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METAGENOMIC STUDY OF THE GUT MICROBIAL DIVERSITY OF CARI NIRBHEEK UNDER INTENSIVE SYSTEM OF REARING

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ABSTRACT

The gastrointestinal tract of poultry is densely populated with microorganisms, which are presumed to interact with the host and ingested feed. 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 characterized by higher genetic resistance against diseases. In this experiment, these birds are reared under intensive system, which is entirely different from the backyard system of rearing. Metagenomic analysis of the gut microbiome of CARI Nirbheek revealed that Firmicute was the dominant phylum followed by Proteobacteria. Bacilli was the dominant class followed by Clostridia. Lactobacillus, Faecalibacterium and Enterococcus were the dominant genera. Modern techniques like NGS 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 . The beneficial bacterial strains can be isolated and used as a potential probiotic for broiler.

Keywords : CARI Nirbheek, Metagenomics, Probiotics, Firmicutes, Lactobacillus

INTRODUCTION

In chicken, colonisation of the gastrointestinal tract is thought to start immediately after hatching, and therefore, the hatching environment has a major influence on a chicken's microbial profile. Previous culture-based studies have established that, as in other vertebrates, the phyla Bacteroidetes and Firmicutes predominated in the chicken cecal microbiota (Salanitro *et al*.,1974; Mead, 1989). In poultry, the metagenomics approaches has been applied for exploring the diversity of GI tract bacterial microflora using 16s

rRNA gene (Zhu *et al*., 2002). These investigations have in general analyzed the diverse bacterial populations in different parts of poultry intestine at different time points during brooding and also under different feed regimes (Mirhosseini *et al*., 2010). 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. Cari Nirbheek, the Desi chicken developed for extensive system of rearing is used for this study and analysis of the whole gut micro-biome of CARI NIRBHEEK reared under intensive system will help in finding out the essential probiotic bacterial strains and its comparison with the whole gut microbiome of broiler will help in understanding the microbiome which modify these birds' response to the rearing system.

MATERIALS AND METHODS

All the experiments were conducted strictly in accordance with the guidelines of "Institutional Animal Ethics Committee" (IAEC). 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 were reared under intensive system for 8 weeks The floor is covered with litter material (Rice Husk). Chicks were reared under standard management and feeding. Birds were fed ad-libitum. Feed formulation for birds reared under this system is given in Table 1

Table -1. Feed formulation for birds reared under intensive system

Feed ingredients	Starter	Finisher
Maize	55.5	62.425
DORB	2.14	2.52
Soya bean	30.6	23.5
Guar korma	4	4
RSM	4	4
Marble chips	0	0.6
Limestone	0.8	0.5
DCP	2	1.6
Salt	0.3	0.3
DL-Meth	0.1	0.07
Lysine	0.135	0.07
TM Premix[1]	0.11	0.1
Vit premix[2]	0.15	0.15
B comp[3]	0.015	0.015
Ch.chloride	.05	.05
Toxin binder	0.05	0.05
Coccidiostats	0.05	0.05
Total	100	100
Crude protein	23.3826	20.65105
M Energy	2816.66	2878.785
Calcium	1.06984	1.058708
Available P	0.49059	0.413803
Lysine	1.24084	1.003825
Methionine	0.490712	0.429441

1. TM Premix supplied mg/kg diet: Mg 300 ; Mn 55;I 0.4; Fe,56; Zn,30; Cu,4
2. Vitamin premix supplied per kg diet : Vit -A,8250 IU; Vit- D3,1200 ICU; Vit- K, 1mg
3. B complex supplied per kg diet: Vit-B1,.2mg; Vit-B2 4mg;Vit-B12,10 mcg;niacin,60mg; pantothenic acid,10mg; choline,500 mg, Vit -E,40IU.

Juvenile body weights of birds were recorded weekly up to 8 weeks. On each week days, the body weight was recorded

using a digital pan balance in morning hours prior to feeding. 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. V3, V4, and V4-V6 hyper variable regions of 16srRNA were amplified using region specific primers and NGS was done using Illumina 300bp paired end platform. The data generated were analyzed using bio-informatics software, MG-RAST, a fully automated service for annotation of metagenomic data.

RESULTS

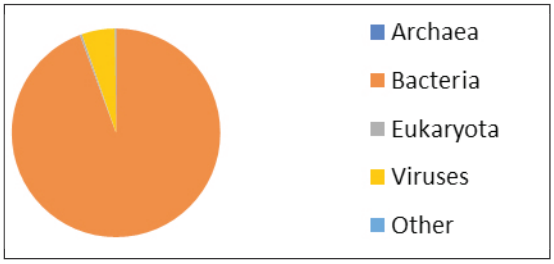
Quality check by MG-RAST filtered 90.2% of sequences and the remaining 34061 sequences represented the gut micro-flora using the V3,V4,and V4-V6 region of the bacterial 16S rRNA. Out of this 87.5% predicted to be protein coding. Sequence similarity searches are computed against a protein database derived from M5NR database.

Diversity of intestinal microbiome at the level of various taxa

Domain

Bacteria were the dominant domain accounting for (94.3%) of total micro-biome followed by Viruses (5%), Eukaryote (0.33%), Others (0.13%) and Archaea (0.006%).

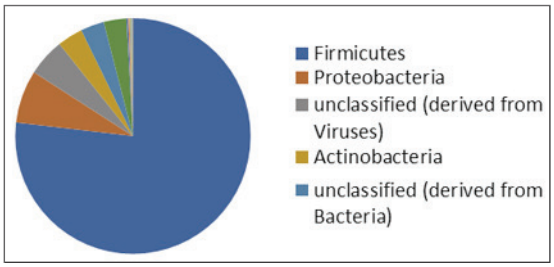
Fig-1. Pie chart- Domain level



Phylum

Dominant phylum was firmicutes (76.8%) followed by Proteobacteria (7.2%), Actinobacteria (3.47%), Unclassified phyla derived from bacteria (3.2%) and Bacteroidetes (3.1%). Firmicutes/ Bacteroidetes ratio= 24.7.

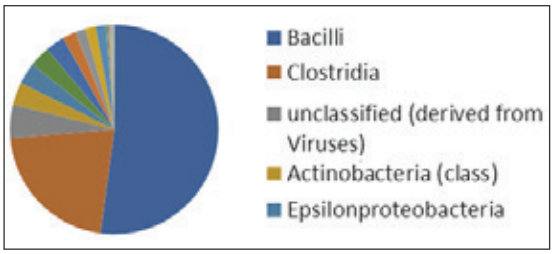
Fig-2. Pie chart- Phylum level



Class

Bacilli (52.1%) formed dominant class followed by Clostridia (21.5%), Actinobacteria (3.4%), Epsilonproteobacteria (3.3%), Unclassified Class derived from Bacteria (3.2%), Bacteroidia (2.9%), Gamaproteobacteria (2%), Erysipelotrichi (1.6%), Deltaproteobacteria (1.6%), and Negativicutes (1.5%).

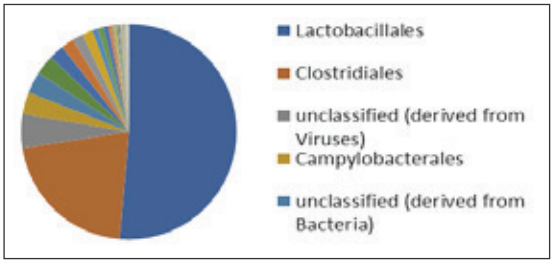
Fig-3. Pie chart – Class level



Order

Lactobacillales (51.3%) and Clostridiales (21.1%) were dominant followed by Campylobacterales (3.2%), unclassified orders derived from Bacteria (3.2%), Bacteroidales (2.9%), Coriobacteriales (2.2%), Enterobacteriales (1.9%), Erysipelotrichales (1.6%), and Selenomonadales (1.5%).

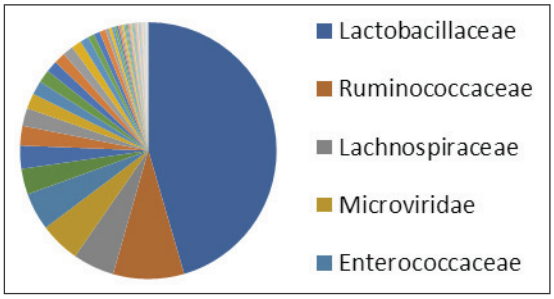
Fig-4 .Pie chart-Order level



Family

Lactobacillaceae (45.1%) was dominant followed by Ruminococcaceae (8.8%), Lachnospiraceae (5.1%), , Enterococcaceae (4.7%), Unclassified family derived from Bacteria (3.2%), Clostridiaceae (2.9%), Campylobacteraceae (2.4%), Coriobacteriaceae (2.2%), Enterobacteriaceae (1.89%), Eubacteriaceae (1.7%), Erysipelotrichaceae (1.62%), Prevotellaceae (1.44%) Streptococcaceae (1.42%), Veillonellaceae (1.34%), Peptostreptococcaceae (1.23%) and Bacteroidaceae (1.08%).

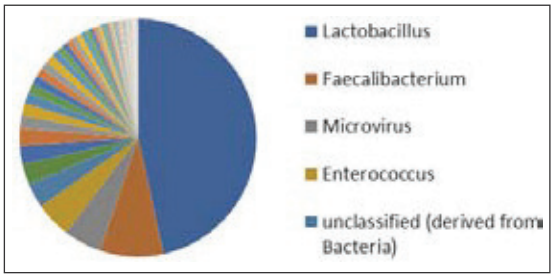
Fig-5. Pie chart Family level



Genus

Dominant genera were Lactobacillus (45%), Faecalibacterium (8.4%), Enterococcus (4.6%), unclassified genus derived from Bacteria (3.2%), Blautia (2.76%), Campylobacter (2.44%), Clostridium (2.26%), Eubacterium (1.72%), unclassified genus derived from Erysipelotrichaceae (1.59%), Prevotella (1.44%), Escherichia (1.24%), unclassified genus derived from Peptostreptococcaceae (1.23%), Collinsella (1.14%), Bacteroides (1.08%) andLactococcus (1.07%) .

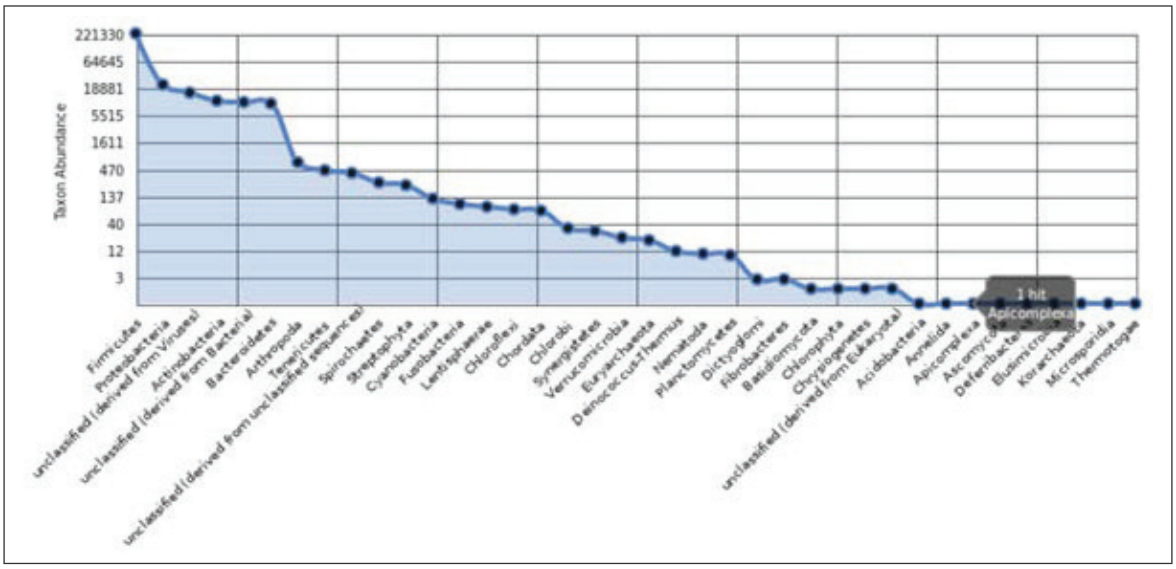
Fig-6. Pie chart – Genus level



Rank abundance plot at species level

The plot below shows the species abundances ordered from the most abundant

Fig-7. Rank abundance plot at species level



to least abundant. Only the top 50 most abundant are shown. The y-axis plots the abundances of annotations in each species on a log scale. Rank abundance plot for gut microbial diversity of broiler reared under intensive system was given in fig-7

DISCUSSION

The gut represents a complex microbial ecosystem consisting of trillions of commensal bacteria living in symbiosis with the host. For chickens, interactions between the host and the gastrointestinal microbiome play a crucial role in host physiological development, health, nutrition, and food safety. The gut microbiota 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 (Abrams *et al*. 1963; Mead, 2000). In chickens, the diets and the environments can affect the microbial status of the gastrointestinal tract (Apajalahti *et al*., 2004). Clostridium sordellii which is the causative organism of ulcerative enteritis like disease in quail (Crespo *et al*., 2013) was found at lower proportions in intensively reared CARI Nirbheek under intensive system. Bacteroides fragilis, used as a potential probiotic and immunobiotic is reported from intensively reared CARI Nirbheek. Scupham *et al*. (2010) reported that Megamonas hypermegale which belongs to phylum Firmicutes is a beneficial bacteria and recent metagenomics work revealed possible association between the presence of a subspecies of Megamonas hypermegale and campylobacter suppression. It is also present in intensively reared CARI Nirbheek. These beneficial

bacterial strains can be isolated and used as a potential probiotic for broiler as reported by Musikasang *et al.,* (2009).

CONCLUSION

The results of our experiment revealed that bacterial strains with probiotic properties were found in gut micro-biome of CARI Nirbheek and this could be contributing to the higher genetic resistance of native chicken in extensive or semi intensive system of rearing. Species level analysis revealed the presence of both beneficial and pathogenic bacterial strain and beneficial bacterial strains like Lactobacilli, Lactococci, Bifidobacteria and Butyric acid producing bacteria immunity besides growth in native chicken.

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