microbiol. 136:180–183.
- 8. Patterson MJ, Hafeez AEB. 1976. Group B streptococci in human disease. Bacteriol. Rev. 40:774–792.

Received 23 August 2012 Accepted 10 September 2012 Address correspondence to Christian M. J. Delannoy, c.m.delannoy@stir.ac.uk. Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JB.01552-12

- Pruitt KD, Tatusova T, Klimke W, Maglott DR. 2009. NCBI reference sequences: current status, policy and new initiatives. Nucleic Acids Res. 37:D32–D36.
- Richards VP, et al. 2011. Comparative genomics and the role of lateral gene transfer in the evolution of bovine adapted *Streptococcus agalactiae*. Infect. Genet. Evol. 11:1263–1275.
- 11. Tatusov R, et al. 2003. The COG database: an updated version includes eukaryotes. BMC Bioinformatics 4:41.
- Tettelin H, et al. 2005. Genome analysis of multiple pathogenic isolates of Streptococcus agalactiae: implications for the microbial "pan-genome." Proc. Natl. Acad. Sci. U. S. A. 102:13950–13955.
- Tettelin H, et al. 2002. Complete genome sequence and comparative genomic analysis of an emerging human pathogen, serotype V Streptococcus agalactiae. Proc. Natl. Acad. Sci. U. S. A. 99:12391–12396.
- Zerbino D, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. 18:821–829.