*Int.J.Curr.Microbiol.App.Sci* (2018) Special Issue-7: 5161-5166

*International Journal of Current Microbiology and Applied Sciences*
*ISSN: 2319-7706* **Special Issue-7 pp. 5161-5166**
Journal homepage: http://www.ijcmas.com

**Original Research Article**

# Effects of Biofilm Forming Probiotics Feeding on Broiler Chicken Performance

**N. Subhashree[1*], M. Rajashree[1] and A. Prajapati[2]**

[1]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Bhubaneswar, India
[2]Indian Veterinary Research Institute, Izatnagar, India
**Corresponding author*

**Keywords**

Biofilm, Lactobacillus, Probiotics, Chicken

**A B S T R A C T**

Present study was conducted to isolate the potential probiotics *Lactobacillus* spp and *Saccharomyces cerevisiae* and evaluate their probiotics efficacy in chickens. A total 104 milk, curd and bread samples were collected and processed for isolation and characterization of biofilm forming *Lactobacillus* spp and, *Saccharomyces cerevisiae* (in bread). Based on salt tolerance, temperature tolerance and antibiotics sensitivity 42 different isolates of *Lactobacillus spp* (*L. plantarum*, *L. acidophilus*, *L. fermentum*, *L. lacti* and *L. casei)* and *Saccharomyces cerevisiae* was found suitable for probiotics preparation. A probiotics oral suspension was prepared by using different percentage of each isolates type and experimental trail was conducted on two groups of day old chicks each containing 10 chicks. One group was fed with probiotics (1gm daily in water) and other without probiotics for 30 days. Results showed that probiotics preparation significantly increase the body weight and feed efficiency of chicken fed with probiotics in comparison to control group without probiotics ($p<0.05$) in 3rd and 4th week.

## Introduction

Poultry production is an important economic business in many countries of world. India ranks 3rd in egg production and 7th in chicken meat production in the world (Watt Executive Guide, 2015). Use of antibiotics in poultry feed is common practice in many countries including India for growth promoter but the extensive use of antibiotics has the possibility to generate antibiotic-resistant bacteria in animal products (Sethiya, 2016). The poultry Industry is now facing a ban for the use of antibiotic feed additives for disease prevention and growth enhancing supplements (Smith, 2014).

One of the safe alternatives of antibiotics in feed is probiotics in order to decrease the antimicrobial resistance. Probiotics are non-pathogenic microorganisms that, when administered in adequate amounts, exert health benefits on the host (Zhang and Kim, 2013). *Lactobacillus, Bacillus, Streptococcus, Bifidobacterium, Clostridium*

and *Saccharomyces* based probiotics are the most frequently used as probiotics (Raja and Imran, 2009) and different strains may affect their efficiency. Probiotics exerts their effects by increasing the digestibility of nutrients in chicken, increasing feed conversion ratio (FCR), immune modulation, enhancement of resistance against pathogens, (Wan *et al*., 2015) and competitive exclusion process (Mountzouris *et al*., 2007). Probiotics have been used for improving immunity to infections with *Salmonellas, Candida albicans, Coccidia* and *Escherichia coli* in poultry. A biofilm is extracellular polysaccharide matrix that enclosed the producing microbial cells and supports the generation of a microenvironment for the metabolic interaction of the population (Shauder *et al.*, 2001). For probiotics biofilm formation is beneficial characteristics of bacteria enabling them successful colonization and maintenance in gut epithelium and resist to gut environmental (Lepargneur and Rousseau, 2002). A mature biofilm has greater antibacterial activity and tolerance to gastric pH than a newly formed biofilm (Phillips *et al*., 2013). Probiotic *Lactobacillus reuteri* biofilms produce antimicrobial and anti-inflammatory factors and modulate Immunity (Jones *et al.,* 2009). Probiotics have promising effects as alternatives for antibiotics as pressure to eliminate growth-promotant antibiotic use (Awad *et al.,* 2009).

## Materials and Methods

### Isolation and characterization of probiotics isolates

A total 104 samples were collected from veterinary clinics of Veterinary College, and local markets comprising of raw milk, Dahi, Bread, and Bakery yeast powder. MRS agar was used to isolate *Lactobacillus* spp and Sabouraud-Dextrose agar for *Saccharomyces cerevisiae* isolation (Barnett, 1990). The identification was performed by battery of biochemical test according to Bergey's manual of determinative of bacteriology. Biofilm forming potency of bacteria was determined by culturing the isolates on modified MRS agar containing 0.3% bentonite clay.

### Determination of probiotics potential

Susceptibility of isolated *Lactobacillus* spp to bile and salt was tested by growing in MRS broth contains 2%, 4% and 6.5% sodium chloride (Bhardwaj *et al*., 2011) separately. Colonies (cfu) were counted by the pour plate method. Similarly effects of temperature was determined by inoculating the bacteria in MRS broth and incubated at different temperatures 25, 30, 35, 40, 50 and 60 $^{0}$C for 24 h and the growth was monitored by plating on MRS agar.

### Antimicrobial activity

The antimicrobial susceptibility testing of different strains of *Lactobacillus* was determined against 9 antimicrobial drugs using the disc agar diffusion methods. The test was carried out according to NCCLS (National Committee on Clinical Laboratory Standards) protocols (CLSI-2009).

### Preparation of probiotics

The composition of probiotics was prepared as per the method described by Adams, 2010 and Bujalance *et al*., 2006 with slight modification. All bacteria isolates were grown in bulk in broth and sedimented by centrifugation. Oral solution (MOS) was prepared (*L. acidophilus, L. plantarm, L. casei, L. latis, L. fermentum, S. cevisiae,* NaCl and Glucose) and included in poultry water (1gm/day).

## Experimental feeding of probiotics to broiler chicken

Twenty commercial broiler day old chicks procured from local market and randomly divided into two equal groups. The chickens were allowed to have free access water and feed. Experimental group was supplied with 1 gm of probiotic oral solution in water daily and control group supplied only normal water without probiotic solution. Body weight gain and feed consumption were monitored weekly.

## Results and Discussion

A total 125 lactic acid bacteria (45 isolates from dahi and 80 isolates from milk) was isolated from the samples. All are found to be Gram positive, rod shaped, non-motile, catalase negative and show absence of endosopres in microscopic examination. Further biochemical characterizations of isolates reveal the presence of *L. plantarum, L. acidophilus, L. fermentum, L. lacti* and *L. casei.* Most of the isolates recovered from Dahi which is concordance with Bhardwaj *et al.* (2011) who isolated and biochemical characterized different *Lactobacillus* species isolated from Dahi. *Lactobacillus* genus has evolved and contains to date more than 80 species and mostly they are present in raw milk and dairy products such as cheeses, yoghurts and fermented milks. Biofilm producing potential of different isolated *Lactobacillus* were tested by growing them on MRS Agar plate with bentonite clay on which *L. fermentum, L. plantarum* and *L. casei* showed enhanced growth (42 isolates). No luxurious growth was observed in *L. acidophilus*. Effects of various NaCl concentration on growth of *Lactobacillus* species in MRS broth showed that *L. plantram and L. acidophillus* grow well in 2% and 4% and, no growth was observed in 6.5%, *L. fermentum* showed growth only at 2% concentration of NaCl whereas, *L. casei* showed no growth in the above three concentration of NaCl in MRS broth. All isolates tolerated temperature between 30-45$^{o}$C on the inoculated culture. So after 45$^{o}$C the growth gradually starts to decline. On analysing the result of antibiotic sensitivity test (AST) for raw milk samples, it was found that the majority of *Lactobacillus* species were sensitive to Gentamycin, Vancomycin followed by Tobramicine, Tetracyclin, Kanamicin, Nalidixic acid, Ampicillin, Azithromycin and Metronidazole on normal MHA agar plate. Generally lactobacilli have a high natural resistance to many antibiotics but present isolates are found to be susceptible to antibiotic tested which eliminate the possibility of antibiotic resistant gene transfer from the probiotics preparation. Members of the genus *Lactobacillus* are particularly suited for development as probiotics, since they confer benefits to their host by improving properties of the indigenous flora resulting in the improvements in health and weight of the chickens (Ouwehand *et al*., 2002). The results obtained of chickens performance (Table 1) showed that the average live weight of the group receiving probiotics on the 3$^{th}$ and 4$^{th}$ weeks (820 g, and 1150 g), was higher ($p<0.05$) than control (680 g, and 950 g respectively). No differences were observed in feed intake between groups. The feed conversion rate (FCR) of the probiotics fed group was significantly higher than the control group on 3$^{th}$ and 4$^{th}$ weeks. Result showed that there is a no significant increase in body weight of probiotics group till 21 days. Mohan *et al.* 1996 also stated that the use of probiotics in feed had a beneficial effect on body weight gain of broiler chicks from 4$^{th}$ to 6$^{th}$ week of age. Jin *et al.* 1996 found that inclusion of probiotics (*Lactobacilli* spp and *Bacillus subtilis*) in diet stimulated favourable microbial balance

in gut and consequently improved feed efficiency and growth performance in broilers. Various workers reported that broilers fed probiotic-supplemented diet had better weight gain and feed efficiency when compared to the broilers fed the unsupplemented diet (Chiang and Hsieh, 1995), improves immune function including natural killer cell activity (Ayoung *et al.*, 2017). Zhang *et al.* 2005 reported that effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Biofilm formation is important attributes to effective probiotics.

**Table.1** Average body wt (gm) of probiotics group and Control group

| Days | Average body wt (gm) probiotics group | Average body wt (gm) Control group |
|---|---|---|
| 1 | 45 | 42 |
| 7 | 156 | 145 |
| 14 | 380 | 340 |
| 21 | 820 | 680 |
| 30 | 1150 | 950 |

In conclusion, conventional strategy in poultry production includes hygienic methods, vaccination and use of antimicrobial agent to reduce the disease in animal production aim to reduce disease causing pathogen. Biofilm forming probiotics is newer and safer approach to reduce the impact of antibiotics resistance and residue in poultry product. Present study showed that direct-fed microbial or probiotics products can be best alternative solution to the use of antibiotics in poultry diet.

## References

Adams, A.C. (2010). The probiotic paradox: live and dead cells are biological response modifiers, *Journal of Nutrition Research*, 23: 37–46

Awad, W.A., Ghareeb, K., Abdel-Raheem S. and Bohm J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights and intestinal histomorphology of broiler chickens. *Poultry Science*, 88: 49-56.

Ayoung, L., Young, J.L., Hye, J.Y., Minkyung, K., Yeeun, C., Dong, S.L. and Jong, H.L. (2017). Consumption of dairy yogurt containing *Lactobacillus paracasei* ssp. *paracasei*, *Bifidobacterium animalis* ssp. lactis and heat-treated *Lactobacillus plantarum* improves immune function including natural killer cell activity. *Journal of nutrients*, 9: 1-9.

Barnett, J.A. (1975). The entry of D-ribose into some yeasts of the genus *Saccharomyces. Journal of Genera Micribiology*, 90(1): 1-12.

Bhardwaj, A., Puniya, M., Sangu, P.S., Kumar, S. and Dhewa, T. (2011). Isolation and biochemical characterization of *Lactobacillus* species isolated from dahi. *A Journal of Dairy Science and Technology*, 1:1-15.

Bujalance, C., Jiménez-Valera, M., Moreno, E. and Ruiz-Bravo, A. (2006). A selective differential medium for *Lactobacillus plantarum*. *Journal of Microbiological Methods*, 66: 572–575

Chiang, S.H. and Hsieh,W.M. (1995). Effect of direct-fed microorganisms on broiler growth performance and litter ammonia level. *Asian-Aust. Journal of Animal Science*, 8(2):159-162.

Jin, L.Z., Ho, Y.W., Abdullah N. and Jalaludin. S. (1996). Influence of dried *Bacillus subtilis* and lactobacilli cultures on intestinal microflora and performance in broilers. *Asian-Aust. Journal of Animal Science*, 9:397-40.

Jones, S.E. and Versalovic, J. (2009). Probiotic *Lactobacillus reuteri* biofilms produce antimicrobial and anti-inflammatory factors. *BMC Microbiology*, 11(9): 35.

Lebeer, S., Verhoeven, Y.L.A., Velez, M.P., Vanderleyden, J. and De Keersmaecker, S.C.J. (2007). Impact of Environmental and Genetic Factors on Biofilm Formation by the Probiotic Strain *Lactobacillus rhamnosus* GG. *Journal of Applied and environmental microbiology*, 73: 6768–6775

Lepargneur J.P. and Rousseau V. (2002). Protective role of the Doderlein flora. *The Journal of Gynecology Obstetrics and Human Reproduction*, 31:485–494.

Mohan, B., Kadirvel, R. Natarajan, A. and Bhaskaran, M. (1996). Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers. *British Poultry Science*, 37:395-40.

Mountzouris, K.C., Tsirtsikos, P., Kalamara, E., Nitsch, S., Schatzmayr, G. and Fegeros, K. (2007). Evaluation of the efficacy of a probiotic containing *Lactobacillus, Bifidobacterium, Enterococcus* and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. *Poultry Science*, 86: 309-317.

Ouwehand, M., Kimoto, H., Ohmomo, S. and Okamoto, T. (2002). Impact of probiotic Lactobacillus Strains feeding on fecal Lactobacilli and weight gains in chicken. *The Journal of General and Applied Microbiology*, 49: 1.

Phillips, P.L., Yang, Q., Davis, S., Sampson, E.M., Azeke, J.I. and Hamad, A. (2013). Antimicrobial dressing efficacy against mature *Pseudomonas aeruginosa* biofilm on porcine skin explants. *International Wound Journal*, 12, 469–483.

Raja, M.M. and Imran, M.M. (2009). Lactobacillus as a probiotic feed for chickens. *International Journal of Poultry Science*, 8 (8): 763-767.

Sethiya, N.K. (2016). Review on natural growth promoters available for improving gut health of poultry: An alternative to antibiotic growth promoters. *Asian Journal of Poultry Science*, 10: 1-29.

Shauder, S., Shokat, K., Surette, M.G. and Bassler, B.L. (2001). The luxS family of bacterial autoinducers: Biosynthesis of a novel quorum-sensing signal molecule. *Molecular Microbiology*, 41, 463–476.

Smith, J.M. (2014). A review of avian probiotics. *Journal of Avian Medicine & Surgery*, 28: 87-94.

Wan, L.Y.M., Chen, Z.J., Shah, N.P. and El-Nezami, H. (2015). Modulation of intestinal epithelial defense responses by probiotic bacteria.

*Critical Reviews in Food Science and Nutrition*, 56(16):2628-41.

Watt Executive Guide. (2015). Poultry Trends. www.wattagnet.com.

Zhang, A.W., Lee, B.D., Lee, S.K., Lee, K.W., An, G.H., Song, K.B. and Lee. C.H. (2005). Effects of yeast cell components on growth performance, meat quality and ileal mucosa development of broiler chicks. *Poultry Science,* 84:1015–1021.

Zhang, Z.F. and Kim, I.H. (2013). Effects of probiotics supplementation in different energy and nutrient density diets on performance, egg quality, excreta microflora, excreta noxious gas emission and serum cholesterol concentrations in laying hens. *Journal of Animal Science*, 91: 4781-4787.