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## *Pasteurella multocida* genome assembly of Indonesian local isolates using long-read Oxford Nanopore Technology sequencing

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### ABSTRACT

**Background:** *Pasteurella multocida* is a Gram-negative bacterium responsible for various diseases in animals, including hemorrhagic septicemia (HS). Oxford Nanopore Technologies' (ONT) platform streams the sequencing data immediately, allowing for on-the-fly analysis. This capability enables researchers to monitor experiments in real time and make prompt decisions, which is particularly beneficial for clinical diagnostics and outbreak surveillance.

**Aim:** This study presents the genome assembly of two *P. multocida* isolates using long-read sequencing technology provided by ONT.

**Methods:** This method involves passing DNA strands through nanopores and detecting changes in electrical current to determine nucleotide sequences. The long-read capability of ONT technology allows for the sequencing of extensive DNA fragments, which is particularly beneficial for resolving complex genomic regions and achieving high-contiguity genome assemblies.

**Results:** The study generated high-quality single-contig genomes with sizes of 2.35 and 2.29 Mbp and completeness scores exceeding 99% (BUSCO). These assemblies provide valuable resources for functional analyses, enhancing the understanding of virulence factors and the pathogenesis of HS in Indonesian livestock.

**Conclusion:** This study successfully assembled the genomes of two *P. multocida* isolates from Lampung (2,952) and Kupang, Nusa Tenggara Timur, Indonesia, using Oxford Nanopore Technologies' long-read sequencing. The resulting single-contig genomes, measuring 2.35 and 2.29 Mbp, respectively, exhibited high completeness scores (BUSCO: >99%). These high-quality assemblies enhance genomic contiguity, providing valuable resources for functional analyses aimed at understanding the virulence factors and pathogenesis of HS in Indonesian livestock.

**Keywords:** Genome assembly, Hemorrhagic septicemia, Oxford Nanopore Technologies, *P. multocida*, Whole-genome sequencing.

### Introduction

*Pasteurella multocida* is a Gram-negative pathogen bacterial of veterinary significance that affects livestock and causes substantial economic losses worldwide (Lestari *et al.*, 2025). *P. multocida* is a major causative agent of hemorrhagic septicemia (HS), a severe, often fatal disease affecting cattle and buffalo (Hasnan *et al.*, 2022). HS outbreaks have significant economic impacts on smallholder farmers and the livestock industry throughout the Indonesian archipelago, particularly in regions such as Sumatra and Nusa Tenggara (Yohanes and Theresia, 2012). This is because both locations are livestock warehouses in Indonesia and HS-

endemic areas. This disease has long been recognized in Indonesia as a strategic infectious animal disease. Young livestock are usually more susceptible to disease than adult animals. Buffalo are more susceptible to HS than cattle (Almoheer *et al.*, 2022).

This disease attacks various organs, causing bleeding in the digestive system, under the skin, and even the respiratory tract (Wei *et al.*, 2025). Clinical signs usually begin with fever, lethargy, lacrimation, excessive salivation, subcutaneous edema, and nasal discharge, followed by respiratory distress, snoring, and septic shock with extensive bleeding (Lestari *et al.*, 2025). In addition, swelling and edema can be seen on

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the head, lower chest, legs, or base of the tail (Doyle-Baker *et al.*, 2020). Lesions in the esophagus cause shortness of breath and difficulty swallowing. Affected animals appear depressed. Death may occur 1-3 days after clinical signs begin (Shivachandra *et al.*, 2011). Antibiotic treatment can be effective in the early stages, but because acute clinical signs are usually very rapid, mortality is almost 100% (Almoheer *et al.*, 2022). Effective and efficient prevention of HS is achieved through vaccination (Gowrakkal *et al.*, 2014).

Proper genome assembly is essential for identifying antimicrobial resistance genes and virulence factors (Zankari *et al.*, 2012). Traditional short-read sequencing methods often produce fragmented assemblies, complicating downstream genomic analysis. Long-read sequencing technologies, such as Oxford Nanopore Technology (ONT), offer improved genome contiguity and completeness by resolving repetitive regions that typically fragment short-read assemblies (Wick *et al.*, 2019).

In this study, we generated genome assemblies for local Indonesian isolates of *P. multocida* from Lampung, Sumatera (labeled 2952) Nusa Tenggara Timur (NTT), and Kupang NTT (labeled NTT) using ONT sequencing. This approach aims to provide high-quality reference genomes to better understand the genetic basis of the virulence and pathogenicity of local *P. multocida* strains, which is crucial for developing improved diagnostic tools and control strategies specific to the Indonesian context.

## Materials and Methods

### Bacterial strains and DNA extraction

*Pasteurella multocida* 2952 was collected from clinical cases of bovine HS in Lampung, Sumatera Island, and *P. multocida* NTT was collected from Nusa Tenggara Timur, Indonesia. Both *P. multocida* isolates were grown on a brain heart infusion (BHI) medium overnight in an incubator at 37°C, and DNA was extracted using Qiagen QIAamp DNA Mini Kits, following the manufacturer's protocol.

To continue DNA extraction, a series of tests were conducted to ensure the feasibility of whole-genome sequencing (WGS). The test included polymerase chain reaction targeting on the *km* gene, a specific marker for *P. multocida* in confirming the identity of the isolates. Initial DNA quantification and purity assessment were performed using an Implen NanoPhotometer N50/N60, followed by visualization via agarose gel electrophoresis. Precise DNA quantification was performed using a QubitFlex Fluorometer (Thermo Scientific), and DNA integrity was assessed using an Agilent TapeStation 4150.

### Library preparation and sequencing

A library preparation kit from ONT was used to prepare whole-genome DNA libraries from *P. multocida* isolates NTT and 2952. The prepared libraries were quantified using a Qubit Fluorometer before being loaded onto the

flow cell. DNA repair was performed using an end-prep enzyme mix, resulting in 5'-phosphorylated and 3'-dA-tailed ends. The repaired DNA was then ligated with ONT-compatible adapters.

Sequencing was conducted using the PromethION 2 Solo platform (ONT) until the required sequencing yield was achieved. The sequencing process was managed using MinKNOW software, and base calling was performed using Dorado in high-accuracy mode (HAC). The sequencing run was configured with the following parameters: Flow cell type FLO-PRO114M, Kit type SQK-NBD114-24, MinKNOW version 24.02.16, Bream version 7.9.8, Configuration 5.9.18, Dorado version 7.3.11, and MinKNOW Core version 5.9.12.

### Data analysis

Filtering was performed using Filtlong (<https://github.com/rrwick/Filtlong>), and data transformation was performed using Nanoplot, Canu, and Flye. The read quality was evaluated using Nanoplot (De Coster *et al.*, 2018). Reads correction and assembly were performed using Canu and Flye (Koren *et al.*, 2017). The assembled sequence was polished four times with Racon (Luan *et al.*, 2024) and three times with Medaka (<https://github.com/nanoporetech/medaka>) for further refinement. Racon was chosen for initial polishing due to its efficiency in correcting errors from long-read assemblies, while Medaka was used for final polishing because it is specifically optimized for Oxford Nanopore data. The polishing was mapped using minimap2 v2.2-r1122 (Li *et al.*, 2021).

The quality of the assembled sequence was determined using Quast v5.2.0 and Qualimap v2.2.2. Gene annotation was conducted using NCBI's Prokaryotic Genome Annotation Pipeline (PGAP) (Li *et al.*, 2021), and genome visualization was performed using Circos v0.69-9. Taxonomy check verification was performed using dfast\_qc (Tanizawa *et al.*, 2018), as shown in Tables 3 and 4. Genome completeness was evaluated using BUSCO v5.4.3 (Levy and Boone, 2019) and CheckM (integrated in dfast\_qc) (Simão *et al.*, 2015).

### Ethical approval

This research was approved by the ethics committee for animal care and use of the National Research and Innovation Agency (BRIN) (approval number: 139/KE.02/SK/07/2023).

## Result

### Sequencing output and assembly statistics

The two isolates yielded approximately 2.82 and 1.43 Gb of sequencing data for isolates 2952 and NTT, respectively. The mean read length exceeded 10 kb (10,776.78 bp for 2952 and 10,183.36 bp for NTT), facilitating high-contiguity assemblies. Both genomes were assembled into single, complete circular chromosomes with no gaps or ambiguities.

### Assembly metrics

Both isolates were successfully assembled into single-contig genomes with high completeness scores

**Table 1.** Summary of recent genome assembly statistics of bovine *P. multocida*.

| Isolate             | Lampung 2952 | NTT        |
|---------------------|--------------|------------|
| Assembly statistics |              |            |
| Number of contig    | 1            | 1          |
| Total length (Mbp)  | 2.35         | 2.29       |
| Number of reads     | 261,742      | 139,998    |
| N50/L50             | 118284/7Kb   | 115853/8Kb |
| Total percentage GC | 40.1         | 40.25      |
| N50 length (bp)     | 12,662.0     | 17,255.0   |
| BUSCO score (%)     | 99.2         | 99.8       |
| Mean read length    | 10,776.78    | 10,183.36  |

(BUSCO: 99.2% and 99.8%), indicating high-quality assemblies. The genome sizes (2.34 Gb for 2952 and 2.29 Gb for NTT; 2.35 Mbp for 2952 and 2.29 Mbp for NTT) and GC content (40.1% and 40.25%) were consistent with other *P. multocida* strains reported in the literature. The mean read length exceeded 10 kb, facilitating high-contiguity assemblies (Table 1).

### Genome annotation features

Annotation of the genomes using NCBI's PGAP identified 2,104 coding sequences (CDS), 19 rRNAs (5S, 16S, 23S), and 57 tRNAs in isolate 2952, and 2,065 CDS, 19 rRNAs, and 56 tRNAs in isolate NTT. The genomes of NTT and 2952 contained genes associated with virulence, including capsular biosynthesis genes (*capD*), outer membrane proteins (*ompA\_1* and *ompA\_2*), iron acquisition systems (*futA1* and *fur*), and adhesions (*fhaC*), as shown in Table 2.

Taxonomy check based on the percentage of sequence similarity between the sample and reference genomes. The strain most closely related to the *P. multocida* NTT

**Table 2.** Virulence-associated genes identified in *P. multocida* NTT and 2952 isolates using the Prokka annotation tool

| Category                              | Gene(s)                               | Function                                                                                             | <i>P. multocida</i> NTT<br>(Gene, length, product)                                                     | <i>P. multocida</i> 2952<br>(Gene, length, product)                                                          |
|---------------------------------------|---------------------------------------|------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|
| <b>Capsule</b>                        | <i>capD</i>                           | Capsule biosynthesis; determines serotype                                                            | <i>capD</i> , 1035 bp; UDP-glucose 4-epimerase                                                         | <i>capD</i> , 1035 bp; UDP-glucose 4-epimerase                                                               |
|                                       | <i>hyaD</i>                           | Hyaluronic acid production (capsular component)                                                      | N/A                                                                                                    | <i>hyaD</i> , 2919-bp, Hyaluronan synthase                                                                   |
| <b>Toxin</b>                          | <i>tdeA</i><br>Type VI DNase effector | Type VI DNase effector A; involved in interbacterial competition and is a potential virulence factor | <i>tdeA</i> , 1368 bp; toxins and drug export protein A                                                | <i>tdeA</i> , 1368 bp; toxins and drug export protein A                                                      |
| <b>Outer membrane proteins (OMPs)</b> | <i>ompA</i>                           | Adhesion; immune evasion                                                                             | <i>ompA_1</i> , 819 bp; outer membrane protein A<br><i>ompA_2</i> , 1077 bp; outer membrane protein P5 | <i>ompA_1</i> , 1062 bp; outer membrane protein P5<br><i>ompA_2</i> , 819 bp; outer membrane protein A       |
|                                       | <i>fhaC</i>                           | Hemagglutinin; promotes host cell attachment                                                         | <i>fhaC</i> , 1719 bp; filamentous hemagglutinin transporter protein                                   | <i>fhaC1</i> , 1731 bp<br><i>fhaC_2</i> , 1722 bp; filamentous hemagglutinin transporter protein <i>fhaC</i> |
| <b>Iron acquisition</b>               | <i>futA</i>                           | Iron transport across the membrane                                                                   | <i>futA1</i> , 1014 bp; iron uptake protein A1                                                         | <i>futA1</i> , 1014 bp; iron uptake protein A1                                                               |
|                                       | <i>fur</i>                            | Regulators of iron uptake                                                                            | <i>fur</i> , 441 bp, Ferric uptake regulation protein                                                  | <i>fur</i> , 441 bp, Ferric uptake regulation protein                                                        |
| <b>Defense enzymes and regulation</b> | <i>sodA</i> ,<br><i>sodC</i>          | Superoxide dismutase; protection against reactive oxygen species from immune cells                   | <i>sodA</i> , 645 bp, Superoxide dismutase [Mn]<br><i>sodC</i> (561 bp, Superoxide dismutase [Cu-Zn]   | <i>sodA</i> , 645 bp, Superoxide dismutase [Mn]<br><i>sodC</i> (561 bp, Superoxide dismutase [Cu-Zn]         |
|                                       | <i>tad</i><br>genes                   | Type IV secretion system (T4SS)-like pili structure; involved in colonization                        | <i>tadA</i> , 501-bp tRNA-specific adenosine deaminase                                                 | <i>tadA</i> , 501-bp tRNA-specific adenosine deaminase                                                       |

N/A = not available.

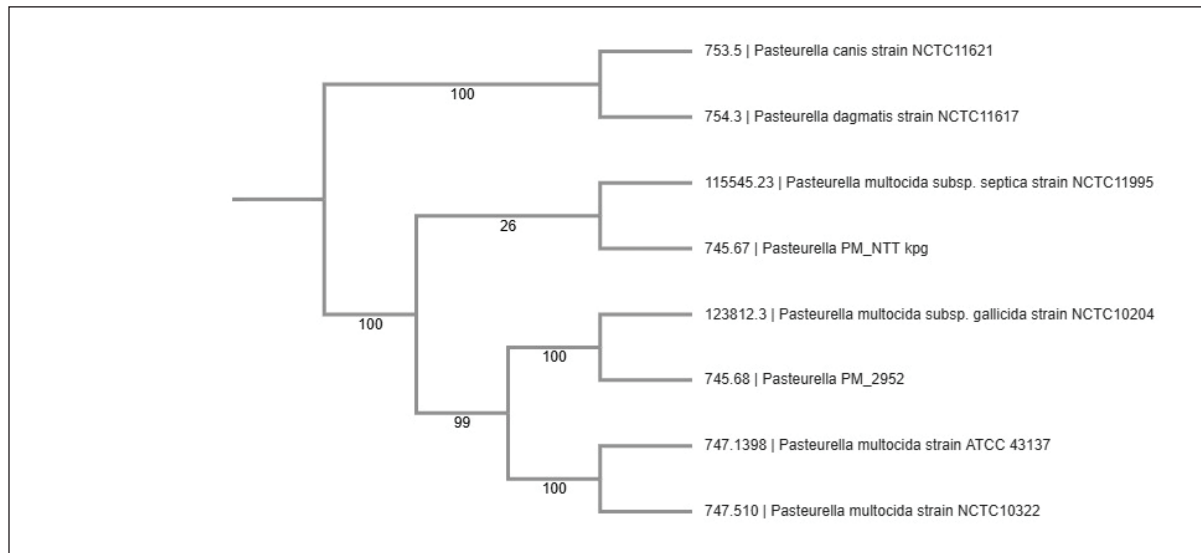
Table 3. Comparison of the *P. multocida* NTT isolate with several other *P. multocida* strains available in GenBank.

| Organism name                                        | Strain                   | Accession       | Taxid  | Species taxid | Relation to type | Validated | Ani     | Matched fragments | Total fragments | Ani threshold | Status          |
|------------------------------------------------------|--------------------------|-----------------|--------|---------------|------------------|-----------|---------|-------------------|-----------------|---------------|-----------------|
| <i>Pasteurella multocida</i>                         | Strain NCTC 10322 (2019) | GCA 900187275.1 | 747    | 747           | Type             | True      | 98.5599 | 703               | 764             | 95            | Conclusive      |
| <i>Pasteurella multocida</i>                         | Strain ATCC 43137        | GCA 000754275.1 | 747    | 747           | Type             | True      | 98.5351 | 707               | 764             | 95            | Conclusive      |
| <i>Pasteurella multocida</i> subsp. <i>gallicida</i> | Strain NCTC 10204        | GCA 900636625.1 | 123812 | 747           | Type             | True      | 98.4535 | 704               | 764             | 95            | Conclusive      |
| <i>Pasteurella multocida</i> subsp. <i>septica</i>   | Strain NCTC 11995        | GCA 900454845.1 | 115545 | 747           | Type             | True      | 96.5909 | 702               | 764             | 95            | Conclusive      |
| <i>Pasteurella dagmatis</i>                          | Strain NCTC 11617        | GCA 900186835.1 | 754    | 754           | Type             | True      | 80.4206 | 431               | 764             | 95            | Below threshold |
| <i>Pasteurella canis</i>                             | Strain NCTC 11621        | GCA 900454865.1 | 753    | 753           | Type             | True      | 80.2324 | 459               | 764             | 95            | Below threshold |

Table 4. Comparison of the *P. multocida* Lampung 2952 isolate with several other *P. multocida* strains available in GenBank.

| Organism name                                        | Strain                   | Accession       | Taxid  | Species taxid | Relation to type | Validated | Ani     | Matched fragments | Total fragments | Ani threshold | Status          |
|------------------------------------------------------|--------------------------|-----------------|--------|---------------|------------------|-----------|---------|-------------------|-----------------|---------------|-----------------|
| <i>Pasteurella multocida</i> subsp. <i>gallicida</i> | Strain NCTC 10204        | GCA 900636625.1 | 123812 | 747           | Type             | True      | 98.7547 | 733               | 782             | 95            | Conclusive      |
| <i>Pasteurella multocida</i>                         | Strain ATCC 43137        | GCA 000754275.1 | 747    | 747           | Type             | True      | 98.6145 | 709               | 782             | 95            | Conclusive      |
| <i>Pasteurella multocida</i>                         | Strain NCTC 10322 (2019) | GCA 900187275.1 | 747    | 747           | Type             | True      | 98.6092 | 712               | 782             | 95            | Conclusive      |
| <i>Pasteurella multocida</i> subsp. <i>septica</i>   | Strain NCTC 11995        | GCA 900454845.1 | 115545 | 747           | Type             | True      | 96.5983 | 702               | 782             | 95            | Conclusive      |
| <i>Pasteurella dagmatis</i>                          | Strain NCTC 11617        | GCA 900186835.1 | 754    | 754           | Type             | True      | 80.6103 | 413               | 782             | 95            | Below threshold |
| <i>Pasteurella canis</i>                             | Strain NCTC 11621        | GCA 900454865.1 | 753    | 753           | Type             | True      | 80.4265 | 453               | 782             | 95            | Below threshold |





**Fig. 1.** Phylogenetic tree of 8 species reconstructed from a comparison of whole-genome sequences using PATRIC.

genome was NCTC 10322 (Table 3). According to the ANI analysis, the genome of *P. multocida* Lampung 2952 shared the highest similarity (98.754%) with *P. multocida* subsp. *gallicida* NCTC 10204 (Table 4). Long-read sequencing effectively resolved repetitive regions, enabling improved genome assemblies compared with short-read methods. The sequencing produced complete circular chromosomes (2.35 Mbp with 261,742 reads) with 100% mapped reads for both isolates, demonstrating the effectiveness of the ONT platform for bacterial genome assembly.

The whole genome dendrogram representation of *P. multocida* genomes illustrates a comparative analysis between our two isolates, one from East Nusa Tenggara (NTT) and the other from Lampung (2952) against reference isolates obtained from GenBank conducted in PATRIC (Wattam *et al.*, 2017), as shown in Figure 1.

### Discussion

High-quality genome assemblies of *P. multocida* isolates from Indonesia provide valuable genomic resources for understanding the molecular basis of virulence and host adaptation in these local strains. The use of long-read ONT sequencing allowed us to achieve complete single-contig assemblies, overcoming the limitations of short-read sequencing in resolving repetitive regions that are commonly present in bacterial genomes (Li, 2021; Hall *et al.*, 2024). This approach is particularly valuable for *P. multocida* genomics because accurate assembly enables precise identification of virulence determinants (Wick *et al.*, 2019).

The genome sizes and GC contents of both isolates were consistent with previously reported *P. multocida* genomes, supporting the accuracy of our assemblies (Boyce *et al.*, 2012). The slight differences in genome size between the two isolates (2.35 Mbp vs. 2.29 Mbp)

suggest potential genomic variations associated with adaptation to different geographical regions within Indonesia. Such variations could influence virulence, host specificity, and environmental persistence and warrant further investigation (Tarigan *et al.*, 2010).

A preliminary analysis of the annotated genomes revealed the presence of genes associated with virulence, including those involved in capsule biosynthesis, lipopolysaccharide production, and iron acquisition (Harper *et al.*, 2006; Hatfaludi *et al.*, 2010). These virulence factors are likely important for the pathogenesis of HS in Indonesian cattle (Mariana and Hirst, 2000). Capsule biosynthesis genes are of particular interest because capsule type correlates with host specificity and disease manifestation in *P. multocida* (Hatfaludi *et al.*, 2010; Harper *et al.*, 2012). The lipopolysaccharide structure, which varies among strains, also plays a crucial role in host-pathogen interactions and immune evasion (Harper and Boyce, 2017).

The high BUSCO scores (>99%) indicate that our assemblies capture nearly complete gene sets, providing a robust foundation for comparative genomics and functional studies (Levy and Boone, 2019). Future work will focus on an in-depth analysis of virulence factors, antimicrobial resistance determinants, and comparative genomics with isolates from other geographical regions (Zankari *et al.*, 2012; Boyce *et al.*, 2012). To date, >1,800 genomes of *Pasteurella* spp. have been deposited in public genomic repositories. *P. multocida* NTT isolate exhibits an ANI of 98.56% with *P. multocida* NCTC10322 (GCA\_900187275.1), as shown in Table 3. The *P. multocida* Lampung 2952 strain had an ANI of 98.76%, whereas the *P. multocida* subsp. *gallicida* strain NCTC10204 (GCA\_900636625.1) as presented in Table 4.

Indonesia's diverse geography and livestock management practices may influence the evolution and spread of *P. multocida* strains (Tarigan *et al.*, 2010; Peng *et al.*, 2018). The availability of complete genomes will facilitate molecular epidemiological studies to track transmission patterns and evolutionary relationships among Indonesian isolates (OIE Terrestrial Manual, 2021). This information is crucial for developing targeted control strategies specific to the Indonesian context (Benkirane and de Alwis, 2002), where HS remains a significant economic concern for the livestock industry (Kawasaki *et al.*, 2015).

### Conclusion

This study presents high-quality complete genome assemblies of *P. multocida* isolates from Lampung and Kupang, Indonesia. The assemblies, achieved through long-read Oxford Nanopore sequencing, provide valuable resources for studying the molecular epidemiology and pathogenesis of HS in Indonesian cattle. The single-contig assemblies with high completeness scores demonstrate the effectiveness of ONT sequencing for bacterial genome assembly.

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### Author's contributions

SSP, IWTW, SMN, and SS: Conceived, designed, and coordinated the study. SSP, SS, IWTW, and SW: Designed data collection tools, and supervised the field sample and data collection, laboratory work, and data entry. ARK, SS, and YA: Validation, supervision, and formal analysis. AA, SS, HN, TA, and SS: Contributed reagents, materials, and analysis tools. SSP, SS, HK, ARK, and ARRP: Statistical analysis and interpretation and preparation of the manuscript. All authors have read, reviewed, and approved the final manuscript.

### Conflict of interest

The authors declare no conflict of interest.

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### Data availability

The research is ongoing and several datasets from the WGS results are still in use; therefore, not all data are available in the manuscript. Once the study has been completed, we will promptly submit the data to GenBank.

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