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Whole Genome Shotgun Sequencing Of *Pasteurella Multocida* Strain Skn1 Isolated From Sheep.

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Abstract:

Pasteurella multocida represents a highly diverse group of bacteria infecting wide range of animals and birds like the cattle, buffalo, sheep, goat and fowl leading to huge financial loss to both the cattle and poultry industry. Genome sequences of Pasteurella multocida, isolated from other animals, are available and accessible in public databases. We report one additional whole genome sequences of the Pasteurella multocida (accession number NIOC00000000) isolated from sheep lung to further facilitate pathology, pathogenicity and vaccinology for prevention and control of disease as well as evolution-related genomic and genetic studies of Pasteurella multocida.

Keywords: *Pasteurella multocida*, Sequencing, Sheep

Introduction:

Pasteurella multocida is a gram- negative, coccobacillus and an important pathogenic bacterium that causes a wide range of disease, such as hemorrhagic septicemia in bovine and buffaloes, enzootic bronchopneumonia in bovine, sheep and goat, fowl cholera in poultry, atrophic rhinitis in swine and snuffles in rabbits (El-Jakee *et al.* 2016; Garcia-Alvarez *et al.* 2017; Ewers *et al.* 2006, Patel *et al.* 2016). In temperate climates, *P. multocida* rarely causes pneumonia and little is known of the epidemiology of the infection in sheep. Also, it has been mentioned that, whereas much is known regarding the prevalence and pathogenesis of *Manhemia hemolytica* infection, *P. multocida* has been infrequently cited as the causal agent of researchers reporting ovine pneumonia outbreak (Odugbo et al., 2005).

In the present study, *P. multocida* was isolated from sheep lung and was investigated by whole genome sequence analysis. Genomic data is submitted at National Center for Biotechnology Information (NCBI) for further analysis.

Materials and methods:

Sample collection and sequencing:

Sheep was brought from village situated near to Palanpur, District-Banasthantha to the department of Veterinary Pathology, SDAU, Sardarkrushinagar, Gujarat. Samples were collected from sheep at the time of necropsy examination and isolated *Pasteurella multocida* on blood agar from lung. Colonies of *Pasteurella multocida* was used for Genomic DNA

(gDNA) extraction using a commercially available DNA isolation kit (DNeasy blood and tissue kit, Qiagen) according to the manufacturer's instructions. All procedures were carried out inside a sterile cabinet.

Briefly, 50-100 ng gDNA were fragmented into blunt-ended fragments by Ion shear plus reagents method. Adapter-ligated and nick-translated DNA was purified by AMPure bead (Beckman Coulter). The amplified libraries were purified using AMPure beads (Beckman Coulter) and the concentration of the library determined using the Qubit flourometer (Invitrogen, USA) instruments. Sequencing was undertaken using 318 chips on the Ion Torrent PGM and barcoding was used for this sample. The Ion PGM™ Hi-Q™ Sequencing Kit was used for sequencing reactions, following the recommended protocol. All reads were assembled by SPAdes 5.0.0.0 to the total sequence length of 70,749,798 base pairs.

Data accessibility:

The whole genome shotgun sequence of *Pasteurella multocida* strain SKN1 have been deposited in the National Center for Biotechnology Information (NCBI) under the accession number NIOC00000000 (https://www.ncbi.nlm.nih.gov/nuccore/NIOC00000000). Study ID in the MG-RAST metagenomic analysis server is 7a4a70fe4d6d676d343734373634382e33 (http://metagenomics.anl.gov/mgmain.html?mgpage=project&project=9480622bae6d67703830363130).

Results and Discussion:

The genome sequence was then annotated with RAST and the NCBI Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). A total of 2472 genes were predicted, including 2010 protein-coding genes, 44 tRNAs, 62 rRNAs, five copies of 5SRNA, four copies of 16SRNA, and five copies of 23SRNA (Lainson *et al.*, 2013). The whole genome sequences were compared with some closely related sequences data predicted by RAST showing slight variations. These minute difference may result from adaptation, and/or immune stress and status of the hosts.

This whole genome shotgun sequencing revealed taxonomic as well as functional analysis of the *Pasteurella multocida*. This data describes various pathways and gene signalling responsible for causing pathology and pathogenesis of hemorrhagic septicemia and

enzootic bronchopneumonia. Meta Genome Rapid Annotation using Subsystem Technology (MG-RAST) was used for functional analysis of sequence as shown in Fig. 1. These diseases cause severe economical losses in livestock industry, especially in tropical countries. Since several decades, conventional vaccines have been used as a control strategy, but major limitation of these vaccines is their ineffectiveness in inducing long acting cross protective immunity (Tang, *et al.* 2009). Therefore, subunit vaccine has been prepared from several outer membrane proteins (OMPs) as candidate antigen (Shivachandra *et al.* 2011; Hatfaludi *et al.* 2010).

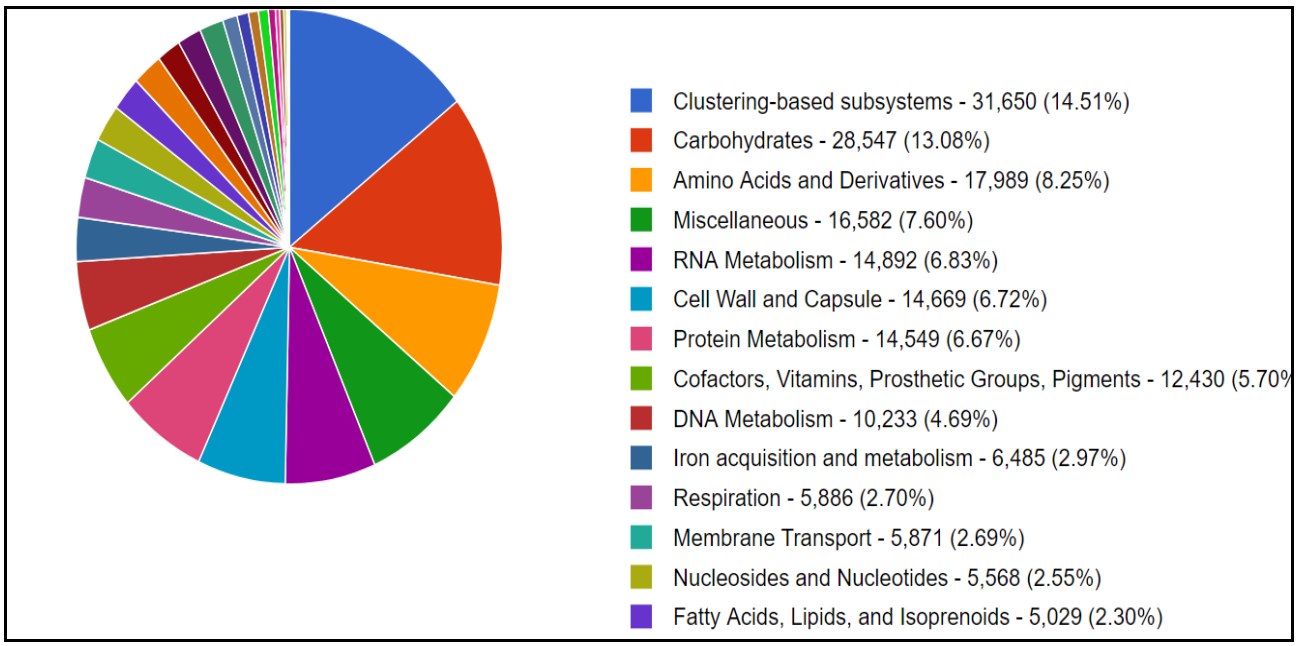

Figure 1: Meta Genome Rapid Annotation using Subsystem Technology (MG-RAST) based functional analysis of *Pasteurella multocida* genome sequences.

Value of the data:

- Previously, only few genome sequence data of *Pasteurella multocida* isolated from sheep is available in the public databases.
- The whole genome shotgun sequences of *Pasteurella multocida* isolated from sheep are now made available.
- The additional genome data will facilitate the pathology, pathogenicity and vaccinology for prevention and control of the disease.

- This data allows other researchers for evolution-related studies of *Pasteurella multocida*, through comparative genomic studies of *Pasteurella* species and related species.

Conclusions and Recommendations:

Pasteurella multocida is isolated from sheep lung and genome sequence of *Pasteurella multocida* is deposited at National Center for Biotechnology Information (NCBI) with accession number NIOC00000000 which will facilitate the pathology, pathogenicity and vaccinology for prevention and control of the disease.

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Conflicts of interest:

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

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