



Draft Genome Sequence of *Hafnia paralvei* Strain VBC_1714, Isolated from Frozen Cod Fillet Imported from Russia to Norway

💿 Julia E. Storesund, a Didrik H. Grevskott, a 💿 Nachiket P. Marathe, a Bjørn Tore Lunestad, a Cecilie Smith Svanevika

^aNorwegian Institute of Marine Research, Bergen, Norway

ABSTRACT Hafnia spp. have the potential to cause opportunistic infections in humans and animals. This announcement describes the draft genome sequence of an H₂S-positive Hafnia paralvei strain that was isolated as a presumptive Salmonella sp. from a frozen cod fillet.

afnia spp. are common gut bacteria that are generally considered nonpathogenic and are associated with cheese production and food spoilage (1). They are, however, infrequently reported as opportunistic pathogens causing infections in humans and animals and have been associated with a range of food products (2, 3). *Hafnia* strains can carry multiple antimicrobial resistance and virulence genes (1, 4–6) and are therefore highly interesting from a human health perspective.

Strain VBC_1714 was isolated from a sample of frozen cod (Gadus morhua) imported from Russia to Norway in 2020 that was analyzed for Salmonella according to ISO:6579-1:2017 (7) as part of the national surveillance of imported seafood. Briefly, 25 g of muscle fillet was aseptically transferred to 225 ml buffered peptone water, homogenized (Stomacher 400 circulator; Seward, UK), and incubated aerobically at 37°C for 18 h. From the incubated enrichment broth, 1 ml was transferred into 10 ml Rappaport-Vassiliadis soy peptone broth (RVS broth) and incubated aerobically at 42°C for 24 h, after which $10 \,\mu$ l was plated on xylose lysine deoxycholate (XLD) agar. One isolate grew as dark pink colonies with a black center and was thus identified as a putative Salmonella strain. Hafnia spp. can be mistaken for Salmonella on certain specific growth media (8), but they do not commonly produce H_2S (9). The strain was purified by restreaking on blood agar and further identified as Hafnia alvei using a Bruker MALDI Biotyper. Genomic DNA was extracted using the Qiagen DNeasy blood and tissue kit (Germany) following the manufacturer's protocol. The extracted DNA was quantified using a Qubit doublestranded (dsDNA) broad-range (BR) assay kit (Thermo Scientific), and sequencing libraries were prepared using a 2S Turbo DNA library preparation kit (Swift Biosciences, USA). Sequencing was performed using the MiSeg platform (Illumina, USA) with 2×300 -bp sequencing technology at the Norwegian Sequencing Centre (Oslo University Hospital, Norway).

The barcodes were removed from 1,488,640 raw reads and the reads were quality trimmed using BBDuk v38.63 (10). The contigs were assembled from 1,260,024 quality-filtered reads using SPAdes v3.13.1 with default parameters (11). The assembled genome had an N_{50} value of 581,084, a total length of 4,558,914 bp, and a G+C content of 48.1% and consisted of 29 contigs (>500 bp; average coverage, 17.1×), 4,428 coding DNA sequences, 15 rRNAs, and 78 tRNAs. Strain VBC_1714 was further identified as *Hafnia paralvei* using Kraken2 v2.0.8-beta (12). Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline v5.0 (13).

The genome of strain VBC_1714 contained two genes associated with H_2S production in *E. coli*, the 3-mercaptopyruvate sulfurtransferase gene, which has been associated with H_2S production under aerobic conditions (14, 15), and *lscS* cysteine desulfurase, which is associated with H_2S production under anaerobic conditions (14), as well as with sulfur and iron assimilation and Fe-S assembly (16).

Citation Storesund JE, Grevskott DH, Marathe NP, Lunestad BT, Svanevik CS. 2021. Draft genome sequence of *Hafnia paralvei* strain VBC_1714, isolated from frozen cod fillet imported from Russia to Norway. Microbiol Resour Announc 10:e00624-21. https://doi.org/ 10.1128/MRA.00624-21.

Editor Frank J. Stewart, Montana State University

Copyright © 2021 Storesund et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Julia E. Storesund, Julia.Storesund@hi.no.

Received 16 June 2021 Accepted 7 July 2021 Published 19 August 2021 Strain VBC_1714 contained one CRISPR array with five spacers and the *arnA* gene, which has been linked to polymyxin resistance (1). The class C β -lactamase gene $bla_{ACC-1b'}$ which confers resistance to aminopenicillins and narrow-spectrum cephalosporins (4), was identified using AMRFinder v3.1.1b (17). Four complete and two partial prophages were identified using PHASTER (18). One plasmid of 5,216 bp was identified and displayed 100% nucleotide identity to plasmid pAlvB, found in *Hafnia alvei* strain MISC261 (19).

Data availability. This whole-genome shotgun project has been deposited in DDBJ/ ENA/GenBank under the accession number JAEAGQ00000000.1. The raw sequences are available in the SRA under the accession number SRR14280049 and the BioProject accession number PRJNA682044.

ACKNOWLEDGMENTS

The samples were acquired and the strain was isolated as a part of the National Surveillance of Imported Fish and Seafood, funded by the Norwegian Food Safety Authority (project number 15220/OK-program: 43387). The DNA preparation and genome sequencing were funded by the Institute for Marine Research as a part of the internally funded Ocean Health program (project number 15495). The genomic analyses and manuscript preparation were funded by the Institute for Marine Research through the internally funded Antimicrobial Resistance in Marine Environments program (project number 15319).

We are grateful to Betty Irgens for her valuable help with the laboratory work.

REFERENCES

- Bean DC, Wigmore SM, Abdul Momin MHF, Wareham DW. 2020. Polymyxin resistant bacteria in Australian poultry. Front Sustain Food Syst 4:550318. https://doi.org/10.3389/fsufs.2020.550318.
- 2. Gunthard H, Pennekamp A. 1996. Clinical significance of extraintestinal Hafnia alvei isolates from 61 patients and review of the literature. Clin Infect Dis 22:1040–1045. https://doi.org/10.1093/clinids/22.6.1040.
- Janda JM, Abbott SL. 2006. The genus Hafnia: from soup to nuts. Clin Microbiol Rev 19:12–28. https://doi.org/10.1128/CMR.19.1.12-28.2006.
- Stock I, Rahman M, Sherwood KJ, Wiedemann B. 2005. Natural antimicrobial susceptibility patterns and biochemical identification of Escherichia albertii and Hafnia alvei strains. Diagn Microbiol Infect Dis 51:151–163. https://doi.org/10.1016/j.diagmicrobio.2004.10.008.
- Jayol A, Saly M, Nordmann P, Ménard A, Poirel L, Dubois V. 2017. Hafnia, an enterobacterial genus naturally resistant to colistin revealed by three susceptibility testing methods. J Antimicrob Chemother 72:2507–2511. https://doi.org/10.1093/jac/dkx154.
- Abbott SL, Moler S, Green N, Tran RK, Wainwright K, Janda JM. 2011. Clinical and laboratory diagnostic characteristics and cytotoxigenic potential of Hafnia alvei and Hafnia paralvei strains. J Clin Microbiol 49:3122–3126. https://doi.org/10.1128/JCM.00866-11.
- International Organisation for Standardization. 2017. ISO 6579-1:2017: microbiology of the food chain—horizontal method for the detection, enumeration and serotyping of Salmonella—part 1: detection of Salmonella spp.
- Cooney S, O'Brien S, Iversen C, Fanning S. 2014. Bacteria: other pathogenic Enterobacteriaceae—Enterobacter and other genera, p 433–441. *In* Encyclopedia of food safety. Academic Press, Cambridge, MA.
- Huys G, Cnockaert M, Abbott SL, Janda JM, Vandamme P. 2010. Hafnia paralvei sp. nov., formerly known as Hafnia alvei hybridization group 2. Int J Syst Evol Microbiol 60:1725–1728. https://doi.org/10.1099/ijs.0.018606-0.
- 10. Bushnell B. 2019. BBMap. https://sourceforge.net/projects/bbmap/.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV,

Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.

- Wood DE, Lu J, Langmead B. 2019. Improved metagenomic analysis with Kraken 2. Genome Biol 20:257. https://doi.org/10.1186/s13059-019-1891-0.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10 .1093/nar/qkw569.
- Wang J, Guo X, Li H, Qi H, Qian J, Yan S, Shi J, Niu W. 2019. Hydrogen sulfide from cysteine desulfurase, not 3-mercaptopyruvate sulfurtransferase, contributes to sustaining cell growth and bioenergetics in E. coli under anaerobic conditions. Front Microbiol 10:2357. https://doi.org/10.3389/ fmicb.2019.02357.
- Tanizawa K. 2011. Production of H₂S by 3-mercaptopyruvate sulphurtransferase. J Biochem 149:357–359. https://doi.org/10.1093/jb/mvr018.
- Blanc B, Gerez C, Ollagnier de Choudens S. 2015. Assembly of Fe/S proteins in bacterial systems: biochemistry of the bacterial ISC system. Biochim Biophys Acta 1853:1436–1447. https://doi.org/10.1016/j.bbamcr.2014 .12.009.
- Feldgarden M, Brover V, Haft DH, Prasad AB, Slotta DJ, Tolstoy I, Tyson GH, Zhao S, Hsu C-H, McDermott PF, Tadesse DA, Morales C, Simmons M, Tillman G, Wasilenko J, Folster JP, Klimke W. 2019. Validating the AMRFinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. Antimicrob Agents Chemother 63: e00483-19. https://doi.org/10.1128/AAC.00483-19.
- Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44:W16–W21. https://doi.org/10.1093/nar/gkw387.
- 19. Wertz JE, Riley MA. 2004. Chimeric nature of two plasmids of Hafnia alvei encoding the bacteriocins alveicins A and B. J Bacteriol 186:1598–1605. https://doi.org/10.1128/JB.186.6.1598-1605.2004.