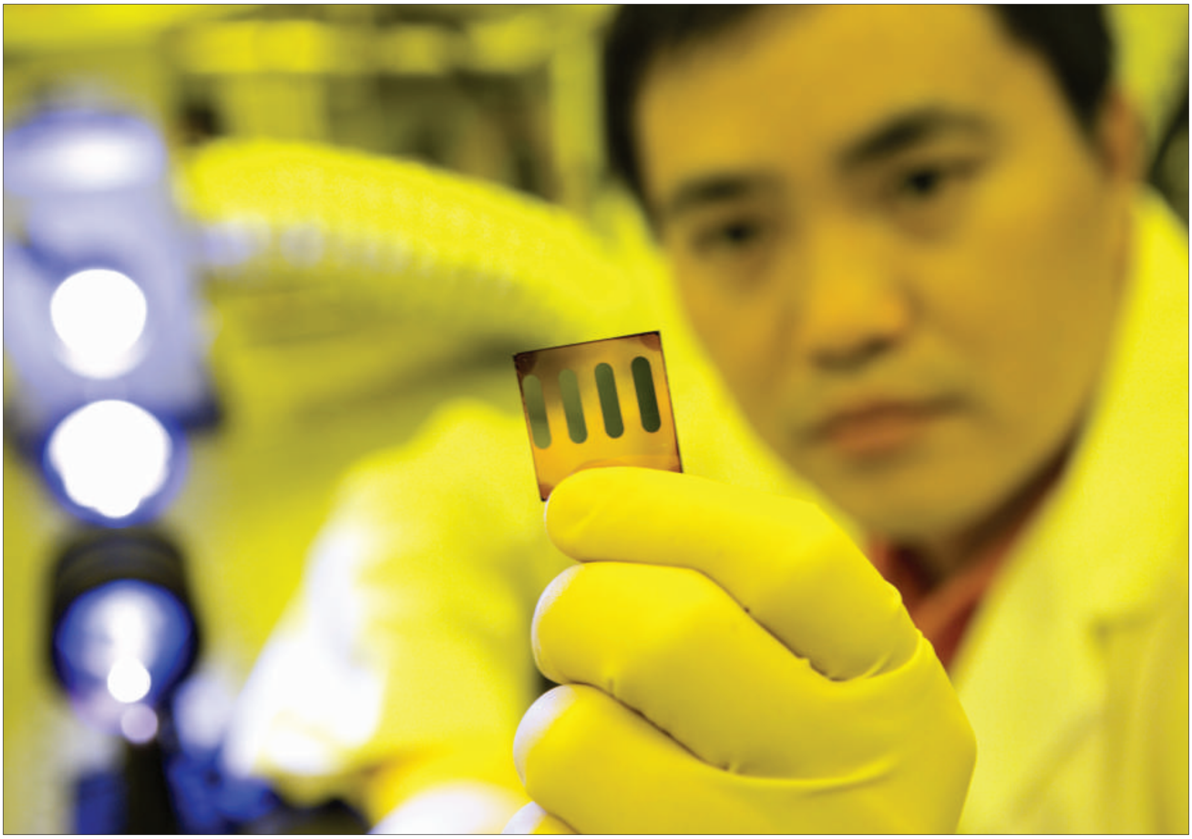

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Research Article
Effect of Probiotics on Diversity of Bacterial Community in the Gastrointestinal Tract of Chickens

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Abstract

Background and Objective: Probiotics as alternatives to antibiotics, have potential advantage because they are thought to promote gastrointestinal tract (GIT) health of the host. The aim of this experiment was to investigate the effect of probiotics on diversity of bacterial community (BC) in the GIT of chickens. **Materials and Methods:** Three hundred one-day-old Ross broilers were allocated to 4 experimental treatments for 28 days as a control treatments (birds were given no probiotics) and probiotics treatment (supplemented probiotics with water) along with feed of corn and soya diet. Thirty randomly sampled broilers per treatment (10 chicken/replicate of each treatment) were slaughtered and their GIT digesta taken for DNA extraction. The extracted DNA was sequenced using bacterial tag-encoded 16-FLX amplicon pyrosequencing procedures. **Results:** The control treatment samples showed that the BC formed of *Firmicutes* species including *Lactobacillus* species with different pathogenic bacteria along. On the other hand, probiotic treatment samples included *Lactobacillus salivarius* and *Lactobacillus aviarius* as dominant species. **Conclusion:** Probiotic supplements had made a shift in BC in broilers' GIT from pathogenic bacteria species in control treatment to beneficial bacterial species.

Key words: DNA sequence,16S-rRNA pyrosequence, intestinal health, microbiota, poultry, beneficial and pathogenic bacteria, food safety

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Promoting the development of the beneficial bacteria, also known as probiotics, within the gastrointestinal tract (GIT) of chickens could help to reduce food borne pathogen colonization, which could reduce human exposure to these pathogenic organisms and related illness and deaths[1]. Thus it becomes so important monitoring GIT's bacteria because many bacterial species with pathogenicity have been causing various diseases to human[2,3]. It is well established now that poultry performance is closely interrelated with bacteria that are present in GIT[4]. Moreover, they believed to play an important role in host health and productivity[5]. These microorganisms may be located in the gut lumen, buried in the mucus layer or adhered to the digestive mucosa where they can form very important cell layer[6]. On the other hand, the GIT of newly hatched chick is usually sterile and is highly susceptible to enteropathogen colonization and infection[7]. Bacteria have not been detected in any of GIT sections at hatching but at day 3 a significant number of bacteria have been isolated from all sites of GIT[8]. The bacteria community is well established in the small intestine within approximately 2 weeks of age[9].

The identification of the digestive bacterial community (BC) is necessary to understand the effect of nutrient and growth-promoting feed[10]. Also understanding the dynamics and diversity of the BC is necessary to establish and develop strategies to improve health, feed efficiency and growth rate of chickens[11]. An understanding of the dynamics of BC in the chicken intestine is very important for selection of diets for optimal nutrition, effective treatment of enteric pathogens and the development of competitive exclusion products to prevent or limit the colonization of pathogenic bacteria in the birds[12]. The nutritional strategies that have been used in the poultry industry to promote GIT health and increase the resistance to pathogen colonization are using antibiotics or recent alternatives such as probiotics[13].

Antibiotics are well known for the inhibition of undesired microbial population and the negative effects of their metabolites as well[14]. However, a consequence of this is the increase concern about the potential for antibiotic resistance strain of bacteria and about transfer of antibiotic resistance genes from animal to human microbial. Other alternatives to antibiotics such as probiotics have potential advantage because they are thought to promote intestinal health[15]. The definition of a probiotic is a product that contains sufficient numbers of viable bacteria that can alter the microflora in the host and exert beneficial health effects in this host[16]. Another definition, they are microorganisms that when administrated in suitable quantities, confer health benefits to the host[17]. Therefore, the aim of this study was reporting the effect of supplementing probiotics on BC of chickens' GIT.

MATERIALS AND METHODS

Experiment design and treatments: Three hundred 1-day-old (Ross-8) broiler chicks were obtained from a commercial hatchery and randomly divided into 2 groups, where each group had 3 replicate pens(50 birds/pen) for 28 days. The birds were raised in traditional poultry house with cages for allocating broilers according to their experimental design of randomized complete design of 3 replicates/treatment. The poultry house is Al-Kharj region of Central region of Kingdom of Saudi Arabia. It has been maintained and managed as experimental house for rearing broiler into cage system for academic staff of Animal production Department at King Saud University. The experiment was executed during the period from July-August, 2016. Temperature was maintained at 32°C for the 1st week and thin was gradually reduced to 24°C until end of week 3, thereafter it was kept at 24°C. A standard management procedure was used throughout the experiment period. All birds had free access to feed and drinking water.

The treatments were as follows, feed without any supplementation (control treatment) from 1-28 days of age and feed with probiotics supplemented in water from 1-28 days of age (probiotics treatment. The feed details were a commercial feed as starter diet from day 1-21 and finisher diet from day 22-28 days of age. The probiotics supplement, commercially known as Primalac®. Primalac® is a multi-strain of probiotics in dry white powder form (1.0×108 CFU g^{-1}) containing *Lactobacillus Acidophilus* (2.5×107 CFU g^{-1}), *Lactobacillus casei* (2.5×107 CFU g^{-1}), *Bifidobacterium thermophilum* (2.5×107 CFU g^{-1}) and *Enteroccocus faecium* (2.5×107 CFU g^{-1}).

Digest collection and DNA extraction: Digest of GIT for thirty chickens was collected from each treatment (10 chicken samples from each replicate taken randomly). Each chicken was killed and its digest of ileum was removed. The animal handling, killing and sampling procedures followed the implementing regulations of the law of ethics of research on Living Creatures (Saudi Arabia National Committee of Bio-Ethics with the approval of the King Saud University Animal Ethics Committee (No.: SG-2555). One gram of the ileum digesta of each chicken was mixed with those of same replicate for each treatment forming 10 g as pooled sample and then suspended in 90 mL of PBS (Phosphate buffer solution) (pH 8.0) using stomacher blender (Seward Medical, London, UK) for 2 min. One milliliter of aliquot of pooled sample was removed into eppendorf tube. The content of 1 g digesta in the eppendorf tube was exposed procedure of extracting DNA. The total genomic pooled DNA was extracted using a stool DNA kit recommended by the manufacturers (a QIAamp- Qiagen®, USA). The details of DNA extraction was described previously by Al-Atiyat[18]. The extracted DNA was then quantified using a Nanodrop spectrophotometer for measuring concentration and purity for further DNA sequencing step.

Bacterial DNA sequencing: The sequencing of bacterial extracted DNA was done using the bTEFAP of 16S rRNA. In this sequencing technique, specific bacterial primers suitable for microbial identification and metagenomic studies were used. Bacterial DNA samples were sequenced with those 16S universal eubacterial primers using PCR reactions. The resulted sequences of each pooled sample were processed using bioinformatics pipeline. The major bioinformatics pipeline used for to identify sequenced genome of bacterial isolates was BLAST (http://www.ncbi.nlm.nih.gov). The sequence of each pooled sample was separately lunched into BLAST database for finding the closest match with best bacterial identification level at portion more of 98% as threshold value.

RESULTS

The results showed that a dramatic shift in bacteria populations in GIT of chicken (Table 1). Table 1 shows a bacterial species in chicken received no probiotics, control treatment, in a comparison with those species found in chicken received probiotics in their feed as described in details in the following sections.

Control treatments of no probiotics: The digesta of chicken which received the control treatment contained firmicutes and pathogenic bacteria. The results of pyro sequencing clearly identified the pathogenic bacteria species in control treatment samples. They were *Streptococcus* sp., *Streptococcus alactolyticu*, *Nocardiopsis* sp., *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Serratia* sp., *Aeromonas* sp., *Micromonospora* sp., *Microbacterium testaceum*, *Serratia* sp., *Leptothrix* sp., *Acinetobactor* sp., *Alpha proteobacterium* and *Bacillus flexus* (Fig. 1-4). These results showed the

Table 1: Name of identified bacteria in the 4 treatments samples

Control treatments	Probiotic treatments
Streptococcus alactolyticus	*Lactobacillus aviarius*
Streptococcus sp.	*Lactobacillus salivarius*
Pseudomonas aeruginosa	*Lactobacillus inermedius*
Pseudomonas sp.	
Aeromonas sp.	
Firmicutes	
Microbacterium testaceum	
Micromonospora sp.	
Nocardiopsis sp.	
Serratia sp.	
Leptothrix sp.	
Acinetobactor sp.	
Bacillus flexus	

predominant cultured flora included in control samples of broiler's GIT. Furthermore, Fig. 1-3 showed the identified bacterial species in Operational taxonomic units (OTUs) defined as clones that shared more than 98% sequence similarity. The OTUs formed the dendrogram construction for each pooled sample representing those sampled digesta for each replicate in the control treatment. The dendrogramin form of phylogenetic tree of Fig. 1 showed the identified bacteria species were *Streptococcus alactolyticus*, *Lactobacillales*, *Streptococcus* sp. and *Firmicutes*. Figure 2 showed the identified bacteria species were *Pseudomonas* sp., *Pseudomonas aeruginosa*, *Leptothrix* sp. and *Nocardiopsis* sp. Finally, Fig. 3 showed the identified bacteria species were *Leptothrix* sp., *Aeromonas* sp., *Nocardiopsis* sp., *Pseudomonas* sp., *Pseudomonas aeruginosa*, *Micromonospora* sp., *Microbacterium testaceum*, *Bacillus flexus*, *Acinetobactor* sp., *Proteobacterium* and *Serratia* sp.

Probiotics treatments: Bacterial species found in the chicken received Probiotics were only *Lactobacillus aviarius*, *Lactobacillus salivarius* and *Lactobacillus inermedius*. The results highlighted the shift from different pathogenic BC in digesta of control samples to be only *Lactobacillus aviarius*, *Lactobacillus inermedius*, *Lactobacillus salivarius*. Moreover, the identification of the BC in digesta of probiotic samples was illustrates by phylogenetic trees. The phylogenetic trees analysis were utilized in identification BC diversity for the Probiotic treatment samples (Fig. 4-6). As stated earlier in Table 1 that identified bacteria species were *Lactobacillus aviarius* (Fig. 4, 5), *Lactobacillus salivarius* (Fig. 6), *Lactobacillus inermedius* (Fig. 6). Finally, all samples of the probiotic treatments associated with *Lactobacillus* sp.

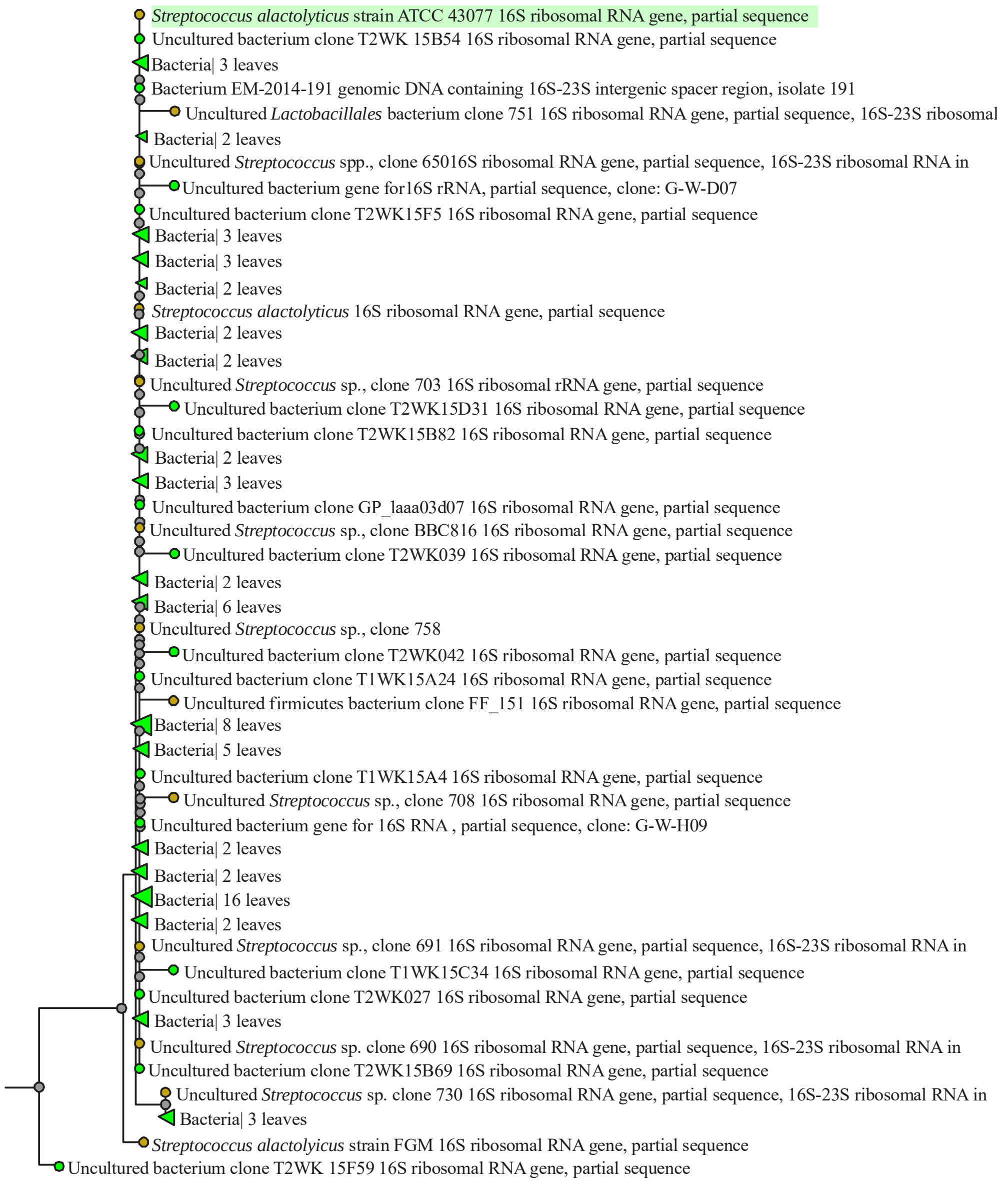

Fig. 1: Phylogenetic tree of identified bacterial species of control treatment
Identifying *Streptococcus alactolyticus*, *Streptococcus* sp., Firmicutes

DISCUSSION

The BC in GIT of chicken shifted towards beneficiary and functional bacteria in chicken received probiotic supplements in a comparison with those chicken received control treatment with no probiotic supplements. The BC in digesta of those chicken received the control treatment indicated that firmicutes and pathogenic bacteria were found. The firmicutes are a phylum of bacteria, most of which have Gram-positive cell wall structure[19]. Firmicutes include *Bacilli* (e.g., *Lactobacillales*, *Bacillus*, *Staphylococcus*, *Enterococcus*, *Streptococcus*), Clostridia and Erysipelotrichia[19]. The results of pyrosequencing clearly identified the pathogenic bacteria species such as *Streptococcus* sp., *Streptococcus alactolyticu, Nocardiopsis* sp., *Pseudomonas aeruginosa, Pseudomonas* sp., *Leptothrix* sp. and *Acinetobactor* sp. in control treatment samples (Fig. 1-4). These results were in agreement of very earlier study investigation BC in chickens which showed that the predominant cultured flora of included *Streptococcus*, *E. coli* and eubacteria[20]. Recent study, based on DNA sequencing detection, showed also similar findings in which *Lactobacillus* and *Streptococcus*, Clostridiaceae and proteobacteria were identified[21].

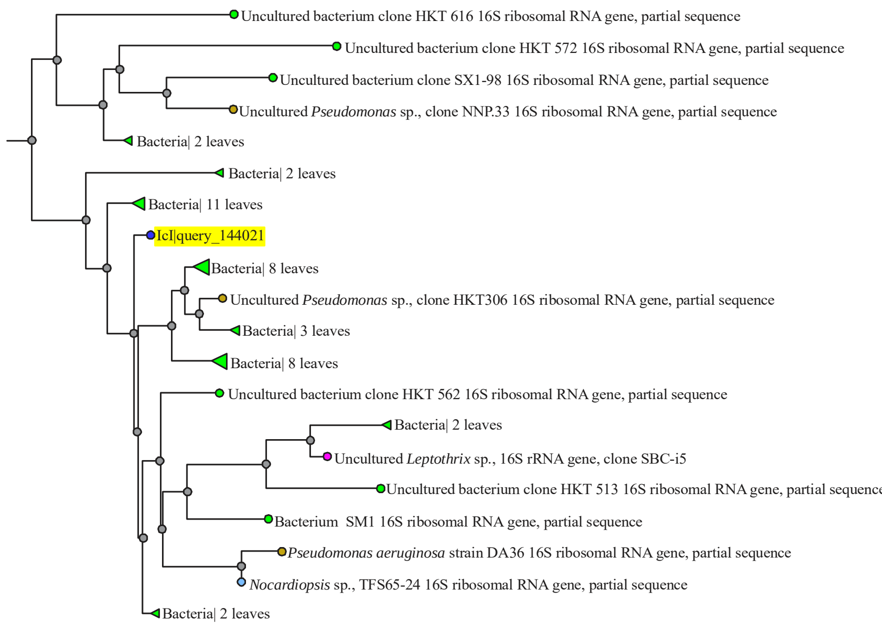

Fig. 2: Phylogenetic tree of identified bacterial species of control treatment

Identifying *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Aeromonas* sp., *Leptothrix* sp., *Nocardiopsis* sp.

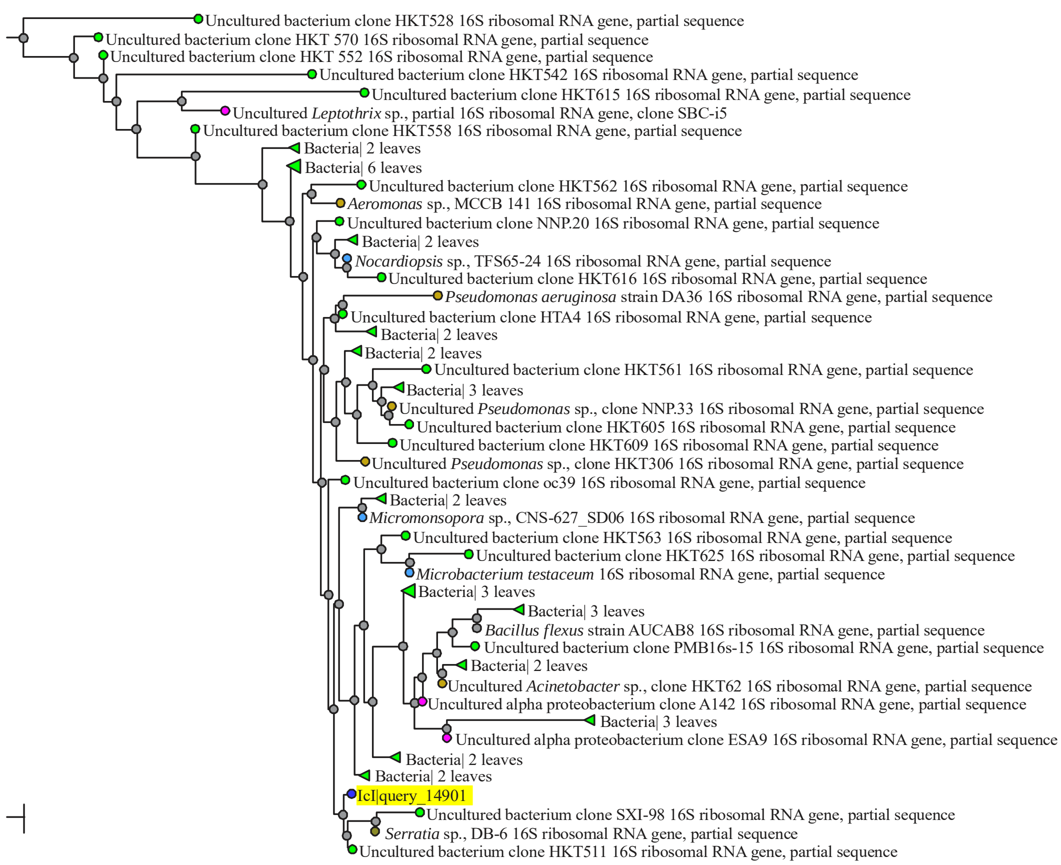

Fig. 3: Phylogenetic tree of identified bacterial species of control treatment

Identifying *Leptothrix* sp., *Aeromonas* sp., *Nocardiopsis* sp., *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Micromonospora* sp., *Serratia* sp., *Microbacterium testaceum*, *Leptothrix* sp., *Acinetobactor* sp., *Alpha proteobacterium* sp., *Bacillus flexus*, *Serratia* sp.

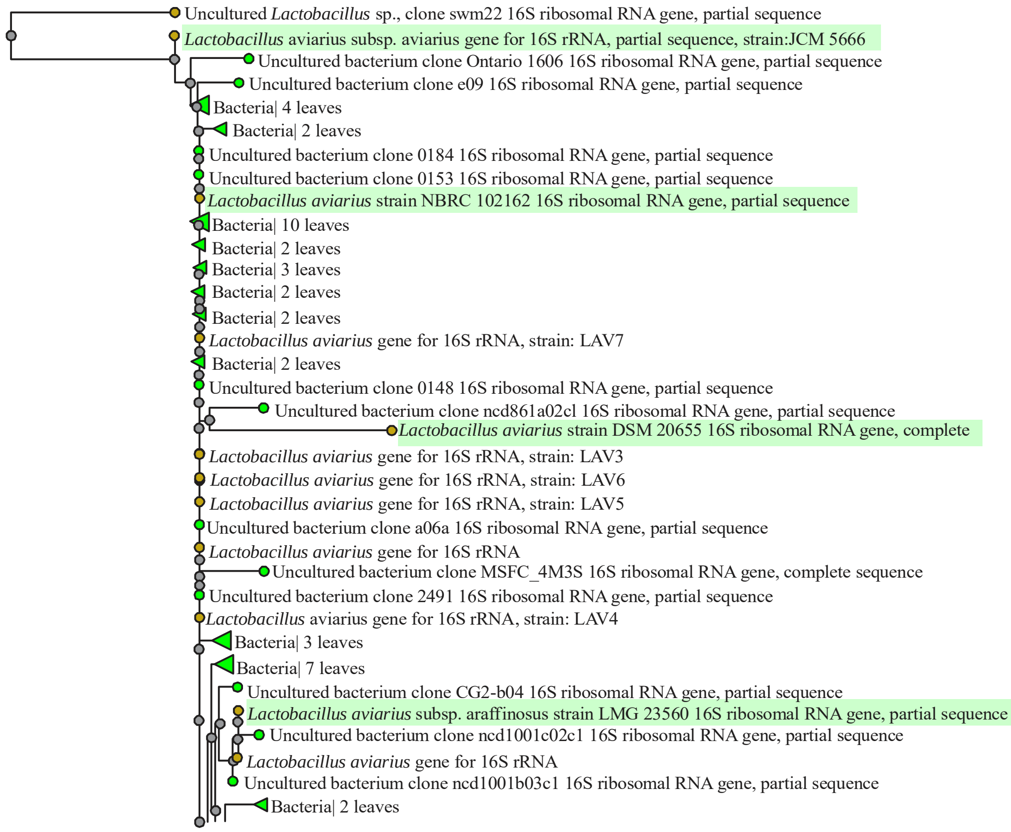

Fig. 4: Phylogenetic tree of identified bacterial species of probiotic treatment
Identifying *Lactobacillus aviaries*

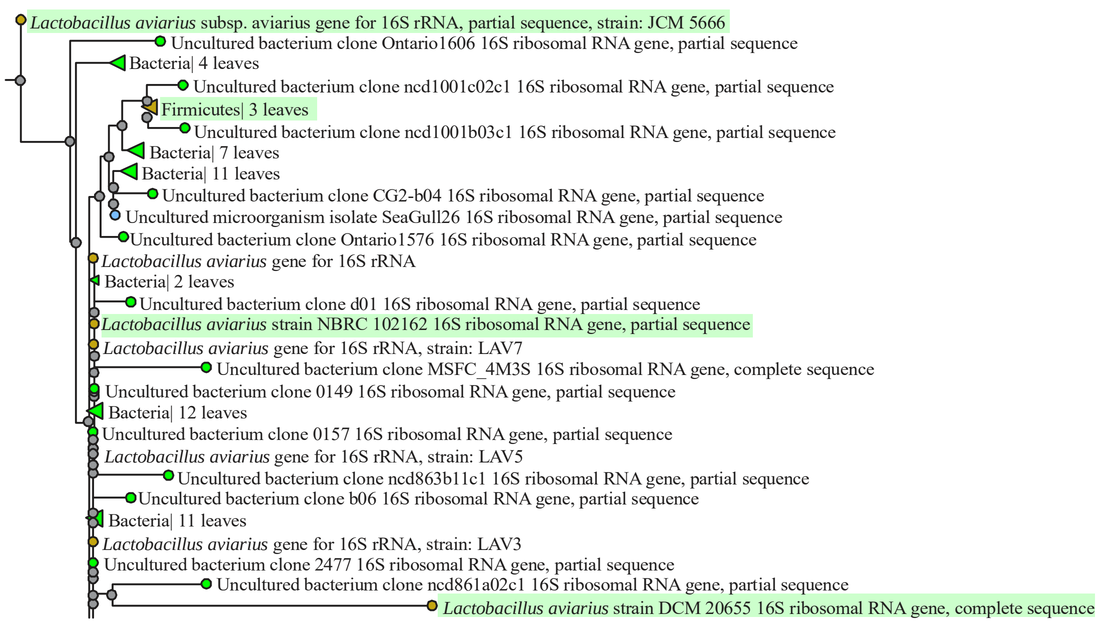

Fig. 5: Phylogenetic tree of identified bacterial species of probiotic treatment
Identifying *Lactobacillus aviaries*

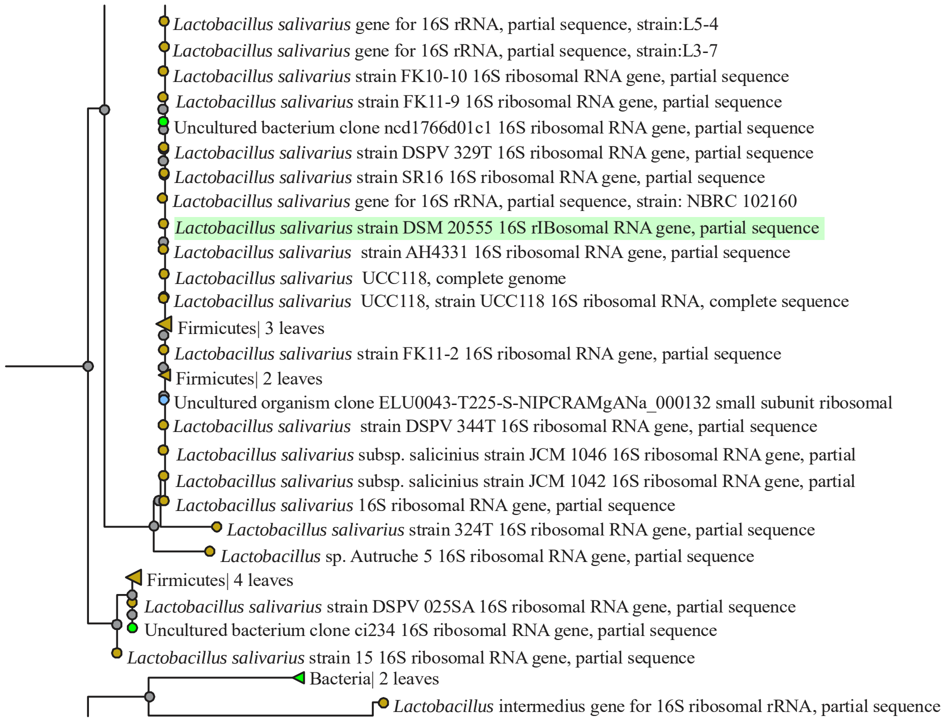

Fig. 6: Phylogenetic tree of identified bacterial species of probiotic treatment
Identifying *Lactobacillus* sp., *Lactobacillus salivarius* and *Lactobacillus inermedius*

The notable shifting towards beneficial bacteria such as *Lactobacillus aviarius*, *Lactobacillus inermedius* and *Lactobacillus salivarius* in the chicken received probiotics was unexpected. Theses bacteria species are very useful enhancing immunity system of the organism. The mode of action of probiotics in poultry includes maintaining normal intestinal microflora by competitive exclusion and antagonism[22]. This mode of action explain why BC reported in control treatment samples were shift to be only *Lactobacillus aviarius*, *Lactobacillus inermedius*, *Lactobacillus salivarius*. Furthermore, probiotics represent biological alternatives in the control of enteric pathogens such as *Campylobacter*, *Salmonella* and *Escherichia coli*[16]. In agreement with our results, Amit-Romach *et al.*[23] reported that only *Lactobacillus* was consistently detected in all intestinal regions of poultry. It has been proposed that dietary fiber can be used by Bifidobacteria and *Lactobacillus* species, leading to the production of lactic acid of which is inhibitory to *Salmonella*[24]. Moreover, Rocha *et al.*[25] reported that *Lactobacillus* have immune modulatory actions in the chicken, they suggested that the oral introduction of *Lactobacillus* can increase the nonspecific resistance of the host to pathogenic bacteria such as *Salmonella*, *Coliform* and *Campylobacter* in birds of various ages. Finally, Pourabedin *et al.*[26] stated that BC taxonomy showed taxa significantly associated with cladogram under neutral situations and with beneficial bacteria when probiotics used. Thus, eliminating pathogenic BC in broiler's GIT would reduce human exposure to those pathogenic organisms into its meat. Furthermore, researcher and producer would be urged to start replacing antibiotics with probiotics which is beneficial for both livestock and human. The growing pressure to reduce antibiotics usage in livestock production help researchers to explore benefits of probiotics in feed industry, animals products, food chain and safety and finally human health.

CONCLUSION

The findings of this study reporting that *Lactobacillus* species solely accounting for probiotic treatment and other pathogenic bacteria species such as *Streptococcus* and *Pseudomonas* accounting majority for control treatment of no probiotics application. In other words, it is conclude that probiotics would be able to make desirable shift in BC of broiler's GIT by eliminating pathogenic bacteria community. Thus, it is recommended using probiotics into feeding broiler in order to reduce human exposure to these pathogenic organisms and related illness and deaths.

SIGNIFICANCE STATEMENT

This study discover supplementing the probiotics into broiler feed eliminating pathogenic BC in broiler's GIT and thus by reduce human exposure to pathogenic organisms into its product. Furthermore, this study will help the researcher and producer to start replacing antibiotics with probiotics which is beneficial for both livestock and human. The critical areas that many researchers were not yet able to explore that strong demand for probiotics in feed come under growing pressure to reduce antibiotics usage in the food chain. Thus a new theory on using probiotics for producing broilers' meat may be arrived at improvements in human overall health.

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