

Complete Genome Sequence of *Gallibacterium anatis* Strain UMN179, Isolated from a Laying Hen with Peritonitis[∇]

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***Gallibacterium anatis* is a member of the normal flora of avian hosts and an important causative agent of peritonitis and salpingitis in laying hens. Here we report the availability of the first completed *G. anatis* genome sequence of strain UMN179, isolated from an Iowa laying hen with peritonitis.**

Gallibacterium anatis (formerly the [*Pasteurella haemolytica*]-*Actinobacillus salpingitidis* complex) is a Gram-negative, nonmotile, rod-shaped bacterium belonging to the family *Pasteurellaceae* (7). This bacterium is known to be a member of the normal upper respiratory tract and lower reproductive tract of chickens but has also been implicated in peritonitis and salpingitis in commercial laying hens (3–5, 16). To this end, *G. anatis* has been experimentally shown to induce natural infection in the chicken model (2). While studies aimed at the molecular pathogenesis of *G. anatis* are limited, some potential virulence factors/functions have been identified, including the production of secreted metalloproteases (9), hemagglutinating activity (9), and the production of a cytolytic RTX toxin known as GtxA (11). *G. anatis* isolates have also been shown to possess decreased antimicrobial susceptibility toward multiple antimicrobial agents (6).

Here we report the complete genome sequence of *G. anatis* strain UMN179, isolated from a peritonitis lesion of a laying hen in Iowa. Sequencing was performed at the University of Minnesota Biomedical Genomics Facility using 454 Life Sciences pyrosequencing (Roche Diagnostics Corporation). The following three data sets were used: (i) GS-FLX, with 270,555 shotgun reads with an average length of 247 bp, yielding 67 Mbp; (ii) the GS-FLX 3-kb paired-end library, with 236,132 shotgun reads with an average length of 251 bp, yielding 59 Mbp; and (iii) the GS-FLX Titanium 8-kb paired-end library, with 245,660 reads with an average length of 348 bp, yielding 86 Mbp. Reads were *de novo* assembled into scaffolds using Newbler 2.0 (15). The genome was closed using standard PCRs, followed by Sanger sequencing. The final genome assembly was validated using paired-end checking and targeted PCR at selected regions throughout the genome.

Annotation was automated using publicly available tools. Putative coding regions were predicted using GeneMarkS (1),

tRNA genes using tRNAscan-SE (13), and rRNA genes using RNAmmer (12). Gene function was assigned using HMMER3 (10) against Pfam-A 24.0 (8), RPS-BLASTp against CDD (14), and BLASTp against all microbial proteins.

The genome of *G. anatis* UMN179 consists of a single, circular chromosome (2,687,335 bp; 40% GC content) and one plasmid (pUMN179; 6,804 bp). The chromosome contains 2,528 protein-encoding genes, 57 tRNA-carrying genes, and 6 rRNA-carrying operons. The alternative GUG and UUG codons are each used at the rate of 3.6%.

With respect to virulence-associated traits, *G. anatis* UMN179 contains the *gtxAC* genes encoding its RTX toxin. Additionally, strain UMN179 contains a number of novel genes that might be associated with virulence functions, including those encoding a predicted metalloendopeptidase, carrying several putative fimbrial operons, and encoding several novel ABC-type transport systems.

Work is ongoing to compare the genomes of virulent and avirulent *Gallibacterium* isolates toward the identification of additional genetic loci implicated in their pathogenesis.

Nucleotide sequence accession numbers. The sequences determined in this study have been deposited in GenBank and assigned accession numbers CP002667 and CP002668.

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REFERENCES

1. Besemer, J., A. Lomsadze, and M. Borodovsky. 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. *Nucleic Acids Res.* **29**:2607–2618.
2. Bojesen, A. M., O. L. Nielsen, J. P. Christensen, and M. Bisgaard. 2004. In vivo studies of *Gallibacterium anatis* infection in chickens. *Avian Pathol.* **33**:145–152.
3. Bojesen, A. M., S. S. Nielsen, and M. Bisgaard. 2003. Prevalence and transmission of haemolytic *Gallibacterium* species in chicken production systems with different biosecurity levels. *Avian Pathol.* **32**:503–510.
4. Bojesen, A. M., and H. L. Shivaprasad. 2007. Genetic diversity of *Gallibacterium* isolates from California turkeys. *Avian Pathol.* **36**:227–230.
5. Bojesen, A. M., M. Torpdahl, H. Christensen, J. E. Olsen, and M. Bisgaard. 2003. Genetic diversity of *Gallibacterium anatis* isolates from different chicken flocks. *J. Clin. Microbiol.* **41**:2737–2740.
6. Bojesen, A. M., et al. 2011. Antimicrobial susceptibility and tetracycline

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- resistance determinant genotyping of *Gallibacterium anatis*. *Vet. Microbiol.* **148**:105–110.
7. **Christensen, H., M. Bisgaard, A. M. Bojesen, R. Mutters, and J. E. Olsen.** 2003. Genetic relationships among avian isolates classified as *Pasteurella haemolytica*, 'Actinobacillus salpingitidis' or *Pasteurella anatis* with proposal of *Gallibacterium anatis* gen. nov., comb. nov. and description of additional genomospecies within *Gallibacterium* gen. nov. *Int. J. Sys. Evol. Microbiol.* **53**:275–287.
 8. **Finn, R. D., et al.** 2010. The Pfam protein families database. *Nucleic Acids Res.* **38**:D211–D222.
 9. **Garcia-Gomez, E., et al.** 2005. *Gallibacterium anatis*-secreted metalloproteases degrade chicken IgG. *Avian Pathol.* **34**:426–429.
 10. **Johnson, L. S., S. R. Eddy, and E. Portugaly.** 2010. Hidden Markov model speed heuristic and iterative HMM search procedure. *BMC Bioinformatics* **11**:431.
 11. **Kristensen, B. M., D. Frees, and A. M. Bojesen.** 2010. GtxA from *Gallibacterium anatis*, a cytolytic RTX-toxin with a novel domain organisation. *Vet. Res.* **41**:25.
 12. **Lagesen, K., et al.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* **35**:3100–3108.
 13. **Lowe, T. M., and S. R. Eddy.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* **25**:955–964.
 14. **Marchler-Bauer, A., et al.** 2011. CDD: a conserved domain database for the functional annotation of proteins. *Nucleic Acids Res.* **39**:D225–D229.
 15. **Margulies, M., et al.** 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**:376–380.
 16. **Neubauer, C., M. De Souza-Pilz, A. M. Bojesen, M. Bisgaard, and M. Hess.** 2009. Tissue distribution of haemolytic *Gallibacterium anatis* isolates in laying birds with reproductive disorders. *Avian Pathol.* **38**:1–7.