Boss, JT; Chaudhuri, RR; Li, Y; Leanse, LG; Fernandez Crespo, R; Coupland, P; Holden, MT; Bazzolli, DM; Maskell, DJ; Tucker, AW; Wren, BW; Rycroft, AN; Langford, PR (2016) Complete Genome Sequence of MIDG2331, a Genetically Tractable Serovar 8 Clinical Isolate of Actinobacillus pleuropneumoniae. Genome announcements, 4 (1). ISSN 2169-8287 DOI: https://doi.org/10.1128/genomeA.01667-15

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DOI: 10.1128/genomeA.01667-15

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Complete Genome Sequence of MIDG2331, a Genetically Tractable Serovar 8 Clinical Isolate of Actinobacillus pleuropneumoniae

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We report here the complete annotated genome sequence of a clinical serovar 8 isolate Actinobacillus pleuropneumoniae MIDG2331. Unlike the serovar 8 reference strain 405, MIDG2331 is amenable to genetic manipulation via natural transformation as well as conjugation, making it ideal for studies of gene function.

Received 4 December 2015 Accepted 7 December 2015 Published 28 January 2016


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Actinobacillus pleuropneumoniae is a respiratory tract pathogen of swine that causes significant economic losses worldwide. Fifteen serovars of A. pleuropneumoniae vary in geographic distribution (1). Serovar 8, found in many countries around the world (1), is the predominant serovar in the United Kingdom (2) and southeastern Brazil (3). A United Kingdom clinical serovar 8 isolate, MIDG2331, was shown to be highly competent for natural transformation (4) and amenable to conjugal transfer of plasmids (5). This isolate is ideally suited for facile construction of gene deletions as well as complementation using shuttle vectors such as pMC-Express and pMK-Express (6).

The complete genome of sequence of MIDG2331 was determined using a combination of Illumina GAII and PacBio RS II platforms. Illumina sequencing yielded a total of 1,121,724 read pairs of 2 × 76 bp, of which 1,118,490 were retained after adapter trimming using Cutadapt version 1.8.1 (7). PacBio sequencing yielded 13,273 circular consensus sequence (ccs) reads, with a mean length of 2,861.4 bp (longest 13,273 bp), and 15,276 corrected long reads, with a mean length of 5,997.7 bp (longest 17,412 bp).

Individual assembly of the Illumina and PacBio datasets using SPAdes (8) and HGAP (9), respectively, resulted in multiple-contig assemblies (65 Illumina contigs; 2 PacBio contigs). A hybrid SPAdes assembly using both Illumina and PacBio datasets resolved into a single contig that was circularized using Circlator (10) and corrected based on the PacBio ccs reads using Quiver (9), giving a final assembly of 2,337,633 bp. Automated sequence annotation was performed using Prokka version 1.11 (11), which predicted 2,174 putative open reading frames (ORFs), 63 tRNAs, and 6 rRNA operons. The average GC content of the genome is 41.1%, similar to other members of the Pasteurellaceae.

No plasmid DNA was found in MIDG2331, although plasmids have been described in other strains of this species. However, we have identified a 56-kb sequence in the genome showing a high degree of similarity to previously reported Integrative Conjugative Elements of the ICEHin1056 family (12, 13).

We previously characterized the reference strains of A. pleuropneumoniae with regard to competence for natural transformation. Serovars 1, 3, 4, 5, and 8 all showed low frequencies of transformation (10^-8 to 10^-5), whereas the serovar 15 reference strain, HS143, had a transformation frequency of 10^-4 (14). Subsequently, we identified MIDG2331 as highly competent, with a transformation frequency of 10^-3 (4). The genome of MIDG2331 contains a full set of known competence genes (14) as well as 770 copies of the 9-bp sequence ACAAGCGGT, a DNA uptake signal sequence (USS) which is highly overrepresented in the genomes of the ApI subclade of the Pasteurel- laceae (15). Although the serovar 5-120 genome contains the same complete set of competence genes and 742 copies of the A. pleuro- pneumoniae USS (16), natural transformation is not efficient in this strain. More detailed analysis of the MIDG2331 genome, the first from a highly competent A. pleuropneumoniae isolate, may help identify factor(s) which contribute to transformation efficiency in this bacterium.

Nucleotide sequence accession number. The complete genome sequence of MIDG2331 was deposited in GenBank under the accession number LN908249.
ACKNOWLEDGMENTS

We are grateful to Susanna Williamson of the Animal and Plant Health Agency (UK) for supplying MIDG2331. The BRaDPIT Consortium comprises: Duncan J. Maskell, Alexander W. (Dan) Tucker, Sarah E. Peters, Lucy A. Weinert, Jinhong (Tracy) Wang, Shi-Lu Luan, Roy R. Chaudhuri (University of Cambridge; present address for R. Chaudhuri is: Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, United Kingdom); Andrew N. Rycroft, Gareth A. Maglennon, Jessica Beddow (Royal Veterinary College); Brendan W. Wren, Jon Cucui, Vanessa S. Terra (London School of Hygiene and Tropical Medicine); and Paul R. Langford, Janine T. Bossé, Yanwen Li (Imperial College London).

FUNDING INFORMATION

Wellcome Trust provided funding to Paul Coupland and Matthew Holden under grant number 098051. Biotechnology and Biological Sciences Research Council (BBSRC) provided funding to Janine T. Bossé, Roy R. Chaudhuri, Yanwen Li, Leon G. Leanse, Roberto Fernandez Crespo, Paul Coupland, Matthew Holden, Denise Mara Soares Bazzolli, Duncan J. Maskell, Dan Tucker, Brendan W. Wren, Andrew N. Rycroft, Paul R. Langford, and BRaDPIT consortium under grant numbers BB/G020744/1, BB/G019177/1, BB/G019274/1, and BB/G018553/1. Biotechnology and Biological Sciences Research Council (BBSRC) provided funding to Paul R Langford under grant number BB/K021109/1. MCTI | Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) provided funding to Denise Mara Soares Bazzolli under grant number PDE 201840/2011-1. This work was supported by a Longer and Larger (LoLa) grant from the Biotechnology and Biological Sciences Research Council (grant numbers BB/G020744/1, BB/G019177/1, BB/G019274/1, and BB/G018553/1), the UK Department for Environment, Food and Rural Affairs, and Zoetis Biotech UK, BB/G020744/1, BB/G019177/1, BB/G019274/1, and BB/G018553/1. Biotechnology and Biological Sciences Research Council (BBSRC) provided funding to Janine T. Bosse et al. under grant number BB/K021109/1. MCTI | Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) provided funding to Denise Mara Soares Bazzolli under grant number PDE 201840/2011-1.

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