Draft Genome Sequences of Actinobacillus pleuropneumoniae Serotypes 2 and 6^{∇}

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Actinobacillus pleuropneumoniae is a bacterial pathogen that causes highly contagious respiratory infection in pigs and has a serious impact on the production economy and animal welfare. As clear differences in virulence between serotypes have been observed, the genetic basis should be investigated at the genomic level. Here, we present the draft genome sequences of the A. pleuropneumoniae serotypes 2 (strain 4226) and 6 (strain Femo).

Previous studies of different serotypes of Actinobacillus pleuropneumoniae showed that there are significant variations among them at the DNA sequence level, supposed to cause differences in pathogenicity and immunogenicity (2, 8). However, it is difficult to carry out more general studies of the immunity mechanisms of different serotypes, typing-based diagnosis, and multivalent genetically engineered vaccines due to the lack of complete genome sequences of the different serotypes (11).

Draft genome sequences of serotypes 2 and 6 were assembled by combining Roche 454-FLX reads with Illumina Genome Analyzer IIx paired-end reads. The final assembly (CLC-GenomicsWorkbench version 3.6, CLCbio) of the serotype 2 genome has a length of 2,314,315 bp (38 contigs), while the assembly of serotype 6 has a length of 2,375,501 bp (36 contigs). The average GC contents are 41.17% and 40.95% in serotypes 2 and 6, respectively, similar to those reported for other serotypes (1, 12). Using EasyGene (6, 9), 2,100 putative open reading frames were predicted for serotype 2 and 2,168 for serotype 6. Approximately 86% of the nucleotides were predicted to be involved in coding sequences, which is similar to results reported for other finished A. pleuropneumoniae genomes (1, 12). There are 86 and 136 genes found to be specific for serotypes 2 and 6, respectively, that do not have any homologue in other reported serotypes. rRNA genes were identified by RNAmmer (5). Serotype 2 harbors six rRNA operons (16S-23S-5S rRNA) and three additional 5S rRNA genes, while six rRNA operons and one additional 5S rRNA gene are present in serotype 6. Using the tRNAscan-SE server (7), 61 and 59 tRNA operons were predicted for serotypes 2 and 6, respectively.

Since porcine pleuropneumonia caused by A. pleuropneumo*niae* leads to large economic losses for the swine industry (3), it is of considerable interest to investigate the virulence factors of the different serotypes. We compiled a list of 105 known and putative virulence genes of A. pleuropneumoniae from pub-

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lished literature and performed a diversity study of these genes at the nucleotide level, comparing serotypes 2 and 6. Sixty-two conserved virulence genes (>99% identity or fewer than three mismatches) were found between these two serotypes, while 28 virulence genes showed a larger degree of dissimilarity (<95%) identity or more than 20 mismatches), including candidates like the apxIVA gene encoding RTX toxin and the cysI gene encoding NADPH-sulfite reductase hemoprotein. Capsular polysaccharides (CPS) produced by A. pleuropneumoniae are considered to be important virulence factors (10). Investigation of CPS genes among different A. pleuropneumoniae serotypes, performed by Jessing et al. (4), was based mainly on partial sequences of CPS operons; here, the full-length sequences of CPS-related genes of serotypes 2 and 6 provide additional information for a better understanding of the role of this antigen.

The genomic sequences of A. pleuropneumoniae serotypes 2 and 6 have been included in the construction of a DNA microarray (Nimblegen, Roche) (Klitgaard et al., unpublished data), thus providing a valuable tool for transcriptional profiling studies and typing-based diagnostics.

Nucleotide sequence accession numbers. Genome sequences have been deposited in GenBank under the project identification number 49597 with accession number ADXN00000000 for A. pleuropneumoniae serotype 2 and under project identification number 49599 with accession number ADXO00000000 for A. pleuropneumoniae serotype 6.

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REFERENCES

- 1. Foote, S. J., J. T. Bosse, A. B. Bouevitch, P. R. Langford, N. M. Young, and J. H. Nash. 2008. The complete genome sequence of Actinobacillus pleuropneumoniae L20 (serotype 5b). J. Bacteriol. 190:1495-1496.
- 2. Jacobsen, M. J., J. P. Nielsen, and R. Nielsen. 1996. Comparison of virulence of different Actinobacillus pleuropneumoniae serotypes and biotypes using an aerosol infection model. Vet. Microbiol. 49:159-168.
- 3. Jacques, M. 2004. Surface polysaccharides and iron-uptake systems of Actinobacillus pleuropneumoniae. Can. J. Vet. Res. 68:81-85
- 4. Jessing, S. G., P. Ahrens, T. J. Inzana, and O. Angen. 2008. The genetic

organisation of the capsule biosynthesis region of *Actinobacillus pleuropneumoniae* serotypes 1, 6, 7, and 12. Vet. Microbiol. **129:**350–359.

- Lagesen, K., P. Hallin, E. A. Rodland, H. H. Staerfeldt, T. Rognes, and D. W. Ussery. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35:3100–3108.
- 6. Larsen, T. S., and A. Krogh. 2003. EasyGene—a prokaryotic gene finder that ranks ORFs by statistical significance. BMC Bioinformatics 4:21.
- 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.
- Maas, A., J. Meens, N. Baltes, I. Hennig-Pauka, and G. F. Gerlach. 2006. Development of a DIVA subunit vaccine against *Actinobacillus pleuropneumoniae* infection. Vaccine 24:7226–7237.
- Nielsen, P., and A. Krogh. 2005. Large-scale prokaryotic gene prediction and comparison to genome annotation. Bioinformatics 21:4322–4329.
- Perry, M. B., E. Altman, J.-R. Brisson, L. M. Beynon, and J. C. Richards. 1990. Structural characteristics of the antigenic capsular polysaccharides and lipopolysaccharides involved in the serological classification of *Actinobacillus* (*Haemophilus*) pleuropneumoniae strains. Serodiagn. Immunother. Infect. Dis. 4:299–308.
- Xie, F., L. Lei, C. Du, S. Li, W. Han, and Z. Ren. 2010. Genomic differences between *Actinobacillus pleuropneumoniae* serotypes 1 and 3 and the diversity distribution among 15 serotypes. FEMS Microbiol. Lett. 303:147–155.
- 2. Xu, Z., Y. Zhou, L. Li, R. Zhou, S. Xiao, Y. Wan, S. Zhang, K. Wang, W. Li, H. Jin, M. Kang, B. Dalai, T. Li, L. Liu, Y. Cheng, L. Zhang, T. Xu, H. Zheng, S. Pu, B. Wang, W. Gu, X. L. Zhang, G. F. Zhu, S. Wang, G. P. Zhao, and H. Chen. 2008. Genome biology of *Actinobacillus pleuropneumoniae* JL03, an isolate of serotype 3 prevalent in China. PLoS One 3:e1450.