

Genome Sequences of *Mannheimia haemolytica* Serotype A2: Ovine and Bovine Isolates[∇]

Paulraj K. Lawrence,¹ Weerayuth Kittichotirat,² Roger E. Bumgarner,² Jason E. McDermott,³ David R. Herndon,⁴ Donald P. Knowles,^{1,4} and Subramaniam Srikumaran^{1*}

Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164-7040¹; Department of Microbiology, University of Washington, Seattle, Washington 98195-7242²; Pacific Northwest National Laboratory, Richland, Washington 99352³; and Animal Disease Research Unit, USDA Agricultural Research Service, Washington State University, Pullman, Washington 99164-6630⁴

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This report describes the genome sequences of *Mannheimia haemolytica* serotype A2 isolated from pneumonic lungs of two different ruminant species, one from *Ovis aries*, designated ovine (O), and the other from *Bos taurus*, designated bovine (B).

Mannheimia haemolytica is a Gram-negative weakly hemolytic coccobacillus and is the principal bacterial pathogen of respiratory disease in cattle and other domestic and wild ruminants (15, 1, 14). *M. haemolytica* is a commensal found in the upper respiratory tract of healthy ruminants (6), but in conjunction with certain viral infections and stress factors, this organism multiplies, reaches the lungs, and causes acute fibrinonecrotic pleuropneumonia (16). This disease is commonly known as shipping fever in cattle and costs more than \$1 billion annually in economic losses to the U.S. cattle industry alone (2). *M. haemolytica* produces several virulence factors (3), including leukotoxin (Lkt). Lkt is a member of the RTX (repeats-in-toxin) family of pore-forming exotoxins produced by bacteria, including *Actinobacillus actinomycetemcomitans* (11), *Actinobacillus pleuropneumoniae*, (6) and *Escherichia coli* (18).

Of the 12 different serotypes of *M. haemolytica* that have been identified, serotypes A1 and A2 are the most prevalent worldwide (10, 19). These serotypes colonize the upper respiratory tract of healthy cattle and sheep and are generally (but not exclusively) species specific in their ability to cause pneumonia (10, 19)—serotype A1 being responsible for pneumonia in cattle and serotype A2 being responsible for pneumonia in sheep. The molecular basis for differential susceptibility of sheep and cattle to serotypes A1 and A2 has not been elucidated. The genome of *M. haemolytica* serotype A1 has been sequenced (9), and the availability of the genome sequence of serotype A2 will facilitate the genomic and proteomic analysis aimed at elucidating the molecular basis for differential species susceptibility. The A2 genomic sequence will provide for the potential identification of additional virulence factors involved in host specificity.

High-throughput sequencing was performed via 454 pyrosequencing (12) with an average read length of 300 nucleotides, resulting in approximately 20× coverage for each strain. Qual-

ity filtered reads were assembled into contigs by using the Newbler assembler (454 Life Sciences) and produced 82 large contigs (defined as >500 bp) for *Bos taurus* (bovine [B]) and 14 large contigs for *Ovis aries* (ovine [O]). The total number of base pairs in large contigs was 2,478,004 for B and 2,584,200 for O, which is in agreement with the published size of the *M. haemolytica* A1 genome.

Contigs were aligned to the genome of serotype A1 by using in-house software. Automated annotation was performed using a protocol similar to the annotation engine service at The Institute for Genomic Research (J. Craig Venter Institute) with some in-house modifications. The protein-coding regions were identified using Glimmer3 (5). BLAST Extend-Repraze was applied to predicted genes to identify truncations due to frameshift mutations or premature stop codons. tRNA and rRNA genes were identified by using tRNAscan-SE (13), and a similarity search was performed using our in-house database. We identified 3,818 protein-coding regions (64% G+C), which is approximately 1,000 more than in the *M. haemolytica* A1 genome. Homologs between coding regions in A1 and the two A2 strains share 95 to 99% nucleotide identity. Coding regions were annotated using BLASTP (<http://blast.ncbi.nlm.nih.gov>) with a stringency of 80% identity over 90% lengths. This resulted in fewer genes with assigned function compared to A1 (1,966). The numbers of genes with assigned function were 1,649 (B) and 1,717 (O) with 173 genes unique to O and 57 to B. A large proportion of phage (11.5 and 12.1% for B and O, respectively) and pseudogenes (9.1% and 9.3% for B and O, respectively) were found, indicating the presence of pathogenic islands conducive for lateral gene transfer (for B, COK_0537-COK_0547 and COK_1260-COK_1276; and for O, COI_0076-COI_0079 and COI_1005-COI_1045). In contrast with strain A1, both A2 strains have all secondary metabolism biosynthesis and transport and catabolism pathways, including leukotoxin and fatty acyl coenzyme A (CoA) needed for toxin activation. The first 35 amino acids of the chief virulence factor (Lkt), which is involved in pore formation (4, 7), are 100% identical in O and B. However, LktA of serotype A1 has 51% amino acid substitution in this region but shares 98% overall identity with O and 88% with B, respectively. The host colonization en-

* Corresponding author. Mailing address: Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040. Phone: (509) 335-4572. Fax: (509) 335-8529. E-mail: ssrikumaran@vetmed.wsu.edu.

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zyme *O*-sialoglycoprotein endopeptidase, outer membrane lipoprotein precursor, *N*-acetyl-hexosamine, dUTP diphosphatase heptosyl transferase, and UDP-glucose 4-epimerase share 98 to 100% identity in all three strains. These are key enzymes involved in lipopolysaccharide (LPS) modification and complex carbohydrate synthesis potentially involved in host cell recognition. We discovered evidence of type III or IV secretion systems in B and O genomes by using a novel computational method (17), which demonstrated homology with conjugal DNA-protein, transfer VirB and pilin (Pilc). However, there is currently no experimental evidence that *M. haemolytica* possesses systems to chaperone these effector proteins. Collectively, the genomes of B and O are largely in synteny with A1, even though we identified large-scale inversions and rearrangements that will be subsequently confirmed by optical mapping and primer walking.

The availability of three *M. haemolytica* genome sequences will enable a more thorough genome-wide comparison across pathogenic bacteria, especially among RTX toxin-secreting species, and, in conjunction with proteomics, will further enable the design of subunit vaccines.

Sequence accession numbers. The complete genome sequences of *Mannheimia haemolytica* serotype A2 were deposited into the GenBank database and assigned accession numbers ACZX01000000 (O) and ACZY01000003 (B).

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REFERENCES

- Ackermann, M. R., and K. A. Brogden. 2000. Response of the ruminant respiratory tract to *Mannheimia (Pasteurella) haemolytica*. *Microbes Infect.* **2**:1079–1088.
- Bowland, S. L., and P. E. Shewn. 2000. Bovine respiratory disease: commercial vaccines currently available in Canada. *Can. Vet. J.* **41**:33–48.
- Confer, A. W., R. J. Panciera, K. D. Clinkenbeard, and D. A. Moiser. 1990. Molecular aspects of virulence of *Pasteurella haemolytica*. *Can. J. Vet. Res.* **54**:S48–S52.
- Cruz, W. T., R. Young, Y. F. Chang, and D. K. Struck. 1990. Deletion analysis resolves cell-binding and lytic domains of the *Pasteurella* leukotoxin. *Mol. Microbiol.* **4**:1933–1939.
- Delcher, A. L., K. A., Bratke, E. C. Powers, and S. L. Salzberg. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* **23**:673–679.
- Devenish, J., S. Rosendal, R. Johnson, and S. Hubler. 1989. Immunoserological comparison of 104-kilodalton proteins associated with hemolysis and cytolysis in *Actinobacillus pleuropneumoniae*, *Actinobacillus suis*, *Pasteurella haemolytica*, and *Escherichia coli*. *Infect. Immun.* **57**:3210–3213.
- Forestier, C., and R. A. Welch. 1991. Identification of RTX toxin target cell specificity domains by use of hybrid genes. *Infect. Immun.* **59**:4212–4220.
- Reference deleted.
- Gioia, J., X. Qin, H. Jiang, K. Clinkerbeard, R. Lo, Y. Liu, G. E. Fox, S. Yerrapragada, M. P. McLeod, T. Z. McNeill, L. Hemphill, E. Sodergren, Q. Wang, D. M. Muzny, F. J. Homsy, G. M. Weinstock, and S. K. Highlander. 2006. The genome sequence of *Mannheimia haemolytica* A1: insight into virulence, natural competence, and *Pasteurellaceae* phylogeny. *J. Bacteriol.* **188**:7257–7266.
- Highlander, S. K. 2001. Molecular genetic analysis of virulence in *Mannheimia (Pasteurella) haemolytica*. *Front. Biosci.* **6**:1128–1150.
- Kolodrubetz, D., T. Dailey, J. Ebersole, and E. Kraig. 1989. Cloning and expression of the leukotoxin gene from *Actinobacillus actinomycetemcomitans*. *Infect. Immun.* **57**:1465–1469.
- Margulies, M., M. Egholm, W. E. Altman, S. Attiya, J. S. Bader, L. A. Bembem, J. Berka, M. S. Braverman, Y. J. Chen, Z. Chen, S. B. Dewell, L. Du, J. M. Fierro, X. V. Gomes, B. C. Godwin, W. He, S. Helgesen, C. H. Ho, G. P. Irzyk, S. C. Jando, M. L. Alenquer, T. P. Jarvie, K. B. Jirage, J. B. Kim, J. R. Knight, J. R. Lanza, J. H. Leamon, S. M. Lefkowitz, M. Lei, J. Li, K. L. Lohman, H. Lu, V. B. Makhijani, K. E. McDade, M. P. McKenna, E. W. Myers, E. Nickerson, J. R. Nobile, R. Plant, B. P. Puc, M. T. Ronan, G. T. Roth, G. J. Sarkis, J. F. Simons, J. W. Simpson, M. Srinivasan, K. R. Tartaro, A. Tomasz, K. A. Vogt, G. A. Volkmer, S. H. Wang, Y. Wang, M. P. Weiner, P. Yu, R. F. Begley, and J. M. Rothberg. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**:376–380.
- Lowe, T. M., and S. R. Eddy. 1997. tRAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* **25**:955–964.
- Miller, M. W. 2001. Pasteurellosis, p. 330–339. In E. S. Williams and I. K. Barker (ed.), *Infectious diseases of wild mammals*. Iowa State University Press, Ames, IA.
- Mosier, D. A. 1997. Bacterial pneumonia. *Vet. Clin. N. Am. Food Anim. Pract.* **13**:483–493.
- Rehmtulla, A. J., and R. G. Thomson. 1981. A review of the lesions in shipping fever of cattle. *Can. Vet. J.* **22**:1–8.
- Samudrala, R., F. Heffron, and J. E. McDermott. 2009. Accurate prediction of secreted substrates and identification of a conserved putative secretion signal for type III secretion systems. *PLoS Pathog.* **4**:e1000375.
- Strathdee, C. A., and R. Y. Li. 1989. Cloning, nucleotide sequence, and characterization of genes encoding the secretion function of the *Pasteurella haemolytica* leukotoxin determinant. *J. Bacteriol.* **171**:916–928.
- Zecchinon, L., T. Fett, and D. Desmecht. 2005. How *Mannheimia haemolytica* defeats host defense through a kiss of death mechanism. *Vet. Res.* **2**:133–156.