AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

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

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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 porcitonsillarum*," 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

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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. Raw sequence reads have been deposited in the ENA under accession numbers ERS134282 and ERS134597, respectively. The ENA accession numbers for the assembled contigs are GCA_901764975 (CABFKH010000001 through CABFKH010000022) and GCA_901764995 (CAAGST010000001 through 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

1. 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.

2. Møller K, Fussing 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.
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 porcinus* 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.