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Original Research Paper

Assessment of the Abundance of Rumen Microflora in Black Bengal Goat by Metagenomic Analysis

Engineering

KEYWORDS : Abundance, Black Bengal goat, metagenomics, microflora

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ABSTRACT

A crucial area of scientific research is the genetic as well as biological diversity of microorganisms. Efforts are needed to improve the ability of small ruminant like goat to convert feed stuffs available locally to animal products taking into consideration the importance of Black Bengal goat in livestock strategy. Due to the recent advancement in the field of molecular biology and genomics there has been development of metagenomics. This in turn can enable researchers; scientists; as well as post graduate students to conduct a more holistic examination of the structure as well as function of rumen microflora of goat. In the present metagenomic profiling has revealed the abundance of Prevotella sp. & Bacteroides sp. (pectin digesting bacteria) compared to other microbes. Based on such kind of studies future work on genogrouping of the rumen microflora of Black Bengal goat can also be undertaken.

INTRODUCTION

The first chamber of the stomach of goat like other ruminants is the rumen. There are presence of microorganisms in the rumen that remain in symbiotic relationship and break down food that is ingested. Nutrients like volatile fatty acids as well as bacterial proteins are provided by these microorganisms to the host animal and such microflora include representatives from Eukarya; Bacteria; and Archea (Deng et al., 2008). By means of various studies investigations have been conducted to identify the rumen microbiota of small ruminants due to their link to traits that are economically as well as environmentally important viz., feed conversion efficiency; production of methane; and discovery of microbes and enzymes (more recently) enabling biomass fermentation for production of biofuel (Guan et al., 2008; Hegarty, 1999; Zhou et al., 2009 & 2010; Hess et al., 2011).

Handelsman et al. (1998) first coined the term 'metagenomics' for studying all microbial genomes in a specific environment. An exercise in metagenomic analysis is any kind of study that includes all the individuals in a community of microbes as a single genomic pool (Kowalchuk et al., 2007). The aim of the present study is to assess the abundance of rumen microflora in Black Bengal goat in the north-eastern hilly state of Tripura by metagenomic analysis as little is known about the microbial ecology in the rumen of this particular variety of goat.

MATERIALS AND METHODS

Collection of samples:- The size of the sample was 15 ranging in age group between 3 month to 48 months (Mean=15.73 months), collected from the rumen of Black Bengal goats population from West Tripura district during the year 2015. The age range of 15 rumen samples is described in (Figure-1). The rumen samples were collected in the sterile 50 ml container.

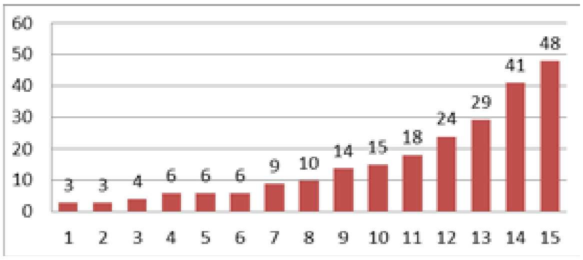

Figure 1. Age (in Month) & total number of rumen samples collected from the Black Bengal

Software used for the analysis:
FastQC tool, Rap-Search 2.0, Megan 5.

FastQC
A series of tests is run on FastQ file by an application called FastQC for generating a QC report comprehensively. Any unusual kind of sequence is detected by it. The test result is flagged as a pass; warning or fail that depends on how far it departs from what is expected from a large dataset normally with no significant biases.

Rap-Search 2.0
RAPSearch stands for Reduced Alphabet based Protein similarity Search. It is a tool for fast protein similarity search and is 100 times faster than BLASTX. Multi-threading is supported by it enabling faster protein similarity search. In comparison to Blastx RAPSearch is little less sensitive. But for discovering other homologous proteins that BLASTX misses it also helps us.

MEGAN (v-5.1.4):
MEGAN stands for MEtaGenome Analyzer. Taxonomic classification; functional classification using SEED; KEGG; COG/EGGNOG classification for the meta-genomic data are calculated by this programme. An interactive view based on a tree (in which each node shows the number of reads assigned to it for each of the datasets) is provided by it. Pie

chart, heatmap; histograms can be generated for the classification. In the present study we have generated a pie chart by using MEGAN.

Workflow

Metagenomic reads are usually assembled into contigs and subjected to BLASTX analysis. The accuracy of the results are directly affected by it since there is always a possibility of generating chimeric contigs. Hence for identification of the taxonomic diversity of the bacteria in the samples reads are directly subjected Rap2Search alignment. For the better resolution of the microbial community the non-redundant database and other 16s related databases reads are exhaustively aligned. The same approach has been adopted in the analysis of the present data.

RESULTS AND DISCUSSION

In the present study different types of microorganisms are isolated and characterized from the 15 Rumen samples collected from the Black Bengal goats from the North Eastern state of Tripura. The distribution of rumen microorganisms (percentage wise) is shown in figure. 2. Different types of phylum were identified within the 15 rumen samples, among them 4 Phylum were most abundant -Bacteroidetes, actinobacteria,Firmicutes, Spirochaetes.

- Bacteroidetes Phylum- Prevotella and Bacteroides genus are most abundant.
- Actinobacteria Phylum- Actinobacteridae & Mesangiospermae genus.
- Firmicutes phylum- Bacillaceae, Lactobacillales genus.
- Spirochaetes Phylum- Treponema genus.

In this study, most abundant microorganism are Prevotella sp. & Bacteroides sp. (pectin degrading bacteria) as is shown by graphical representation (figure.3). They are important because pectin represents 10-20% of total carbohydrates in rumen nutrition (Kamra, 2005).

The correlation between Prevotella sp. & Bacteroides sp. are (r = 0.64). The age is also correlated with this study. The Prevotella sp. and the Bacteroides sp. increase with the increase of the age of the specimen. Here the correlation between the age of the specimens and increase of the Prevotella sp. (r= 0.75) has been described. Both the correlation shows positive relation.

It is interesting to note that the present metagenomic study based on the determination quantitatively of the total microflora of the rumen has got implication in nutrition of Black Bengal goat (Firkins et al., 2007).

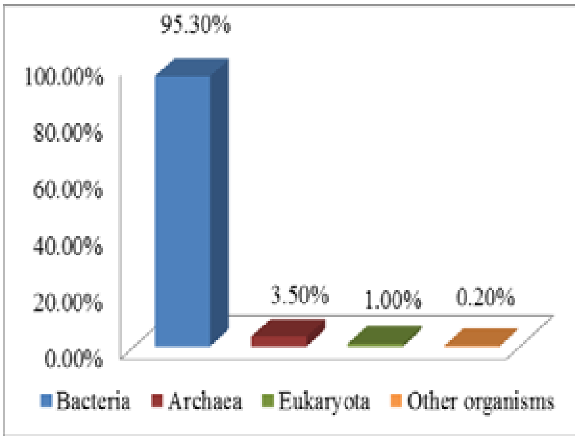

Figure 2. Percentage wise distribution of rumen microorganisms

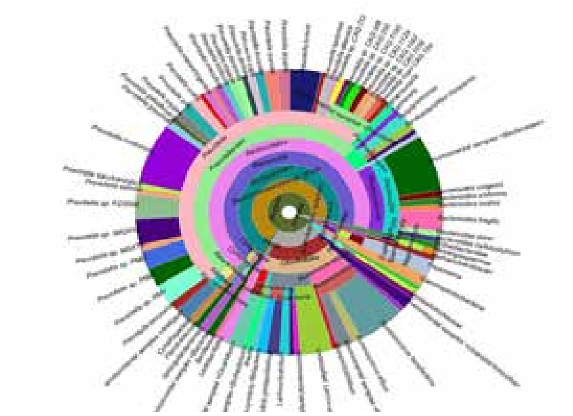

Figure 3. Pie chart representation of abundance of rumen microflora

CONCLUSION

It can be concluded from the present study that there is more versatility in the metagenome profiles of rumen microflora of Black Bengal goat. In this study, pronounced differences in the microbiota composition among different age groups are evident. In the 48 month-old group Prevotella sp. and Bacteroides sp. are significantly higher than in the 3-month-old group specimen. This fact can form the basis of the phylogenetic analysis (genogrouping) of the plethora of rumen microbes of this particular variety of goat in near future.

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REFERENCES

1. Deng, W., Xi, D., Mao, H., Wanapat, M. (2008): "The use of molecular techniques based on ribosomal RNA and DNA for rumen microbial ecosystem studies: a review". Mol. Biol. Rep.; 35:265-74.
2. Hegarty, R. (1999): "Reducing rumen methane emissions through elimination of rumen protozoa". Aust. J. Agric. Res.; 50:1321-28.
3. Guan, L.L., Nkrumah, J.D., Basarab, J.A., Moore, S.S. (2008): "Linkage of microbial ecology to phenotype: correlation of rumen microbial ecology to cattle's feed efficiency". FEMS Microbiol. Lett.; 288:85-91.
4. Zhou, M., Hernandez-Sanabria, E., Guan, L. (2009): "Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies". Appl. Environ. Microbiol.; 75:6524-10.
5. Zhou, M., Hernandez-Sanabria, E., Guan, L.L. (2010): "Characterization of variation in rumen methanogenic communities under different dietary and host feed efficiency conditions, as determined by PCR-denaturing gradient gel electrophoresis analysis". Appl. Environ. Microbiol.; 76:3776-86.
6. Hess, M., Sczyrba, A., Egan, R., Kim, T-W., Chokhawala, H., Schroth, G., Luo, S., Clark, D.S., Chen, F., Zhang, T. (2011): "Metagenomic discovery of biomass-degrading genes and genomes from cow rumen". Sci.; 331:463-67.
7. Handelsman, J., Rondon, M.R., Brady, S.F., Clardy, J., Goodman, R.M. (1998): "Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products". Chem. Biol.; 5:245-49.
8. Kowalchuk, G.A., Specksnijder, A.G., Zhang, K., Goodman, R.M., Van Veen, J.A. (2007): "Finding the needles in the metagenome haystack". Microbial Ecol.; 53:475-85.
9. Kamra, D.N. (2005): "Rumen microbial ecosystem". Curr. Sci.; 89(1):124-35.
10. Firkins, J.L., Yu, Z., Morrison, M. (2007): "Ruminal nitrogen metabolism: perspectives for integration of microbiology and nutrition for dairy". J. Dairy. Sci.; 90:1-16.