genomeA
Journals.ASM.org

Complete Genome Sequence of *Mannheimia haemolytica* Strain 42548 from a Case of Bovine Respiratory Disease

Christopher Eidam,[a] Anja Poehlein,[b] Geovana Brenner Michael,[a] Kristina Kadlec,[a] Heiko Liesegang,[b] Elzbieta Brzuszkiewicz,[b] Rolf Daniel,[b] Michael T. Sweeney,[c] Robert W. Murray,[c] Jeffrey L. Watts,[c] Stefan Schwarz[a]

Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut (FLI), Neustadt-Mariensee, Germany[a]; Department of Genomic and Applied Microbiology, Goettingen Genomics Laboratory, Institute of Microbiology and Genetics, Georg-August-University of Goettingen, Goettingen, Germany[b]; Zoetis, Kalamazoo, Michigan, USA[c]

***Mannheimia haemolytica* is the major bacterial component in the bovine respiratory disease complex, which accounts for considerable economic losses to the cattle industry worldwide. The complete genome sequence of *M. haemolytica* strain 42548 was determined. It has a size of 2.73 Mb and contains 2,888 genes, including several antibiotic resistance genes.**

Received 19 April 2013 **Accepted** 23 April 2013 **Published** 30 May 2013

Citation Eidam C, Poehlein A, Brenner Michael G, Kadlec K, Liesegang H, Brzuszkiewicz E, Daniel R, Sweeney MT, Murray RW, Watts JL, Schwarz S. 2013. Complete genome sequence of *Mannheimia haemolytica* strain 42548 from a case of bovine respiratory disease. Genome Announc. 1(3):e00318-13. doi:10.1128/genomeA.00318-13.

Address correspondence to Stefan Schwarz, stefan.schwarz@fli.bund.de.

The gammaproteobacterium *Mannheimia haemolytica* is a facultatively pathogenic, Gram-negative bacterium of the upper respiratory tract and nasopharynx of ruminants. It is considered the major bacterial agent of the bovine respiratory disease (BRD) complex. BRD accounts for over $3 billion in losses to the cattle industry every year (1). Viral pathogens are believed to initiate the disease and pave the way for secondary bacterial infections. While vaccines are used to prevent the onset of BRD, antimicrobial agents are also used, especially in cases when *M. haemolytica*, *Pasteurella multocida*, or *Histophilus somni* is involved (2). The development of antimicrobial resistance among bacterial BRD pathogens is of growing concern (3), as antimicrobial multiresistance conferred by the integrative and conjugative element ICE*Pmu1* has recently been described (4, 5). ICE*Pmu1* carries 12 antimicrobial resistance genes, some of which also confer resistance against gamithromycin and tildipirosin, the latest antimicrobial agents approved for food-animal use (6). The complete genome sequence of the multiresistant *M. haemolytica* isolate 42548 will further our understanding of genome structure and function as well as provide insight into the transfer, acquisition, and genetic basis of antimicrobial multiresistance.

Genomic DNA was isolated from *M. haemolytica* 42548 using the MasterPure Complete DNA and RNA Purification kit (Epicenter, Madison, WI). Whole-genome sequencing was performed using the 454 GS-FLX titanium XL system (titanium GS70 chemistry; Roche Life Science, Mannheim, Germany) and the Genome Analyzer IIx (Illumina, San Diego). We performed 454 shotgun sequencing, which produced 135,530 single-end reads with an average length of 612 bases (28.6× coverage), while Illumina sequencing resulted in 3,696,221 of 112-bp paired-end reads (116× coverage). The initial hybrid *de novo* assembly performed using the MIRA software resulted in 316 contigs. Gaps between contigs were closed using PCR amplification, subsequent Sanger sequencing (7), BigDye 3.0 chemistry, and an ABI3730XL capillary sequencer (Applied Biosystems, Life Technologies GmbH, Darmstadt, Germany). Gap closure was done using the Gap4 (v.4.11) software (8). The genome of *M. haemolytica* 42548 consists of a single chromosome of 2.73 Mb with a GC content of 41.05%. The initial gene prediction was done using YACOP (9) and Glimmer (10), while rRNA and tRNA were identified with RNAmmer and tRNAscan (11, 12). This revealed a total number of 2,888 genes, including 6 rRNA gene clusters and 61 tRNA genes. Protein genes were annotated using Swiss-Prot and TrEMBL (13), followed by manual curation based on comparison with genes predicted by IMG/ER (14). This resulted in 2,807 protein-encoding genes with assigned functions. Additionally, 55 putative transposases and one CRISPR region of the I-C/Dvulg subtype were detected (MHH_c16440-16500) (15). Several antibiotic resistance genes were located within a putative integrative and conjugative element, tentatively designated ICE*Mh1*, which shared high similarities with ICE*Pmu1*. ICE*Mh1* carried the resistance genes *aphA1* (MHH_c22550), *strA* (MHH_c22570), *strB* (MHH_c22560), *sul2* (MHH_c22580), and *tetR-tet*(H) (MHH_c23150-23160), which accounted for the multiresistance phenotype.

Nucleotide sequence accession number. The genome sequence of *M. haemolytica* 42548 has been deposited at DDBJ/EMBL/GenBank under the accession no. CP005383.

ACKNOWLEDGMENTS

We thank Kathleen Gollnow, Kerstin Meyer, and Maik Schlieper for their excellent technical assistance.

This study was supported by grant SCHW382/10-1 of the German Research Foundation (DFG) and Zoetis, Kalamazoo, MI. C.E. received a scholarship from the Gesellschaft der Freunde der Tierärzlichen Hochschule Hannover e.V.

REFERENCES

1. **Griffin D.** 1997. Economic impact associated with respiratory disease in beef cattle. Vet. Clin. North Am. Food Anim. Pract. **13**:367–377.
2. **Edwards TA.** 2010. Control methods for bovine respiratory disease for feedlot cattle. Vet. Clin. North Am. Food Anim. Pract. **26**:273–284.
3. **Watts JL, Sweeney MT.** 2010. Antimicrobial resistance in bovine respiratory disease pathogens: measures, trends, and impact on efficacy. Vet. Clin. North Am. Food Anim. Pract. **26**:79–88.

4. **Michael GB, Kadlec K, Sweeney MT, Brzuszkiewicz E, Liesegang H, Daniel R, Murray RW, Watts JL, Schwarz S.** 2012. ICE*Pmu1*, an integrative conjugative element (ICE) of *Pasteurella multocida*: analysis of the regions that comprise 12 antimicrobial resistance genes. J. Antimicrob. Chemother. **67**:84–90.
5. **Michael GB, Kadlec K, Sweeney MT, Brzuszkiewicz E, Liesegang H, Daniel R, Murray RW, Watts JL, Schwarz S.** 2012. ICE*Pmu1*, an integrative conjugative element (ICE) of *Pasteurella multocida*: structure and transfer. J. Antimicrob. Chemother. **67**:91–100.
6. **Michael GB, Eidam C, Kadlec K, Meyer K, Sweeney MT, Murray RW, Watts JL, Schwarz S.** 2012. Increased MICs of gamithromycin and tildipirosin in the presence of the genes *erm*(42) and *msr*(E)-*mph*(E) for bovine *Pasteurella multocida* and *Mannheimia haemolytica*. J. Antimicrob. Chemother. **67**:1555–1557.
7. **Sanger F, Nicklen S, Coulson AR.** 1992. DNA sequencing with chain-terminating inhibitors. 1977. Biotechnol. **24**:104–108.
8. **Staden R, Beal KF, Bonfield JK.** 2000. The Staden package, 1998. Methods Mol. Biol. **132**:115–130.
9. **Tech M, Merkl R.** 2003. YACOP: enhanced gene prediction obtained by a combination of existing methods. In Silico Biol. **3**:441–451.
10. **Aggarwal G, Ramaswamy R.** 2002. Ab initio gene identification: prokaryote genome annotation with GeneScan and GLIMMER. J. Biosci. **27**:7–14.
11. **Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. **35**:3100–3108.
12. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. **25**:955–964.
13. **Zdobnov EM, Apweiler R.** 2001. InterProScan—an integration platform for the signature-recognition methods in interpro. Bioinformatics **17**:847–848.
14. **Markowitz VM, Mavromatis K, Ivanova NN, Chen IM, Chu K, Kyrpides NC.** 2009. IMG er: a system for microbial genome annotation expert review and curation. Bioinformatics **25**:2271–2278.
15. **Makarova KS, Haft DH, Barrangou R, Brouns SJJ, Charpentier E, Horvath P, Moineau S, Mojica FJM, Wolf YI, Yakunin AF, van der Oost J, Koonin EV.** 2011. Evolution and classification of the CRISPR-Cas systems. Nat. Rev. Microbiol. **9**:467–477.