AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome Sequences of Two *Pasteurella multocida* Isolates from Seabirds

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ABSTRACT *Pasteurella multocida* is one of the major causes of mass mortalities in wild birds. Here, we report the complete genome sequences of two *P. multocida* isolates from wild populations of two endangered seabird species, the Indian yellow-nosed albatrosses (*Thalassarche carteri*) and the northern rockhopper penguins (*Eudyptes moseleyi*).

Recurrent outbreaks of fowl cholera (FC), caused by *Pasteurella multocida*, have been associated with mass die-offs; threatening the population of nestling albatrosses on Amsterdam Island, in the southern Indian Ocean (1, 2, 15, 16, and 17). Dead birds were necropsied on this island as part of a wildlife health monitoring program conducted since 2013 (1, 3, 4). The fieldwork was approved by the French Regional Animal Experimentation Ethical Committee no. 036 (Ministry of Research permit 10257-2018011712301381) and by the Comité de l'Environnement Polaire (A-2018-123 and A-2018-139 for 2018 to 2019). During necropsy, femur bone marrow was swabbed, and swabs were incubated in liquid Amies transport medium before cultivation (up to 8 h). The swabs were then inoculated on Columbia sheep blood and incubated at room temperature (13 to 20°C) for up to 48 h. Most abundant type colonies were selected and stored in stock culture agar at room temperature during transportation to the laboratory (up to several months). They were then streaked on blood medium plates and incubated at 37°C. Characterization of the obtained clonal populations was performed by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) (5). Isolates identified as *P. multocida* were stored at −80°C until sequencing. Two isolates of *P. multocida*—one from an adult yellow-nosed albatross (*Thalassarche carteri*) and one from an adult northern rockhopper penguin (*Eudyptes moseleyi*)—were sequenced in this study (Table 1).

Selected isolates were sequenced with the Illumina and Nanopore sequencing platforms for the purpose of hybrid assembly. The Circulomics Nanobind CBB Big DNA kit (Circulomics, Baltimore, MD, USA) was used for extraction of high-molecular-weight DNA for Nanopore sequencing. Nanopore libraries were prepared using the SQK-LSK109 and EXP-NBD104 kits according to the manufacturer's protocol (Oxford Nanopore Technologies, United Kingdom). DNA shearing and size selection were not performed. Sequencing libraries were sequenced using MinION Mk1B sequencer (Oxford Nanopore Technologies) with R9.4.1 flow cells. For Illumina sequencing, bacterial DNA extraction was performed using MagMax pathogen RNA/DNA kit (Thermo Fisher Scientific, Waltham, MA, USA), as previously described (6), on

Editor Irene L. G. Newton, Indiana University, Bloomington

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The authors declare no conflict of interest.

Received 29 December 2022
Accepted 7 March 2023
Published 27 March 2023

TABLE 1 Data associated with the two sequenced *P. multocida* strains

Type of data	Parameter[a]	Result for sequenced *P. multocida* strain	
		PM-32985	PM-33011
Metadata	Seabird species	*Eudyptes moseleyi* (adult)	*Thalassarche carteri* (adult)
	Geographical location	Amsterdam Island	Amsterdam Island
	Date of sample collection	30 October 2019	31 October 2019
	Organ of isolation	Femur bone marrow	Femur bone marrow
Raw sequence reads	Illumina paired-end read length (nt)	300	300
	No. of Illumina reads used	117,512	188,312
	Avg Illumina coverage, ×	15	23
	No. of Nanopore reads	12,004	20,863
	Avg Nanopore coverage, ×	72	104
	Nanopore read N_{50} (bp)	35,506	31,401
Assembly statistics for the closed genomes	No. of contigs	2	2
	Total length of the chromosome (bp)	2,428,887	2,443,848
	Total length of the plasmid (bp)[b]	3,741	3,741
	GC content (%)	40.33	40.29
Annotation results	No. of CDSs (with protein)	2,270	2,275
	No. of tRNAs	57	58
	No. of rRNAs	19	19
	No. of ncRNAs	4	4
	No. of CDSs (without protein)	34	14
	No. of CRISPR arrays	3	3
	No. of hypothetical proteins	169	178
	No. of proteins with functional assignments	2,101	2,097
MLST	ST by RIRDC scheme[c]	ST61	ST61
	ST by multiple-host scheme[d]	ST91	ST91
DNA fingerprinting		1,710	1,710
Serotyping	AGID	1, 14	1, 14
ANI (%)[e]	Identity to *P. multocida* type strain NCTC10322 (LT906458.1)	98.62	98.63
GenBank data	BioSample accession no.	SAMN28552975	SAMN28552996
	BioProject accession no.	PRJNA839711	PRJNA839711
	Complete genome assembly accession no.	Chromosome: CP097610 Plasmid: CP097611	Chromosome: CP097612 Plasmid: CP097613

[a] CDSs, coding DNA sequences; ncRNAs, noncoding RNAs; MLST, multilocus sequence typing; RIRDC, Rural Industries Research and Development Corporation; AGID, agar gel immune diffusion.
[b] The presence or absence of plasmids in the genome sequences was determined using the plasmid database (11) available via PLSDB at https://ccb-microbe.cs.uni-saarland.de/plsdb.
[c] MLST was performed using the *P. multocida* RIRDC MLST scheme (12) available via PubMLST (13) at https://pubmlst.org/organisms/pasteurella-multocida.
[d] MLST was performed using the *P. multocida* multiple-host MLST scheme available via PubMLST (13).
[e] The percentage of average nucleotide identity (ANI) was calculated based on BLAST+ (14) available via the JSpeciesWS online service at https://jspecies.ribohost.com/jspeciesws/#home.

a Kingfisher-Flex instrument (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, extracted DNA was used for the preparation of sequencing libraries using Illumina DNA Prep tagmentation kit (previously called Nextera DNA Flex) with IDT for Illumina DNA/RNA UD indexes and set A according to the manufacturer's protocol (Illumina, USA). Sequencing was performed using the Illumina MiSeq system (v3 reagent kit using 2 × 300-bp paired-end reads).

Raw reads from Illumina data were trimmed for quality, and sequencing adapters were removed using Trimmomatic v.0.33 (7). For Nanopore sequencing, raw Nanopore electrical signal (.fast5) was converted to base sequence (.fastq) through high-accuracy basecalling using Guppy (v.4.2.3) within MinKNOW (20.10.6) software. NanoFilt version 2.8.0 was then used to quality-filter Nanopore reads and to filter out sequences less than 1,000 bp in length (8). Hybrid assemblies were performed using Unicycler v.0.5.0 (9). Default parameters were

used for the software mentioned. Each of the sequenced genomes showed 2 circular contigs (1 chromosome and 1 plasmid). The genomes were annotated using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.6.1 (10).

Sequencing, annotation, and typing results for the sequenced isolates are presented in Table 1. The availability of these complete genomes will help us to understand the source of *P. multocida* infections among seabirds through comparison to *P. multocida* genomes from other species.

Data availability. The genomes are available from NCBI BioProject no, PRJNA839711 under the complete genome assembly accession no. (CP097610, CP097611, CP097612, and CP097613) shown in Table 1.

ACKNOWLEDGMENTS

This project was supported by Iowa Livestock Health Advisory Council grant no. PG103652. The research on Amsterdam Island was supported by the French Polar Institute (IPEV ECOPATH-1151/DIVING BIRDS-394), ANR ECOPATHS (ANR-21-CE35-0016), project France relance TVACALBA, Zone Atelier Antarctique et Terres Australes (ZATA), and OSU OREME ECOPOP. The isolates in this article were produced through a contribution of IPEV project ECOPATH-1151 to the Plan National d'Action Albatros d'Amsterdam.

We thank the Iowa State University Veterinary Diagnostic Laboratory faculty and staff for assistance with sample processing and diagnostic testing. We also thank Nicolas Keck, Karine Lemberger, Christophe Barbraud, Charles-André Bost, Aline Fléchet, and Karine Delord for help at different stages of the work. Support is also acknowledged from Réserve Naturelle Nationale des Terres Australes Françaises, notably via Célia Lesage. We thank technical support agents at Ceva Biovac for help with laboratory work. Bioinformatics was supported using tools available from the University of Minnesota's Minnesota Supercomputing Institute.

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