

Short Research Paper

Draft Genome Sequence of a Virulent Strain of *Pasteurella Multocida* Isolated From Alpaca

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Received: 2017.01.22; Accepted: 2017.05.14; Published: 2017.06.28

Abstract

Pasteurella multocida is one of the most frequently isolated bacteria in acute pneumonia cases, being responsible for high mortality rates in Peruvian young alpacas, with consequent social and economic costs. Here we report the genome sequence of *P. multocida* strain UNMSM, isolated from the lung of an alpaca diagnosed with pneumonia, in Peru. The genome consists of 2,439,814 base pairs assembled into 82 contigs and 2,252 protein encoding genes, revealing the presence of known virulence-associated genes (*ompH*, *ompA*, *tonB*, *tbpA*, *nanA*, *nanB*, *nanH*, *sodA*, *sodC*, *plpB* and *toxA*). Further analysis could provide insights about bacterial pathogenesis and control strategies of this disease in Peruvian alpacas.

Key words: Alpaca, genome, pasteurellosis, pneumonia.

Introduction

Pasteurella multocida is a commensal bacteria from the upper respiratory tract [1], which affects a wide range of hosts [2, 3]. This bacteria is the primary agent of many infections such as; avian cholera, hemorrhagic septicemia in ungulates, atrophic rhinitis in pigs and snuffles in rabbits [3], and acts as a secondary agent in infectious pneumonia, including cases of acute or chronic pneumonia in different hosts such as swine, calves, sheep, bovine and alpaca [3-5].

In Peru, alpaca raising represents an important economic activity for the High Andean population. However, acute pneumonia causes high mortality rates in young alpacas, in which *P. multocida* has been principally isolated [6]. *P. multocida* has a large number of virulence factors that play a role in pathogenesis, including capsule, lipopolysaccharide, fimbriae, adhesins, toxins, outer membrane proteins, iron regulated and iron acquisition proteins, acquisition proteins, hyaluronidase and sialidase [6].

In this study, we announce the draft genome of *P. multocida* strain UNMSM isolated from an alpaca lung affected with pneumonia.

Pasteurella multocida strain UNMSM is a gram negative, short rod shaped bacteria, oxidase and catalase positive and nonhemolytic, with approximate measures of 0.3 – 0.6 µm in width and 0.8 – 2.0 µm in length (Figure 1). Genome sequencing was performed using Illumina HiSeq sequencing platform. The paired-end library contained inserts of an average size of 100 bp. *De novo* assembly was performed using Edena v3.131028 and SIMBA v1.4 software [7], which produced 82 contigs, with a N50 value of 70,838, 2.4 Mb of size and mean depth coverage ~400-fold. The genome was annotated using the Rapid Annotations using Subsystems Technology (RAST) [8], following by manual curation of the predicted CDSs (Coding Sequences). The genome presents GC content around 40.2%. A total of 2,434

genes were predicted, of which 2,252 were protein-coding genes, eight rRNA genes (four 16S rRNA, one 23S rRNA and three 5S rRNA genes) and 55 were tRNA genes. In table 1, a few summary statistics of the genome are presented.

Table 1. Genome statistics of *P. multocida* strain UNMSM.

Attribute	Value	% of Total*
Genome size (bp)	2,439,814	100.00%
DNA coding region(bp)	2,066,315	84.69%
DNA G+C content (bp)	979,694	40.15%
Total genes	2,434	100.00%
Pseudogenes	115	4.72%
Genes assigned to COGs	1,921	78.92%
Genes with Pfam domains	1,183	48.60%
Genes with signal peptides	173	7.10%
Genes with transmembrane helices	490	20.13%
CRISPR repeats	1	-

* The total is based on either the size of the genome in base pairs or the total number of genes in the annotated genome.

The Cluster of Orthologous Genes (COG) [8] assignments were done using CGView Comparison Tool (CCT), and the presence of protein domains was predicted by Pfam. The signal peptides were identified with the SignalP 4.0 software [9], transmembrane helices were classified by method of Krogh and collaborators method [10], and the CRISPR motif was detected with a web tool described by Grissa and collaborators [11] (Table 1). The functional analysis obtained from Rapid Annotation using Subsystem Technology (RAST) revealed 395 collections of functionally related protein families (Figure 2). The genome has 54 genes responsible for iron acquisition and metabolism; 42 genes involved in virulence, disease and defense; 74 genes involved in

membrane transport, and 75 genes related to phages, prophages and transposable elements. Analysis of genes showed that this strain belongs to capsular type A, and the presence virulence associated genes, including outer membrane proteins as *ompH* (UR07_03150), and *ompA* (UR07_05380); hyaluronidases as *nanA* (UR07_06210), *nanB* (UR07_07035) and *nanH* (UR07_07095); iron acquisition related genes, *tonB* (UR07_07610) and *tbpA* (UR07_03090); genes involved in sialic acid metabolism as *sodA* (UR07_00380) and *sodC* (UR07_00680); a lipoprotein, *plpB* (UR07_09985) and *toxA* (UR07_03905), a dermonecrotic toxin [5–12].

This work provides a better understanding of *P. multocida* UNMSM, the first genome isolated from alpaca. Future studies *in vitro* and *in vivo* associated with virulence genes predicted in *P. multocida*, will improve understanding of their pathogenicity and provide an assessment of new targets for the use of vaccines and drugs, which may be important for the control of pneumonia case numbers in alpacas. The genome projects of *P. multocida* strain UNMSM have been deposited in GenBank under the following accession numbers, LGRE00000000.

Acknowledgements

This work was conducted by Laboratory of Cellular and Molecular Genetics in Federal University of Minas Gerais and Veterinary Medicine Faculty in National University of San Marcos and present work was financially supported by the Programa Nacional de Innovación para la Competitividad y Productividad (Innovate Perú), under the contract 133-FINCYT-IB-2013, as part of the project “Reverse Vaccinology: Developing a new generation vaccine for the control and/or prevention pasteurellosis pneumonia in alpacas”.

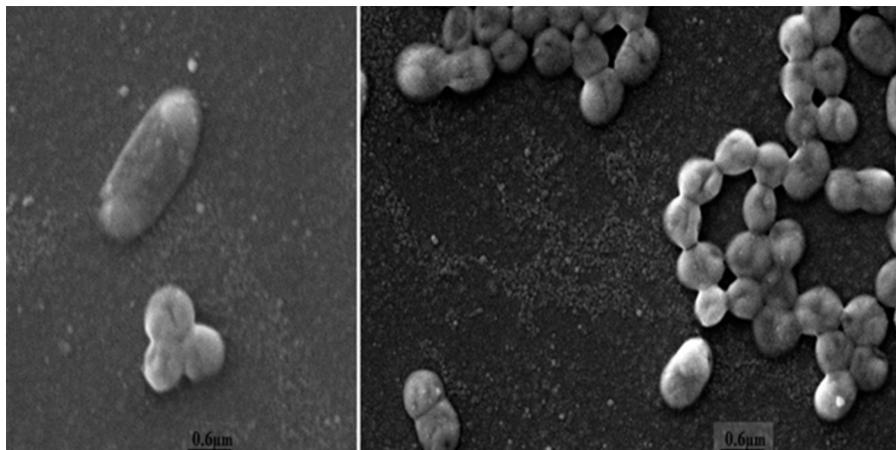


Figure 1. Scanning electron micrograph of cells of *P. multocida* strain UNMSM. The scale bar represents 0.6 μ m.

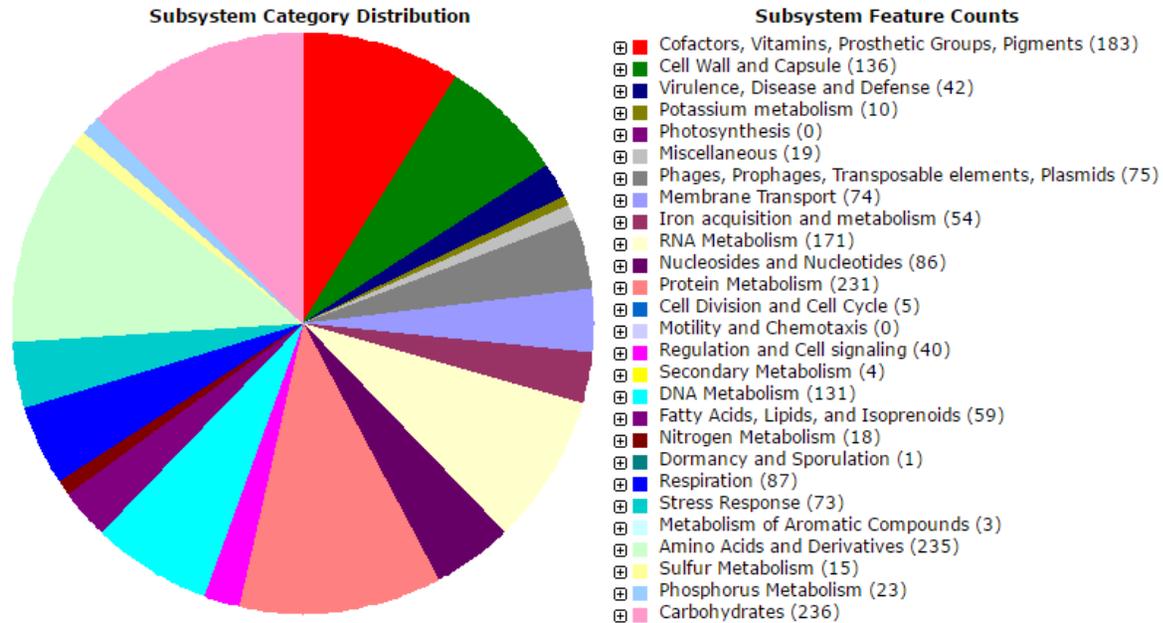


Figure 2. The overview of subsystem category coverage *P. multocida* UNMSM genome based on RAST server.

Competing Interests

The authors have declared that no competing interest exists.

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