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# Closed Genome Sequences of *Providencia alcalifaciens* Isolates from Dogs

Thomas H. A. Haverkamp,[b] Håkon Kaspersen,[b] Øivind Øines,[b] Bjørn Spilsberg,[a] Amar Anandrao Telke,[b] Karin Lagesen,[a] Jannice Schau Slettemeås,[b] Solveig Sølverød Mo,[b] Hannah Joan Jørgensen,[b] Camilla Sekse[b]

aDepartment of Analysis and Diagnostics, Norwegian Veterinary Institute, Ås, Norway
bDepartment of Animal Health and Food Safety, Norwegian Veterinary Institute, Ås, Norway

**ABSTRACT** Eight *Providencia alcalifaciens* isolates from eight different dogs in Norway with acute hemorrhagic diarrhea were sequenced. Based on Illumina and Oxford Nanopore Technologies sequencing, all of the genomes were complete and closed after hybrid assembly.

In the autumn of 2019, there was an outbreak of acute hemorrhagic diarrhea syndrome in dogs in Norway. *Providencia alcalifaciens* was identified as a possible cause of the outbreak (1). To determine whether *P. alcalifaciens* could stem from a common source, isolates from diseased dogs were sequenced. Phylogenetic analysis revealed that several isolates clustered closely together, thus suggesting a common origin (1). *P. alcalifaciens* has previously been associated with cases of enteritis in dogs (2, 3), but a possible role of this pathogen in causing acute hemorrhagic diarrhea in dogs is not well documented.

We aimed to characterize *P. alcalifaciens* genomes from some of the affected dogs. In total, eight *P. alcalifaciens* isolates from eight different dogs were selected for Illumina and Oxford Nanopore Technologies (ONT) sequencing. Here, we report hybrid assemblies of these isolates and characterization of the assemblies.

*P. alcalifaciens* was isolated from stool or gastrointestinal samples from dogs, as described by Jørgensen et al. (1). Samples were obtained with owner consent, which includes a general clause providing permission to use the samples and the secondary material for research. DNA was extracted on Genomic-tip 100/G columns (Qiagen) using the supplier's bacterial protocol. The DNA concentration was determined using the Qubit double-stranded DNA (dsDNA) BR assay kit (Thermo Fisher Scientific), and DNA quality was assessed using the NanoDrop One spectrophotometer (Thermo Fisher Scientific). Libraries for Illumina sequencing were prepared using the Nextera Flex library preparation kit (Illumina), followed by sequencing on a MiSeq system (Illumina) at the Norwegian Sequencing Center, using 300-bp paired-end chemistry. High-quality DNA (~400 ng) from each sample was used for ONT library preparation using the rapid barcoding library preparation kit (SQK-RBK004; ONT) and was indexed using RB1-12 barcodes. Pooled libraries were cleaned with an AMPure XP bead cleanup step. The barcoded library (10 $\mu$L) was loaded onto a FLO-MIN106 R9 flow cell on a MinION device (ONT) and run for 37.5 h. Raw sequence data were base called separately after the run using Guppy v.3.4.5 (ONT) and demultiplexed using qcat v.1.1.0 (ONT) (https://github.com/nanoporetech/qcat). The sequence quality of the demultiplexed data sets was checked with NanoPlot v.1.30.0 (4). Default parameters were used for all software unless otherwise specified.

Filtlong v.0.2.0 (https://github.com/rrwick/Filtlong) was used to filter the long reads based on quality. Hybrid assemblies were generated using Unicycler v.0.4.8 (5), followed by Prokka v.1.14.5 (6) to annotate the hybrid assemblies. The GC content of

**TABLE 1** Overview of *Providencia alcalifaciens* genomes

| Sample | Part of outbreak cluster | Virulence gene | Size of plasmid related to *cdtB* (bp) | No. of Illumina reads (R1 and R2) | No. of ONT reads | ONT read $N_{50}$ (bp) | No. of replicons | Total size (Mbp) | GC content (%) | No. of genes (Prokka) | Coverage (×) | Illumina read accession no. | ONT read accession no. | Assembly accession no. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2019-01-32383-1-1 | No | *cdtB* | 168,972 | 679,898 | 61,938 | 7,965 | 2 | 4,123,814 | 41.8 | 3,864 | 93.0 | ERR6686186 | ERR6716414 | GCA_915401745 |
| 2019-04-28369-1-2 | Yes | *cdtB* | 170,607 | 751,378 | 51,255 | 11,678 | 5 | 4,386,911 | 41.9 | 4,227 | 96.2 | ERR6688396 | ERR6716416 | GCA_915401735 |
| 2019-04-28370-1-5 | Yes | *cdtB* | 171,426 | 723,910 | 39,773 | 6,972 | 6 | 4,386,930 | 41.9 | 4,227 | 93.7 | ERR6688397 | ERR6716417 | GCA_915401765 |
| 2019-04-29290-1-7 | No | | | 727,231 | 69,468 | 5,291 | 2 | 4,218,374 | 41.8 | 4,005 | 97.5 | ERR6688399 | ERR6716419 | GCA_915402005 |
| 2019-04-29291-1-1 | No | | | 337,335 | 138,506 | 4,984 | 2 | 3,933,480 | 42.6 | 3,607 | 48.2 | ERR6688400 | ERR6716420 | GCA_915403165 |
| 2019-04-29292-1-3 | Yes | *cdtB* | 171,398 | 852,192 | 87,265 | 8,597 | 5 | 4,386,609 | 41.8 | 4,229 | 108.8 | ERR6688407 | ERR6716421 | GCA_915402015 |
| 2019-04-27799-1-2 | Yes | *cdtB* | 171,440 | 830,802 | 54,233 | 13,459 | 5 | 4,386,154 | 41.9 | 4,229 | 107.4 | ERR6688302 | ERR6716415 | GCA_915401705 |
| 2019-04-29034-1-3 | Yes | *cdtB* | 171,426 | 624,173 | 94,011 | 10,190 | 5 | 4,386,908 | 41.9 | 4,228 | 80.8 | ERR6688398 | ERR6716418 | GCA_915401715 |

each assembly was calculated using the EMBOSS v.6.6.0 (7) commands union and infoseq. The mean sequencing depth was determined by mapping reads with BWA v.0.7.17 (8) and then calculating the mean coverage using SAMtools v.1.9 (9) depth. MOB-suite v.3.0.1 (10) was used to predict plasmid sequences from the hybrid assemblies and to identify their respective replicon types. Each plasmid FASTA file generated by MOB-suite was examined for the presence of resistance genes, virulence genes, and plasmids by using ResFinder v.4.0 (11), VirulenceFinder v.2.0 (12), and PlasmidFinder v.2.1 (13), respectively. Each isolate was also screened for virulence genes using the web tool VFanalyzer (14), with *Escherichia coli* as the closest reference organism.

All isolates were complete (100% for all isolates, according to CheckM [15]) and closed after hybrid assembly. No antimicrobial resistance genes were identified. Cytolethal distending toxin B (*cdtB*) was identified on a plasmid in six of the isolates. The characteristics of the genomes, including accession numbers, are presented in Table 1.

**Data availability.** All data sets have been deposited in ENA under accession number PRJEB47525 (Table 1).

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