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Case Report

Isolation of *Lactobacillus* sp. from Intestinal Tract of Red Jungle Fowl as an Effective Probiotic for Poultry

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ABSTRACT

Objective: The present study focuses on the use of *Lactobacillus* sp. as probiotics to the Red jungle fowl and to test its growth efficacy. **Materials and Methods:** The isolated colonies were optimized at different pH range and it was found that at pH 7 maximum O.D value of 0.725 was attained and that of Temperature optimization at 30°C showed a maximum O.D value at 0.624 when read at 600 nm. The optimized pH and temperature were used for the mass production of *Lactobacillus* sp. for 3 days in orbital shaker and the biomass. The Biomass was fed to one month grown red jungle fowls , the biomass was mixed with commercial feed and the weight of the fowls were checked every 4 days interval to a total of 28 days. **Results:** A total of five colonies were isolated from the serial dilution performed on the nutrient agar medium and then it was streaked on MRS a specific medium for *Lactobacillus* sp. The isolated *Lactobacillus* sp. was given as a probiotic to the two test fowls and the weight of the fowls was 295 g on the 4th day and which increased gradually to 595 g on the 28th day. This shows that bacteria could be used as a probiotic to enhance the weight and quality of the chickens.

Key words: *Lacto bacillus*, Optimization, Fowls, Probiotic, Biomass.

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INTRODUCTION

Probiotic is a relatively new word meaning 'for life', which is used to name microorganisms that are associated with the beneficial effects for humans and animals. These microorganisms contribute to intestinal microbial balance and play a role in maintaining health. The probiotic microorganisms consist mostly of the strains of the genera *Lactobacillus* and *Bifidobacterium*, but strains of *Bacillus, Pediococcus* and some yeast have also been found as suitable candidates. Together they play an important role in the protection of the organism against harmful microorganisms and also strengthen the host's immunity. Probiotic is a relatively new word meaning 'for life', which is used to name microorganisms that are associated with the benefical effects for humans and animals. These microorganisms contribute to intestinal microbial balance and play a role in maintaining health. The probiotic microorganisms consist mostly of the strains of the genera *Lactobacillus* and *Bifidobacterium*, but strains of *Bacillus, Pediococcus* and some yeasts have also been found as suitable candidates. Together they play an important role in the protection of the organism against harmful microorganisms and also strengthen the host's immunity. Supplementation if probiotics alleviates the problem of lactose intolerance, the enhancement of nutrients bioavailability and prevention or reduction of allergies in susceptible individuals. Probiotics are reported to have also antimutagenic, anticarcinogenic, hypocholesterolemic, antihypertensive, anti-osteoporosis and immune modulatory effects.[1] Moreover, it has been shown that probiotics could protect broilers against pathogens by colonization in the gastrointestinal tract[2] and stimulation of systemic immune responses.[3] The World Health Organization (WHO) has predicted that by 2030, cardiovascular diseases will remain to be the leading causes of death. The intestinal micro biota, epithelium and immune system provide passive and active resistance to enteric pathogens in birds. Inhibition of pathogens by the intestinal micro biota has been called bacterial antagonism or competitive exclusion. Lactobacilli, isolated from chicken gastrointestinal tract, was examined and found useful for their potential probiotic properties and inhibitory activity against enteropathogenic bacteria like Salmonella, *E. coli* and Clostridium perfringens.[4] Currently, the increased bacterial resistance to antibiotics has forced poultry enterprises to eliminate sub-therapeutic use of antibiotics as growth promoting agents.[5]

MATERIALS AND METHODS

Collection of digestive tract of red jungle fowl

The digestive tract of the red jungle fowl was collected from butchery shop in a sterile polythen bag and brought to the laboratory for further processing. The sample was kept in refrigerator at 4°C until the sample was analysed.

Isolation of *Lactobacillus* Strains from Chickens

Small intestine, were collected freshly from red jungle fowl chickens and were stored in sterile, buffered peptone water .The samples were kept refrigerated and were processed on the same day. The samples were homogenized in buffered peptone water with a blender under anaerobic conditions. Mucosal scrapings were derived from the tissue samples and dissolved in buffered peptone water. Further processing was performed under aerobic conditions.

Serial Dilution of the samples

Serial dilutions of the homogenized samples were made in different dilutions ranging from 10^{-1} to 10^{-7}. The dilutions 10^{-4} was plated on were incubated aerobically at 37°C for the observation of growth in anaerobic condition. Well-isolated colonies with different appearances were picked from each plate and transferred at 37°C for 48 h (anaerobic) to obtain pure strains. Finally, the colonies were picked and grown in MRS medium at 37°C for 24 h in anaerobic culture.[6]

Maintenance of cultures

The Bacterial colonies which showed growth on the MRS agar plates were sub-cultured on the same medium and were kept at 37°C for 24 h in anaerobic condition. After incubation the grown pure colonies were kept in refrigerator at 4°C.

Preliminary Identification of the isolates

The preliminary identification of the bacterial isolates was performed by the following biochemical Tests and staining procedures such as gram staining, motility.

Gram Staining

Bacterial smears of 16-18 h old cultures were made on clean grease free slides, heat fixed and stained as follows. The slide was flooded with crystal violet solution for a minutes, drained and rinsed with water; followed by addition of Grams iodine solution for one minute, drained and rinsed one minute and observed under a 100 X Oil immersion microscope.

Motility Test

A drop of bacterial cultures grown in nutrient broth was kept on the center of cover slip and wax was kept at each corners of the cover slip. The cavity slide was placed inverted on the cover slip and then placed in 400 X magnification of the compound microscope. The motility was observed at the corner of the drop for motile organisms.

Biochemical characteristics

Based on the biochemical tests, the suspected isolates were identified according to the Bergey's manual of systemic bacteriology.[7]

Effect of Temperature on the growth of Bacteria

The isolated lacto bacillus strain was grown in different Temperature range (20°C, 25°C, 30°C, 35°C, 40°C) in nutrient broth. The Culture was inoculated in 150 ml of nutrient broth prepared in 250 ml flasks with varying Temperature used. These flasks were kept in different temperatures and were kept for 3 days incubation to analyze the growth.

Effect of pH on the growth of Bacteria

The isolated lacto bacillus strain was grown in different pH range (pH 6, 6.5, 7, 7.5 and 8) in nutrient broth. The Culture was inoculated in 150 ml of nutrient broth prepared in 250 ml flasks with varying pH used. These flasks were kept in shaker at 30°C and were kept for 3 days incubation.

Bacteria as probiotic to Red jungle fowls

The bacterial colonies were grown in nutrient broth and were kept for 3 days incubation. The bacterial colonies at log phase were taken in 50 ml eppendorf tubes and centrifuged at 8000 rpm for 20 min and the pellet was collected. The pellet was then washed with distilled water 2-3 times to remove the nutrients present in the medium. This pellet was then mixed with distilled water, vortexed and was kept in refrigerator.

Preparation of feed and administering the fowls with probiotics

The commercially available feed was mixed with 10 ml of the inoculum and was given to two test fowls and to the control fowl fed with commercially available feed. The fowls were fed two times every day and these fowls were monitored for growth in term of weight every 4 days' time interval.

RESULTS

Isolation of Bacteria from Digest of Fowls

A total of five different colonies were grown on the nutrient agar plates with distinct morphology. These colonies were sub-cultured by quadrant streaking on the solid nutrient agar plates and were stored in refrigerator for further use.

Screening for *Lactobacillus* bacteria

All the five colonies were streaked on MRS medium and it was found that only three organisms were grown able to grow on MRS agar plates. The three colonies which had grown on MRS plates were again subcultured on MRS agar plates by quadrant streaking (Plate 1).

Gram staining

The gram staining of all the three bacteria were performed and it was found that among the three colonies which had grown in MRS medium only one isolate namely R2 showed gram positive rod, whereas the other two bacteria namely R1 and R3 showed gram positive cocci as shown in Table 1 (Plate 2). As *Lactobacillus* is gram positive rod shaped culture R2 is preliminarily confirmed as *Lactobacillus* and this strain is take for further analysis. The motility test of the bacterial culture R2 was carried out and it was found that this bacteria was motile. The catalase and oxidase test of the culture R2 was performed and both results were negative. In the biochemical analysis of the bacterial culture R2, it was found that Methyl red was only positive whereas all the other tests namely Indole, Voges progesur, Triple sugar ion test and citrate test showed negative results (Table 1).

Effect of temperature on bacterial growth

The bacterial culture R2 was grown in different temperature namely 20°C, 25°C, 30°C, 35°C, 40°C were used it was found that temperature 30°C is favorable for the growth of *Lactobacillus* sp., so this temperature is optimized for the growth of isolated bacteria. The optical density value of the broth was taken at 600 nm and a maximum growth of the *Lactobacillus* was obtained as 0.624 followed by 0.574 in temperature 35°C (Figure 1.)

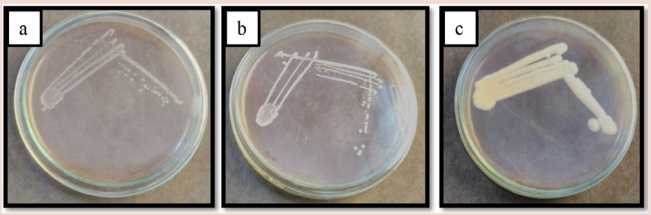

Plate 1: Morphological identification; Grams staining (a). R1. (b)R2. (c).R3.

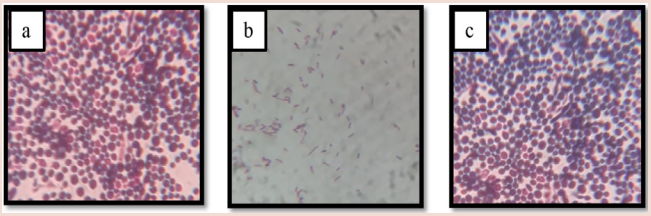

Plate 2: Gram staining of three isolates (a). R1. (b) R2. (c).R3.

Table 1: Biochemical identification of the culture R2.

Strains. No	MR	VP	Citrate	TSI	Oxidase	Catalase	Motility
R2	+	-	_	G-,H_2S-	-	-	+

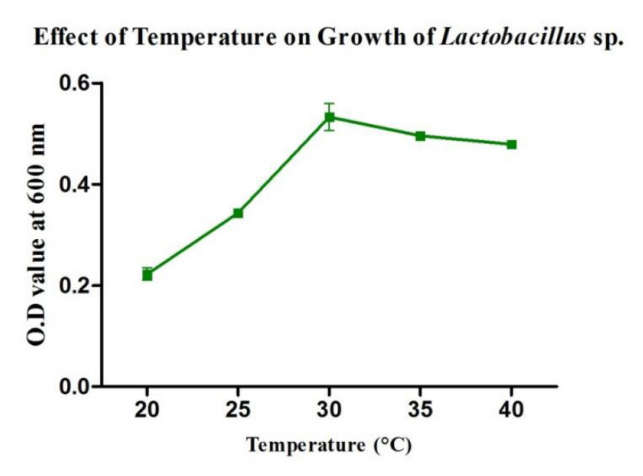

Figure 1: Effect of temperature on growth of strain R2.

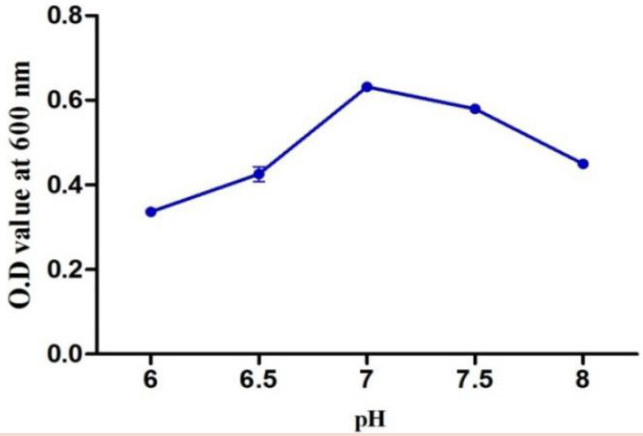

Figure 2: Effect of *pH* on growth of strain R2.

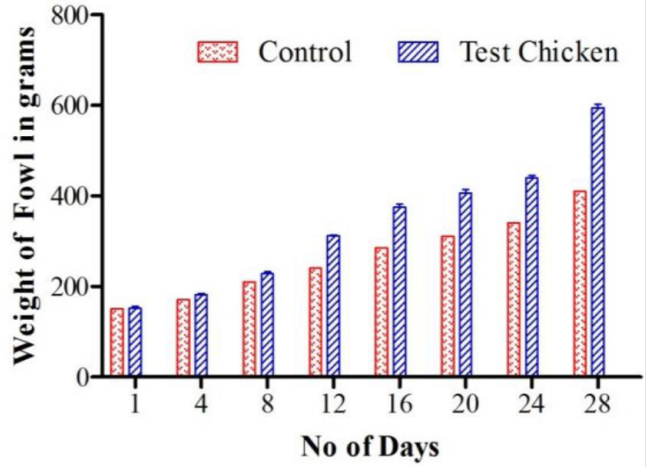

Figure 3: Effect of *Lactobacillus* sp. as a probiotic on the growth of Red jungle Fowls.

Effect of pH on bacterial growth

The bacterial culture r2 was grown in different pH namely 6, 6.5, 7, 7.5 and 8 were used it was found that *pH* 7 is favourable for the growth of *Lactobacillus* sp., so this *pH* is optimized for the growth of isolated bacteria. The optical density value of the broth was taken at 600 nm and a maximum growth of the *Lactobacillus* was obtained as 0.9 followed by 0.725 in pH 6.5. (Figure 2)

Probiotics to the Red jungle fowls

Three fowls were used as a test in the present study where two test fowls were administered with probiotics mixed with commercial feed whereas the control fowl was fed with commercial feed. On monitoring the growth analysis of all the fowls initially the growth was gradual in all the three fowls but after a period of 4 days the test chickens fed with probiotics had shown a better growth at an average of 226 g in test compared to 210 g in control. The growth had gradually increased in test fowls compared to the control and finally at the 28th day an average of 595 g which is nearly a half kilograms whereas in control the weight was only 410 g. As the probiotics fed chickens showed a better growth compared to the control fowl, bacterial probiotics could be recommended to the fowl hatcheries (Figure 3).

DISCUSSION

The beneficial effects of probiotics may be mediated by direct antagonistic effects against specific treatment of organisms, resulting in decrease in number or by an effect on their metabolism or by synthesis of some essential nutrients or by stimulation of immunity. The present study deals with the isolation of *Lactobacillus* from the digestive tract of Red jungle fowls. This bacterium is optimized with different growth conditions in the lab experiments. The isolated bacterium was biochemically identified and it is administered to fowls to determine the effect of probiotics in growth improvement. The mode of action of probiotics was proposed and discussed by many workers.[8-9] A study proposed by Timmerman[10] that involved the collection of Digesta and tissue samples of the crop, small intestine and cecum from layer hens and broiler chickens and were stored in sterile, buffered peptone water.

Rise in feed and water consumption is recorded in laying hens fed with Liquid Probiotics Mixed Culture (LPMC) containing two type micro-organisms, *Lactobacillus* and Bacillus species.[11] Inclusion of probiotic caused no significant increase in feed consumption, egg production and egg weight (P>0.05).[12] Ramasamy[13] reported that supplementation of probiotic *Lactobacillus* cultures did not influence the Feed Intake (FI), egg production or egg mass of hens throughout the 48-week period. Zhang and Kim[14] reported an increase body in FI in chicken fed with multistrain probiotics compared with that in control group fed basal diet. However, feeding viable *Lactobacillus* at 1100 mg kg-1 (4.4 ×107 colony forming units (cfu) kg^{-1}) increased daily feed consumption, egg size, nitrogen and calcium retentions.[15] Yousefi and Karkoodi[16] reported feed consumption was not affected by the dietary probiotic supplementation. Shareef and Dabbagh[17] reported that probiotic (*Saccharomyces cervisiae*) supplementation of broilers had significantly increased feed consumption. Results from a study by Babazadeh[18] indicated that probiotics did not have any significant positive effect on broilers FI, Body Weight (BW) and Feed Conversion Ratio (FCR). Nikpiran[19] reported that Addition of Thepax and *Saccharomyces cerevisiae* significantly increased FI in Japanese quails. Song[20] reported significant increase in body weight gain in broilers fed with probiotics *Lactobacillus, Bifidobacterium*, coliforms and Clostridium species. Results from Kabir Rahman[21] indicated that the live weight gains were significantly ($P<0.01$) higher in birds supplemented with probiotics as compared to the control group at all levels during the period of 2nd, 4th, 5th and 6th weeks of age, both in vaccinated and

non-vaccinated birds. Zhang and Kim[14] reported an overall increase in body weight gain in chicken fed with multi-strain probiotics compared with that in control group fed basal diet. Huang[22] demonstrated that inactivated probiotics, disrupted by a high-pressure homogenizer, have positive effects on the production performance of broiler chickens when used at certain concentrations.

CONCLUSION

The isolated *Lacto bacillus* bacteria isolated from the digestive tract of the red jungle fowls gave promising results in the growth study performed. The results reveal that this bacterium can serve as a probiotic to chicken for their better growth and nourishment. The sensory valuation, quality of meat and other factors will be tested in the future study.

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ABBREVIATIONS

h: Hours, **MRS:** de Man, Rogosa & Sharpe, **rpm:** rotates per minute, **g:** grams.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUMMARY

- *Lactobacillus* sp., was effectively isolated from red jungle fowls
- The bacteria was optimized for growth in varying temperature and pH
- The bacteria was scaled up and the biomass was found effective in growth of fowls when provided as a feed.

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