






Draft Genome Sequences of the Type Strains of *Actinobacillus indolicus* (46K2C) and *Actinobacillus porcinus* (NM319), Two NAD-Dependent Bacterial Species Found in the Respiratory Tract of Pigs

 Janine T. Bossé,^a Yanwen Li,^a Roberto Fernandez Crespo,^a Øystein Angen,^b  Matthew T. G. Holden,^{c*} Lucy A. Weinert,^d Duncan J. Maskell,^d Alexander W. Tucker,^d Brendan W. Wren,^e Andrew N. Rycroft,^f  Paul R. Langford,^a on behalf of the BRaDP1T consortium

^aSection of Paediatric Infectious Disease, Department of Infectious Disease, Imperial College London, London, United Kingdom

^bDepartment of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark

^cThe Wellcome Trust Sanger Institute, Cambridge, United Kingdom

^dDepartment of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom

^eFaculty of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom

^fDepartment of Pathology and Pathogen Biology, The Royal Veterinary College, Hatfield, United Kingdom

ABSTRACT We report here the draft genome sequences of the type strains of *Actinobacillus indolicus* (46K2C) and *Actinobacillus porcinus* (NM319). These NAD-dependent bacterial species are frequently found in the upper respiratory tract of pigs and are occasionally associated with lung pathology.

Bacteria belonging to the family *Pasteurellaceae* that inhabit the porcine respiratory tract and require NAD (also called the V factor) for growth include *Actinobacillus pleuropneumoniae*, *Glaesserella parasuis* (formerly *Haemophilus parasuis*), *Actinobacillus indolicus*, *Actinobacillus porcinus*, *Actinobacillus minor*, and a separate species tentatively designated "*Actinobacillus porcitonissillarum*," which, although phenotypically more similar to *A. pleuropneumoniae*, is phylogenetically closer to *A. minor* (1–5). The first two species are important pathogens, whereas the latter four are considered commensals of the upper respiratory tract (1, 3, 6), though isolates of *A. indolicus* and *A. porcinus*, associated with lung pathology, have been identified (1). For *A. pleuropneumoniae*, there are two biotypes based on the requirement for NAD, with most isolates belonging to the NAD-dependent biovar 1 (7, 8).

The phylogeny of the *Pasteurellaceae* is complicated, and there remain numerous species with uncertain taxonomic status (4, 9, 10). Whole-genome sequences, which can help clarify evolutionary relationships and the development of differential diagnostics, are available for all of the above-mentioned bacteria except *A. indolicus* and *A. porcinus*. We therefore chose to sequence the type strains, 46K2C and NM319, respectively, of these species. Both 46K2C^T and NM319^T were originally isolated from the upper respiratory tract of pigs in Denmark (11) and Canada (12), respectively. They were initially identified as *Haemophilus* taxons D and F, respectively, prior to their designation as the type strains of the newly assigned species in 1996 (1, 2).

Strains 46K2C^T and NM319^T were obtained from the Culture Collection University of Gothenburg (CCUG 39029^T and CCUG 38924^T, respectively), stored frozen at –80°C in 25% glycerol, and minimally passaged (up to four times) on brain heart infusion agar (Difco) supplemented with 0.01% NAD prior to genomic DNA extraction using a FastDNA spin kit (MP Biomedicals), according to the manufacturer's protocol for bacterial cells. Paired-end libraries were prepared from 500 ng of genomic DNA, as

Citation Bossé JT, Li Y, Fernandez Crespo R, Angen Ø, Holden MTG, Weinert LA, Maskell DJ, Tucker AW, Wren BW, Rycroft AN, Langford PR, on behalf of the BRaDP1T Consortium. 2020. Draft genome sequences of the type strains of *Actinobacillus indolicus* (46K2C) and *Actinobacillus porcinus* (NM319), two NAD-dependent bacterial species found in the respiratory tract of pigs. *Microbiol Resour Announc* 9:e00716-19. <https://doi.org/10.1128/MRA.00716-19>.

Editor Steven R. Gill, University of Rochester School of Medicine and Dentistry

Copyright © 2020 Bossé et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Janine T. Bossé, j.bosse@imperial.ac.uk, or Paul R. Langford, p.langford@imperial.ac.uk.

* Present address: Matthew T. G. Holden, School of Medicine, University of St. Andrews, St. Andrews, United Kingdom.

Received 18 June 2019

Accepted 14 November 2019

Published 2 January 2020

previously described (13), using the modified Illumina protocol developed by Quail et al. (14) for sequencing at the Wellcome Sanger Institute (Hinxton, UK) on an Illumina HiSeq 2000 analyzer for 75 cycles. Draft genome sequences were assembled *de novo* using a previously reported pipeline (15). Briefly, the 2×76 -bp paired reads were used to create multiple assemblies for each species' genome using Velvet v1.2 (16) and VelvetOptimiser v2.2.5 (<http://bioinformatics.net.au/software.velvetoptimiser.shtml>). For each genome, the assembly with the best N_{50} value was further improved by scaffolding contigs using SSPACE (17), with any gaps filled by GapFiller (18). Automated annotation was performed for both genome assemblies using Prokka v1.5 (19). Default parameters were used for all software programs.

The draft genome of *A. indolicus* 46K2C^T was assembled from 2,031,716 total reads, with an average quality score of 37.9, into 22 contigs (N_{50} , 183,245; L_{50} , 4), with a total length of 2,103,350 bases. This sequence encodes 1,956 predicted proteins and has a G+C content of 40.1%, which is higher than the 35.5% originally determined (2) but lower than the value of 42.6% calculated by Kuhnert and Korczak based on the G+C content of selected genes (20). The draft genome of *A. porcinus* NM319^T was assembled from 1,733,818 reads, with an average quality score of 37.8, into 29 contigs (N_{50} , 182,117; L_{50} , 5), with a total length of 2,280,774 bases. This sequence encodes 2,095 predicted proteins and has a G+C content of 41.1%, which is similar to values (41.4% and 41.6%) previously reported for this strain (2, 20).

The availability of the draft genome sequences of the type strains of *A. indolicus* and *A. porcinus* will facilitate differentiation of these species from other NAD-dependent bacteria found in the porcine respiratory tract and contribute to the understanding of *Pasteurellaceae* phylogeny and host interactive biology.

Data availability. The draft genome assemblies for *A. indolicus* 46K2C^T and *A. porcinus* NM319^T have been deposited in the European Nucleotide Archive (ENA) under BioProject number [PRJEB31492](https://www.ebi.ac.uk/bioproject/1492). Raw sequence reads have been deposited in the ENA under accession numbers [ERS134282](https://www.ebi.ac.uk/ena/submitter/ERS134282) and [ERS134597](https://www.ebi.ac.uk/ena/submitter/ERS134597), respectively. The ENA accession numbers for the assembled contigs are [GCA_901764975](https://www.ebi.ac.uk/ena/submitter/GCA_901764975) ([CABFKH010000001](https://www.ebi.ac.uk/ena/submitter/CABFKH010000001) through [CABFKH010000022](https://www.ebi.ac.uk/ena/submitter/CABFKH010000022)) and [GCA_901764995](https://www.ebi.ac.uk/ena/submitter/GCA_901764995) ([CAAGST010000001](https://www.ebi.ac.uk/ena/submitter/CAAGST010000001) through [CAAGST010000029](https://www.ebi.ac.uk/ena/submitter/CAAGST010000029)), respectively.

ACKNOWLEDGMENTS

The Bacterial Respiratory Diseases of Pigs-1 Technology (BRaDP1T) Consortium comprises the following members: 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 Cuccui, Vanessa S. Terra (London School of Hygiene and Tropical Medicine); and Paul R. Langford, Janine T. Bossé, and Yanwen Li (Imperial College London).

This work was supported by a Longer and Larger (LoLa) grant from the Biotechnology and Biological Sciences Research Council (BBSRC, grant numbers BB/G020744/1, BB/G019177/1, BB/G019274/1, BB/G018553/1, and, in part, BB/S000103/1 and BB/S005897/1), the UK Department for Environment, Food and Rural Affairs, and Zoetis (formerly Pfizer Animal Health) awarded to the BRaDP1T Consortium. M.T.G.H. was supported by the Wellcome Trust (grant number 098051).

The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

- Kielstein P, Wuthe H, Angen Ø, Mutters R, Ahrens P. 2001. Phenotypic and genetic characterization of NAD-dependent *Pasteurellaceae* from the respiratory tract of pigs and their possible pathogenetic importance. *Vet Microbiol* 81:243–255. [https://doi.org/10.1016/s0378-1135\(01\)00351-0](https://doi.org/10.1016/s0378-1135(01)00351-0).
- Møller K, Füssing V, Grimont PA, Paster BJ, Dewhirst FE, Kilian M. 1996. *Actinobacillus minor* sp. nov., *Actinobacillus porcinus* sp. nov., and *Actinobacillus indolicus* sp. nov., three new V factor-dependent species from the respiratory tract of pigs. *Int J Syst Bacteriol* 46:951–956. <https://doi.org/10.1099/00207713-46-4-951>.

3. Gottschalk M, Broes A, Mittal KR, Kobisch M, Kuhnert P, Lebrun A, Frey J. 2003. Non-pathogenic *Actinobacillus* isolates antigenically and biochemically similar to *Actinobacillus pleuropneumoniae*: a novel species? *Vet Microbiol* 92:87–101. [https://doi.org/10.1016/s0378-1135\(02\)00341-3](https://doi.org/10.1016/s0378-1135(02)00341-3).
4. Korczak B, Christensen H, Emler S, Frey J, Kuhnert P. 2004. Phylogeny of the family *Pasteurellaceae* based on *rpoB* sequences. *Int J Syst Evol Microbiol* 54:1393–1399. <https://doi.org/10.1099/ijs.0.03043-0>.
5. Dickerman A, Bandara AB, Inzana TJ. Phylogenomic analysis of *Haemophilus parasuis* and proposed reclassification to *Glaesserella parasuis*, gen. nov., comb. nov. *Int J Syst Evol Microbiol*, in press. <https://doi.org/10.1099/ijsem.0.003730>.
6. Chiers K, Haesebrouck F, Mateusen B, Van Overbeke I, Ducatelle R. 2001. Pathogenicity of *Actinobacillus minor*, *Actinobacillus indolicus* and *Actinobacillus porcicus* strains for gnotobiotic piglets. *J Vet Med B Infect Dis Vet Public Health* 48:127–131. <https://doi.org/10.1111/j.1439-0450.2001.00433.x>.
7. Sassu EL, Bossé JT, Tobias TJ, Gottschalk M, Langford PR, Hennig-Pauka I. 2018. Update on *Actinobacillus pleuropneumoniae*—knowledge, gaps and challenges. *Transbound Emerg Dis* 65:72–90. <https://doi.org/10.1111/tbed.12739>.
8. Maldonado J, Valls L, Martínez E, Riera P. 2009. Isolation rates, serovars, and toxin genotypes of nicotinamide adenine dinucleotide-independent *Actinobacillus pleuropneumoniae* among pigs suffering from pleuropneumonia in Spain. *J Vet Diagn Invest* 21:854–857. <https://doi.org/10.1177/104063870902100615>.
9. Christensen H, Kuhnert P, Busse H-J, Frederiksen WC, Bisgaard M. 2007. Proposed minimal standards for the description of genera, species and subspecies of the *Pasteurellaceae*. *Int J Syst Evol Microbiol* 57:166–178. <https://doi.org/10.1099/ijs.0.64838-0>.
10. Naushad S, Adeolu M, Goel N, Khadka B, Al-Dahwi A, Gupta RS. 2015. Phylogenomic and molecular demarcation of the core members of the polyphyletic *Pasteurellaceae* genera *Actinobacillus*, *Haemophilus*, and *Pasteurella*. *Int J Genomics* 2015:198560. <https://doi.org/10.1155/2015/198560>.
11. Møller K, Kilian M. 1990. V factor-dependent members of the family *Pasteurellaceae* in the porcine upper respiratory tract. *J Clin Microbiol* 28:2711–2716.
12. Gilbride KA, Rosendal S. 1983. Evaluation of a selective medium for isolation of *Haemophilus pleuropneumoniae*. *Can J Comp Med* 47:445–450.
13. Howell KJ, Weinert LA, Luan S-L, Peters SE, Chaudhuri RR, Harris D, Angen Ø, Aragon V, Parkhill J, Langford PR, Rycroft AN, Wren BW, Tucker AW, Maskell DJ, BRaDP1T Consortium. 2013. Gene content and diversity of the loci encoding biosynthesis of capsular polysaccharides of the 15 serovar reference strains of *Haemophilus parasuis*. *J Bacteriol* 195:4264–4273. <https://doi.org/10.1128/JB.00471-13>.
14. Quail MA, Kozarewa I, Smith F, Scally A, Stephens PJ, Durbin R, Swerdlow H, Turner D. 2008. A large genome center's improvements to the Illumina sequencing system. *Nat Methods* 5:1005–1010. <https://doi.org/10.1038/nmeth.1270>.
15. Page AJ, De Silva N, Hunt M, Quail MA, Parkhill J, Harris SR, Otto TD, Keane JA. 2016. Robust high-throughput prokaryote de novo assembly and improvement pipeline for Illumina data. *Microb Genom* 2:e000083. <https://doi.org/10.1099/mgen.0.000083>.
16. Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <https://doi.org/10.1101/gr.074492.107>.
17. Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. 2011. Scaffolding pre-assembled contigs using SSPACE. *Bioinformatics* 27:578–579. <https://doi.org/10.1093/bioinformatics/btq683>.
18. Boetzer M, Pirovano W. 2012. Toward almost closed genomes with GapFiller. *Genome Biol* 13:R56. <https://doi.org/10.1186/gb-2012-13-6-r56>.
19. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
20. Kuhnert P, Korczak BM. 2006. Prediction of whole-genome DNA–DNA similarity, determination of G+C content and phylogenetic analysis within the family *Pasteurellaceae* by multilocus sequence analysis (MLSA). *Microbiology* 152:2537–2548. <https://doi.org/10.1099/mic.0.28991-0>.