

Complete Genome Sequence of *Actinobacillus suis* H91-0380, a Virulent Serotype O2 Strain

Janet I. MacInnes,^a Joanne Mackinnon,^b Adina R. Bujold,^a Kim Ziebell,^b Andrew M. Kropinski,^{b,c} and John H. E. Nash^{a,b}

Department of Pathobiology^a and Department of Molecular and Cellular Biology,^c University of Guelph, Ontario, Canada, and Public Health Agency of Canada, Laboratory for Foodborne Zoonoses, Guelph, Ontario, Canada^b

Here, we report the first complete genome sequence of *Actinobacillus suis*, an important opportunistic pathogen of swine. By comparing the genome sequence of *A. suis* with those of other members of the family *Pasteurellaceae*, we hope to better understand the role of these organisms in health and disease in swine.

Actinobacillus suis is an important opportunistic pathogen of swine (10) that is able to cause disease in animals of all ages. In addition to a common polysaccharide (6), two O and three K serovars of *A. suis* have been described (11, 12, 14), and there are several lines of evidence to suggest that some strains have greater virulence potential than others (16, 17). *A. suis* shares many virulence factors (e.g., *ApxI* and *ApxII*) with the closely related swine pathogen *Actinobacillus pleuropneumoniae* (7). Both of these organisms can cause an acute hemorrhagic pleuropneumonia, but *A. suis* has a broader host range than *A. pleuropneumoniae* and it can, in addition, cause septicemia, enteritis, meningitis, arthritis, skin lesions, and abortion (10). By comparing the *A. suis* genome sequence with available *A. pleuropneumoniae* sequences, we hope to identify genetic differences that begin to explain the unique tissue and host specificity of these pathogens and members of the family *Pasteurellaceae* that colonize the oropharyngeal cavity.

Shotgun genome sequencing of the virulent *A. suis* serovar O2 strain H91-0380 was done by using 454 pyrosequencing at the McGill University and Génome Québec Innovation Centre and assembled using MIRA 3 (8). The contigs were organized by BLASTX analysis of their 3' and 5' ends (13) and by alignment with an OpGen *AfIII* optical map (9). The gaps were closed by long-range PCR and primer walking. Using these approaches, a single contig totaling 2,484,940 bp was assembled and annotated using the NCBI automated prokaryotic genome annotation pipeline (<http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html>); further analysis was done using RAST (1). A 305-bp region flanked by poly(A) repeats was not able to be sequenced despite numerous attempts.

The *A. suis* H91-0380 genome has a G+C content of 40.24%. It contains 2,249 coding sequences and has six complete rRNA operons. Chromosome alignment using progressiveMauve (5) revealed that the *A. suis* H91-0380 genome is very similar to that of *A. pleuropneumoniae*, especially serovar 3; there are many syntenic regions, but large segments have been rearranged.

In addition to the *apxI* and *apxII* operons, putative virulence factors detected in the *A. suis* genome include 37 open reading frames (ORFs) associated with iron acquisition and metabolism, including hemoglobin receptor (3) and transferrin receptor (2) proteins, and 40 genes encoding 22 putative fimbrial and afimbrial adhesins, including homologues of a type IV fimbriae operon, a low-G+C *tad* locus, genes encoding tangled pili, prepilins, and a fibronectin-binding protein, 11 outer membrane proteins (OMPs), and 5 autotransporters (ATs). Like other members of the

family *Pasteurellaceae*, there is evidence that at least some strains of *A. suis* may be naturally competent (4). *A. suis* H91-0380 contains 827 perfect matches of the *A. pleuropneumoniae* DNA uptake signal sequences and many, but not all, of the reported competence genes (15). *A. suis* has a greater number of cell wall and capsule genes (211 versus 201), complete prophages (2 versus 0), and motility and chemotaxis genes (8 versus 0) but fewer stress response genes (76 versus 92) than *A. pleuropneumoniae*.

Nucleotide sequence accession number. The complete genome sequence of *A. suis* H91-0380 was deposited in GenBank under accession number CP003875.

ACKNOWLEDGMENTS

We gratefully acknowledge the support of the Natural Sciences and Engineering Research Council of Canada, the Ontario Ministry of Food, and the Public Health Agency of Canada and the efforts of Dianne Pillitteri and Jackie Fountain.

REFERENCES

1. Aziz RK, et al. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75.
2. Bahrami F, Ekins A, Niven DF. 2003. Iron acquisition by *Actinobacillus suis*: identification and characterization of transferrin receptor proteins and encoding genes. *Vet. Microbiol.* 94:79–92.
3. Bahrami F, Niven DF. 2005. Iron acquisition by *Actinobacillus suis*: identification and characterization of a single-component haemoglobin receptor and encoding gene. *Microb. Pathog.* 39:45–51.
4. Bossé JT, et al. 2009. Natural competence in strains of *Actinobacillus pleuropneumoniae*. *FEMS Microbiol. Lett.* 298:124–130.
5. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 5:e11147. doi:10.1371/journal.pone.0011147.
6. Deutschmann RR, Boncheff AGA, Daraban L, MacInnes JI, Monteiro MA. 2010. Common sialylated glycan in *Actinobacillus suis*. *Glycobiology* 20:1227–1232.
7. Gottschalk M, Taylor DJ. 2006. *Actinobacillus pleuropneumoniae*, p 563–575. In Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ (ed), *Diseases of swine*. Blackwell Publishing, Ames, IA.
8. Kumar S, Blaxter ML. 2010. Comparing de novo assemblers for 454 transcriptome data. *BMC Genomics* 11:571.
9. Latreille P, et al. 2007. Optical mapping as a routine tool for bacterial genome sequence finishing. *BMC Genomics* 8:321.

Received 28 September 2012 Accepted 1 October 2012

Address correspondence to Janet I. MacInnes, macinnes@uoguelph.ca.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JB.01633-12

10. MacInnes JI, Desrosiers R. 1999. Agents of the “suis-ide diseases” of swine: *Actinobacillus suis*, *Haemophilus parasuis*, and *Streptococcus suis*. *Can. J. Vet. Res.* **63**:83–89.
11. Michael FS, et al. 2004. Structural analysis of the lipopolysaccharide derived core oligosaccharides of *Actinobacillus pleuropneumoniae* serotypes 1, 2, 5a and the genome strain 5b. *Carbohydr. Res.* **339**:1973–1984.
12. Monteiro MA, et al. 2000. The first description of a (1→6)-β-D-glucan in prokaryotes: (1→6)-β-D-glucan is a common component of *Actinobacillus suis* and is the basis for a serotyping system. *Carbohydr. Res.* **329**:121–130.
13. Nash JH, et al. 2010. Genome sequence of adherent-invasive *Escherichia coli* and comparative genomic analysis with other *E. coli* pathotypes. *BMC Genomics* **11**:667.
14. Rullo A, Papp-Szabo E, Michael FS, MacInnes J, Monteiro MA. 2006. The structural basis for the serospecificity of *Actinobacillus suis* serogroup O:2. *Biochem. Cell Biol.* **84**:184–190.
15. Sinha S, Mell JC, Redfield RJ. 2012. Seventeen CRP-S-regulated genes are needed for natural transformation in *Haemophilus influenzae*. *J. Bacteriol.* **194**:5245–5254.
16. Slavic D, Toffner TL, Monteiro M, Perry MB, MacInnes J. 2000. Prevalence of O1/K1- and O2/K3-reactive *Actinobacillus suis* in healthy and diseased swine. *J. Clin. Microbiol.* **38**:3759–3762.
17. Slavic D, DeLay J, Hayes MA, MacInnes JI. 2000. Comparative pathogenicity of different *Actinobacillus suis* O/K serotypes. *Can. J. Vet. Res.* **64**:81–87.