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METAGENOMIC ANALYSIS OF GUT MICROBIAL DIVERSITY OF INDIAN NATIVE CHICKEN (CARI NIRBHEEK) UNDER BACKYARD SYSTEM USING NGS

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ABSTRACT

Metagenomics is the culture independent analysis of microbial diversity in an ecosystem and it can be used to find out the gut microbial diversity of chicken. Native chicken is characterised by good genetic resistance and they are adapted to extensive system of management. NGS (Next generation sequencing) analysis of intestinal micro biome using primers targeting V_3, V_4 and V_4-V_6 region of 16S rRNA helped in revealing the microbial diversity of native chicken CARI Nirbheek (a native chicken breed having the blood of indigenous breed Aseel). Firmicutes was the dominant bacterial phylum and bacilli were the dominant class. Lactobacillus was the dominant genus followed by Bifidobacterium. *Lactobacillus helveticus* and *Lactobacillus delbrueckii* were the dominant bacterial strains having probiotic properties which may help in increasing the genetic resistance and growth in extensive management. Metagenomics studies using NGS helps in exploring the intestinal microbial diversity and identifying the bacterial strains which helps in increasing the genetic resistance and growth of native chicken under backyard rearing.

Keywords: Metagenomics, Illumina, MG-RAST, Firmicutes and CARI Nirbheek

INTRODUCTION

The gastrointestinal micro biota has one of the highest cell densities for any ecosystem and in poultry ranges from 10^7 to 10^{11} bacteria per gram of gut content (Apajalahti *et al*., 2004). The majority of these microbes are uncharacterized and represent an enormous unexplored reservoir of genetic and metabolic diversity. The gut micro-biota has an important role in poultry health and production, which generally affects the health of the host by influencing digestion and nutrient absorption, intestinal morphology, and defence of the host against infection Mead (2000).Metagenomics has been defined as function-based or sequence-

based cultivation-independent analysis of the collective microbial genomes present in a given habitat (Riesenfeld *et al*., 2004). Metagenomics can be used to address the challenge of studying prokaryotes in the environment that are, as yet, unculturable and which represent more than 99% of the organisms in some environments (Amann *et al*., 1995). Recent, advances in high throughput sequencing technologies have increased the number and size of metagenomic sequencing projects (Carola and Rolf, 2009). Bioinformatics tool like Meta Genomic Rapid Annotation using Subsystem Technology (MG-RAST) analysis provides a taxonomic classification and a new pipeline which computes results against many reference databases (GenBank, SEED, IMG, UniProt, KEGG and eggNOGs) (Meyer *et al*., 2008).

Gut micro-biota is highly variable from individual to individual and also affected by several factors *viz*. environment, feed, genetic makeup of host etc. Native chicken are crosses of indigenous chicken developed for backyard system of rearing. They are adapted to extensive management system. There is no report on the whole gut microbial study of native chicken using culture independent methods. Metagenomic analysis of the gut micro-biome of native chicken will help in finding out the beneficial bacterial strains which enhance the growth and immunity of native chicken. Keeping this in view the present investigation was designed to find out the effect of rearing system on the gut microbial regime of CARI Nirbheek (cross of Aseel and Dahlem red) which have been developed and maintained at Desi unit of the institute.

MATERIALS AND METHODS

All the experiments were conducted strictly in accordance with the guidelines of "Institutional Animal Ethics Committee" (IAEC). CARI Nirbheek is a native chicken developed at the Desi unit of the institute, CARI, Izatnagar. The birds are developed for backyard rearing system and are characterised by higher genetic resistance against diseases. Under extensive system day-old chicks (10 chicks) were maintained under rural conditions at farmer's door about 15 km away from institute. The chicks were housed in kaccha houses made of locally available materials like asbestos sheet, card-board, mud etc. and fed on kitchen waste supplemented with broken grains and scavenging. The experiment was conducted during the month of December and February when ambient temperature ranged from 50.6 to 66.2°F and relative humidity 71-98 %. Birds showed a mortality percentage of 20.

Five chicks were humanely slaughtered at 8 weeks age and whole intestine contents were collected and

pooled aseptically. The gut contents were outsourced to M/s Genotypic Pvt Ltd., Bangalore India for Next Generation Sequencing. V_3, V_4, and V_4-V_6 hyper variable regions of 16srRNA were amplified using region specific primers (Table-1) and NGS was done using Illumina 300bp paired end platform. The data generated were analysed using bio-informatics software, MG-RAST, a fully automated service for annotation of metagenomic data.

RESULTS

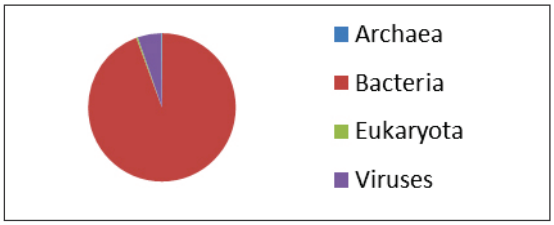
Total number of reads were 256597 and quality check by MG-RAST filtered 1% of total sequences and the remaining 254058 sequences represented the gut micro-flora using the V3,V4,and V4-V6 region of the bacterial 16S rRNA. Out of this 81.8% predicted to be protein coding. Sequence similarity searches were computed against a protein database derived from M5NR database. Remaining 18.2of sequences hit against ribosomal RNA. Source hit distribution of 46238 sequences against Green genes analyzed 60.82 % of sequences. SILVA LSU analyzed 0.02% of sequences and RDP could analyze 72.9% of sequences. 78.6% of sequences were analyzed using SILVA SSU database. Taxonomic analysis was done using an E-value cut off of 1×10^{-5}, minimum identity cut off of 60% and minimum alignment length cut off of 15 amino acid.

Diversity of intestinal micro-biome at the level of various taxa.

Domain

Bacteria were the dominant domain accounted for 92.33% of micro-biome followed by Viruses, Eukaryote, others and Archaea.

Figure-1

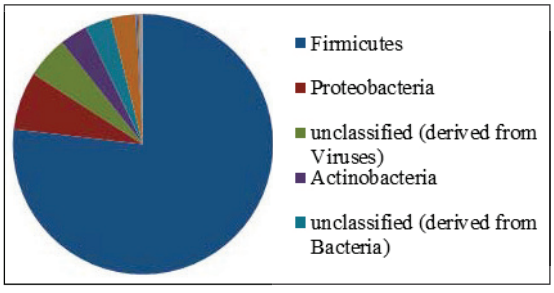


Phylum

Dominant phylum was Firmicutes (82.54%) followed by Actinobacteria (6.74%) and Proteobacteria (1.61%). Among minor phyla which were <1% of best hit annotated reads, Bacteroidetes, Spirochaetes, Tenericutes, Cyanobacteria and Fusobacteria were dominant.

Dominant Archaeal phylum was Euryarchaeota whereas Streptophyta and Arthropoda were the dominant eukaryotic phyla.

Figure 2

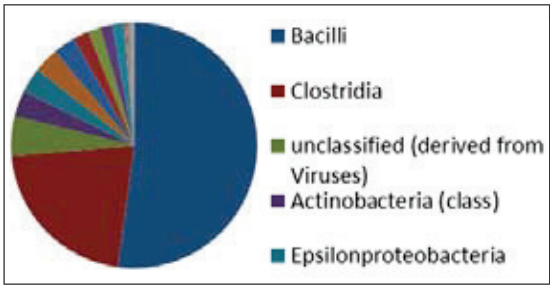


Class

Bacilli (76.96%), Actinobacteria (6.74%), Clostridia (4.39%), Gamma-proteobacteria (1.05%), and Negativicutes (1.01%) were the major classes. Among the minor classes, which were <1% of the best hit annotated reads, unclassified class derived from Bacteria, Bacteroidia, Epsilonproteobacteria, Erysipelotrichi, Deltaproteobacteria and Liliopsida were dominant.

Methanobacteria and Methanomicrobia were the dominant archaeal classes whereas Liliopsida and Insecta were the dominant eukaryotic classes.

Figure 3

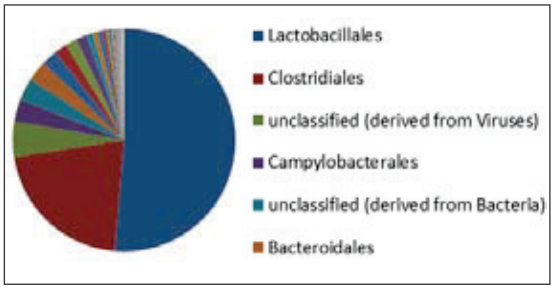


Order

Lactobacillales (76.14%) was the dominant order followed by Clostridiales (4.37%), Bifidobacteriales (4.11%), Coriobacteriales (1.76%) and Selenomonadales (1.01%). Among the minor orders which are less than one percentage of best hit annotated reads, Actinomycetales, Bacillales, Pasteurellales, unclassified order derived from Bacteria, Bacteroidales, Campylobacterales, Enterobacteriales and Erysipelotrichales were dominant.

Thermococcales and Sulfolobales were the dominant archaeal orders whereas Galliformes and Ixodida were the dominant under domain eukaryote.

Figure 4

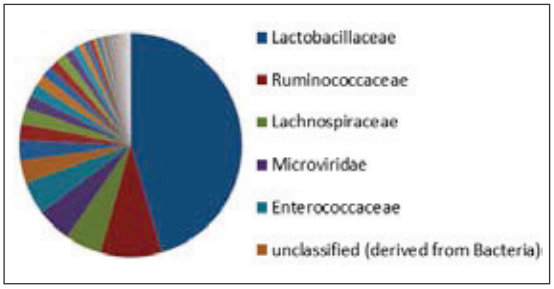


Family

Lactobacillaceae (73.54%) was the dominant family followed by Bifidobacteriaceae (4.11%), Enterococcaceae (1.82%), Coriobacteriaceae (1.76%), Ruminococcaceae (1.4%), and Peptostreptococcaceae (1.05%). Among minor families which were less than one percentage of best hit annotated reads, Veillonellaceae, Pasteurellaceae, Streptococcaceae, unclassified family derived from Bacteria, Lachnospiraceae, Clostridiaceae, Staphylococcaceae, unclassified family derived from Clostridiales, Kineosporiaceae, Enterobacteriaceae, Brevibacteriaceae, Campylobacteraceae and Bacteroidaceae were abundant.

Thermococcaceae and Thermoplasmataceae were the dominant Archaeal families and families Poaceae and Phasianidae were dominant under domain eukaryote.

Figure 5



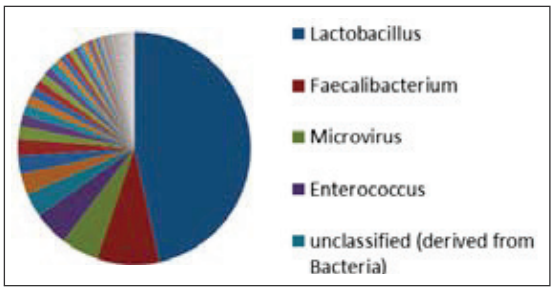
Genus

Lactobacillus (73.44%) was the dominant genus followed by Bifidobacterium (3.9%), Enterococcus (1.68%), Faecalibacterium (1.05%) and unclassified genus derived from Peptostreptococcaceae (1.04%). Among minor genera which were less than one percent of best hit annotated reads, Collinsella, Veillonella, Gallibacterium, unclassified genus derived from Bacteria, Atopobium, Clostridium, Streptococcus, Staphylococcus, Blautia, unclassified genus derived from unclassified sequences, Kineococcus, Brevibacterium, Subdoligranulum Bacteroides, Campylobacter and Heliobacterium were accounted for major proportions.

Genera Sulfolobus and Thermococcus were dominant under domain archaea and Coptotermes and Oryza were the dominant eukaryotic genera.

Dominant bacterial species in the gut microbiome of CARI Nirbheek were *Lactobacillus helveticus, Lactobacillus delbrueckii, Lactobacillus reuteri, Lactobacillus mucosae, Enterobacteria* phage phiX174 sensulato, *Lactobacillus pontis, Lactobacillus acidophilus, Lactobacillus johnsonii, Lactobacillus vaginalis* and *Lactobacillus frumenti.*

Figure 6



DISCUSSION

The initial micro-biota to which chicks are exposed as well as the nutrient composition of diet affect their commensal gut micro biota, host gene expression, and immune system development (Yin *et al*., 2010). Under extensive system, birds were fed on kitchen wastes supplemented with broken grains and reared under backyard condition with Kaccha house/night shelter. CARI Nirbheek is a cross of Aseel and Dahlem red developed for extensive management. Birds are active, large in built, pugnacious in nature with high stamina and majestic gait. They are able to save themselves from their predators due to their fighting characters and activeness

and are adapted to all climatic zones of the country.

For broiler the taxonomic analysis at phylum level showed the dominance of *Firmicutes* followed by *Actinobacteria, Proteobacteria* and *Bacteroidetes* according to Salanitro *et al.* (1974) and Mead (1989). In the case of native chicken also Firmicutes was dominant followed by phyla such as *Actinobacteria* and *Proteobacteria.* Among dominant bacterial strains *Lactobacillus helveticus* is a potential probiotic which modulate host immune response (Borchers et al., 2009; Lebeer et al., 2010) and *L. helveticus* LAT 179 to broiler chickens caused an increase in body weight (Capcarova et al., 2011). *Lactobacillus delbrueckii, Lactobacillus reuteri, Lactobacillus pontis, Lactobacillus acidophilus and Lactobacillus johnsonii* were also characterised by probiotic properties.

The presence of these potential probiotic strains may help the host to perform well under backyard systems of rearing by enhancing growth and immunity.

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