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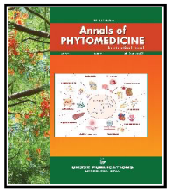

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Probiotic efficacy and antibacterial activity of *Azadirachta indica* A. Juss. leaf endophytes on broiler performance and health

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Abstract

This study explored the use of endophytic bacteria derived from *Azadirachta indica* A. Juss. leaves as probiotics for broiler chickens. The purpose of the study was to compare their effects with those of standard probiotics and a control group on growth performance and resistance to *Salmonella typhimurium* infection. A total of 72 broiler chickens were randomly divided into three experimental groups, control (T1), standard probiotic (T2), and endophytic bacteria (T3). Weekly observations of body weight, feed consumption, and food conversion ratio were recorded for the birds in all treatment groups over the course of the 28-day experimental period. The gain in body weight, feed intake consumption, and FCR were significantly better in the T2 and T3 groups compared to control group, especially during the third week. During the 21st and 28th days, the fecal bacteria counts significantly decreased in the T2 and T3 groups. Compared to the control group, which showed typical *Salmonella typhimurium* symptoms, the T2 and T3 groups developed less severe symptoms and were not associated with mortality. The present study demonstrated that the potential of endophytic bacteria in *A. indica* leaves may be exploited as probiotics in broilers, affirming similar performance benefits to standard probiotics. This can suggest that the endophytic bacteria could become an alternative for antibiotic growth promoters in poultry farming, hence addressing concerns around antibiotic resistance while improving bird health and productivity.

1. Introduction

Endophytic bacteria are classified as endosymbionts, which can live in the host plant's internal cells with (or without) any harm or disease symptom development by its presence (Schulz and Boyle, 2006). All the species of plants on earth contain one or more strains of endophytes (Strobel *et al.,* 2004). There are different pathways by which these bacteria can enter the plant roots (White *et al.,* 2018) and they exist in flowers, leaves, roots seeds as well as stems of plants (Elmagzob *et al.,* 2019). Thus, endophytic bacteria protect plants by producing antimicrobial compounds (lipopeptides, bacillomycin, and 2-4 diacetylphloroglucinol) as well enzymes such as chitinase protease while volatile organic compounds also have the ability to antagonize biofilm formation in plant pathogens (Glick, 2012; Hernández-León *et al.,* 2015; Santoyo *et al.,* 2012). They are considered to be attractive because they possess these activities as antimicrobial, antiviral, and antitumor (Toral *et al.,* 2018). On the other hand, endophytic bacteria also help plants to acquire needed nutrients and regulate phytohormones levels in plant (Calvo *et al.,* 2017).

The *A. indica* tree belong to Meliaceae family, is native to the Indian subcontinent. All parts of the *A. indica* tree have been used in traditional Indian medicine. *A. indica* contains bioactive secondary metabolites limonoids (tetranortriterpenoids), especially azadirachtin (Khanam *et al.,* 2017), that are of great interest in understanding their roles in improving animal performance by modulating the overall physiological and reproductive status of animals, respectively (Wylie and Merrell, 2022). Unfavorably to regular chemotherapy drugs, *A. indica* is pharmacologically proven as an antibacterial, antiviral (Khan *et al.,* 2020), antifungal and antiprotozoal agent. It is accessible promising anticancer properties, hepatoprotective activity at low-toxic doses commonly used in folk medicine also without adverse side effects on liver functions human use excluded so far (Alzohairy, 2016). In broilers, recent works have demonstrated benefits with *A. indica* formulations by providing better weight gain and feed efficiency as well as mortality reduction, and increase immunity that had been synchronized from previous systemic exposure of such active phyto-stuffs to induce partial acclimatization (Deka *et al.,*2019; Ubua *et al.,*2019). *A. indica* is also supplemented as poultry feed ingredient because of its antibacterial, hypocholesterolemic, and antioxidant, hyperglycemic effects (Lin *et al.,* 2003; Debasish and Nath, 2020; Deka *et al.,* 2021). Commonly used in India, *A. indica* leaf extracts

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has been shown to have antibacterial against *Escherichia coli*, *Pseudomonas* spp., Staphylococcus, and *Streptococcus* spp., strains followed by several fungi (Koona and Budida, 2011; Deka *et al.,* 2021)

In the modern broiler industry, feed utilization efficiency is a primary focus for enhancing economic outcomes. Poor growth performance in broiler birds is often caused by pathogenic microorganisms, leading to a range of diseases that impact growth and overall production efficiency. To prevent these microorganisms, low or sub-therapeutic doses of antibiotics, known as antibiotic growth promoters (AGPs), are commonly used. However, the use of AGPs in broilers raises concerns about antibiotic residues in meat, contributing to the entry of these antibiotics into the food chain and the development of antibiotic resistance. Growing awareness of antibiotic resistance has led many countries to impose strict bans on antibiotic-derived growth enhancers in poultry farming. Consequently, there is a growing need to focus on alternative methods for improving poultry health, with probiotics being a promising option. Probiotics can enhance poultry growth and health through various mechanisms. These mechanisms include improving nutrient absorption. Probiotics also increase nutrient availability to the host by altering the intestinal microflora. They reduce harmful gut bacteria. They decrease the production of toxins or metabolites that suppress growth. Endophytic bacteria have been identified as potential "plant probiotics" due to their ability to reconstruct the microbiome. They also improve crop yields (Jayakumar *et al.*, 2020). Bacterial diseases are widespread in India which leads in substantial economic losses in the poultry industry due to high rates of morbidity and mortality. The use of antibiotics to treat these diseases often contributes to the emergence of multidrug-resistant (MDR) bacteria. *A. indica* leaf extract, known for its antibacterial properties (Akhter and Sarker, 2019), does not pose the issue of drug resistance. As a result, *A. indica* leaf extract could be considered a viable alternative to antibiotics for managing MDR bacterial infections. Considering the above beneficial effect, the present study was designed with the objective of isolating endophytic bacteria, sourced from *A. indica* leaves. The study also investigated their probiotic effects on broiler bird's body weight, FCR food consumption and, against *Salmonella typhimurium* infection.

2. Materials and Methods

2.1 *A. indica* leaves collection

Fresh leaves of *A. indica* were procured from the Department of Botany J.N.K.V.V., Jabalpur. Five leaf samples were taken from the tree, which divided into five sub-samples. The collected samples were processed within 24 h for the isolation of endophytic bacteria.

2.2 Sterilization of *A. indica* leaves

The plant samples underwent a thorough sterilization process to eliminate surface contaminants. First, they were briefly rinsed with double-distilled water. Then, they were immersed in a dilute sodium hypochlorite solution for 5 min, followed by another rinse. Next, the samples were treated with a fungicide (bavistin) and soaked in distilled water. After another rinse, the leaves were treated with ethanol (70%) and given a final soak in double-distilled water. To maintain sterility, the leaves were cut inside a laminar air flow chamber, where they were also allowed to air-dry. This multi-step surface sterilization procedure helps to ensure that only true endophytic microorganisms, rather than surface contaminants, were isolated in subsequent steps (Mahajan *et al*., 2014).

2.3 Verification of sterilization

To confirm the effectiveness of sterilization process, we employed two verification methods. In the first method, we took 1 ml of the final rinse water and spread it on nutrient agar plates. These plates were then incubated at room temperature for 24 h to see if any bacteria would grow. As an additional check, we rolled the surface-sterilized leaf segments directly on nutrient agar plates, which were similarly incubated. After 24 h, we examined both sets of plates for bacterial growth. The presence of bacterial colonies would indicate that the sterilization process was not completely effective, while the absence of growth would suggest successful surface sterilization. This two-pronged approach helps us to ensure that any microorganisms isolated in subsequent steps are truly endophytic and not surface contaminants.

2.4 Media preparation and sterilization

The agar was added to three different types of media: King's B (KB) media, Mueller-Hinton media, and blood agar media. These were all prepared using distilled water. To ensure thorough mixing, the media were heated on a hot plate while being stirred. To sterilize the media and prevent any contamination, they were then autoclaved. The autoclave process involved heating the media to 121°C for 15-20 min under 15 pounds of pressure. This sterilization step is crucial for maintaining the purity of bacterial cultures in subsequent experiments (Schaad, 1980).

2.5 Inoculation of leaves

The sterilized media were plated into different autoclaved Petri dishes, after which plant leaves were inoculated into them. The plates were incubated at room temperature 37°C for 24 h. Bacteria were characterized according to their morphology and staining through Gram staining. Inoculation was made from a single colony of the isolated bacteria to BHI broth media, further incubated at room temperature (37°C) for 24 h (Mahajan *et al*., 2014).

2.6 Isolation of endophytic bacteria

To isolate and purify the endophytic bacteria, first took a small amount of the bacteria-containing BHI broth and streaked it onto fresh blood agar plates. These plates were then incubated at room temperature for 24 h. After incubation, individual bacterial colonies were selected and transferred to new BHI broth tubes, which were again incubated under the same conditions. To ensure the purity of these cultures, performed Gram staining was performed. Once confirmed as pure, these bacterial cultures were preserved at –20°C for further use in experiments (Zinniel *et al*., 2002).

2.7 *In vivo* experimentation

The study consisted of three main groups with a total of 72 birds. Group T1 served as the control group and was divided into three replication with 8 birds each. The group T2, which received standard probiotic feeding, was also divided into three replications. The group T3, which was administered endophytic bacteria orally, comprised three replications as well with 8 birds each. This study was approved by institutional animal ethics committee (No. 130/iaec/vety)

2.7.1 Preparation of suspension for endophytic bacteria

Henric and coworker (1956) described method was used for determnination of concentration of endophytic bacteria through Mc-Farlands nephlometer.

2.7.2 Dose and route

One ml of endophytic bacterial culture containing 3.0×10^9 cfu/ml as determined by Mc-Farlands nephlometer was given to the treatment group of birds for 28 days. On the 28th day 8 birds from each group were challenged with 1 ml *Salmonella typhimurium* bacterial culture containing 3.0×10^9 cfu/ml as determined by Mc-Farlands nephlometer.

2.7.3 Clinical symptoms

The experimental birds of the three groups challenged with 1 ml *Salmonella typhimurium* bacterial culture were carefully examined for the clinical symptoms following oral inoculation of *Salmonella typhimurium*. The morbidity and mortality rate in the groups challenged with 1 ml *Salmonella typhimurium* bacterial culture was recorded.

2.7.4 Assessment of treatment effect *in vivo* study

During the course of 4 weeks study, body weight and feed intake was recorded weekly to assess growth of birds. Feed Efficiency Ratio (FCR) was calculated weekly based on the bodyweight gain over feed intake. To monitor the bacterial population dynamics, rectal swabs were taken and the total viable count (TVC) of bacteria was enumerated on 0,7th, 14th, 21st and 28th day. Effect of Endophytic bacteria was assessed against *S. typhimurium* infection in broiler birds on the 28thday. Additionally, a post-mortem examination was conducted on any birds that died during the experimental period.

2.7.5 Total viable count

To assess bacterial populations, fecal samples collected from six randomly selected birds in each group. These samples were collected in sterile containers, homogenized using a sanitized blender, and diluted by mixing 1 gram of feces with 10 ml of sterile water. The samples were then further diluted up to six fold (Fawole and Oso, 2001). To count viable bacteria, 0.1 ml of each diluted sample was cultured on nutrient agar plates using the pour plate technique. These plates were then incubated for 24 h at 37°C. The resulting bacterial colonies were counted and reported as colony-forming units per gram of feces (CFU/g). This method allows for a standardized approach to quantifying fecal bacteria across different treatment groups.

2.7.6 Statistical analysis

The data were presented as means ± standard error (SE). Statistical analysis was conducted using SPSS version 20. ANOVA, followed by Duncan's Multiple Range test was used for multiple comparisons. Statistical differences were determined at the 5% level of significance.

3. Results

3.1 Identification of isolated endophytic bacteria

3.1.1 Culturing of endophytic bacteria

The growth characteristics of endophytic bacteria isolated from *A. indica* (neem) when cultured on Kings B media (Figure 1) provided detailed information on the colony morphology for each isolate, including form, elevation, margin, surface texture, opacity, and chromogenesis. Isolates exhibited a variety of colony shapes, with most being irregular in form, while a few were circular. The elevation of the colonies varied, with some being flat and others raised. The margin of the colonies was predominantly undulated, though some had entire margins. Surface textures were mostly rough, with occasional smooth and dull textures. The majority of the isolates were opaque in opacity, with one isolate displaying a glistening appearance. Most isolates did not produce pigments (chromogenesis absent), except for isolate one, which showed green pigmentation.

Conclusively, 84% had an endophytic bacterium of irregular shape, while 16% were rounded. Concerning elevation on petri plates, 64% had flat elevations and 36% had raised elevations. In a similar fashion, 72% of the colonies presented undulate margins, while 28% of them had entire margins. A surface texture that was rough in 72% of the growth contrasted with being smooth in 16% and glossy in 12%. Furthermore, 96% of such growth was of an opaque color. All the isolates identified were of a non-haemolytic and non-chromogenic nature.

Overall, the characteristics suggest a high level of diversity in colony morphology among the endophytic bacteria isolated from *A. indica* leaves. These bacteria are generally non-chromogenic and non-hemolytic, indicating their potential non-pathogenic nature and possible applications in probiotic development.

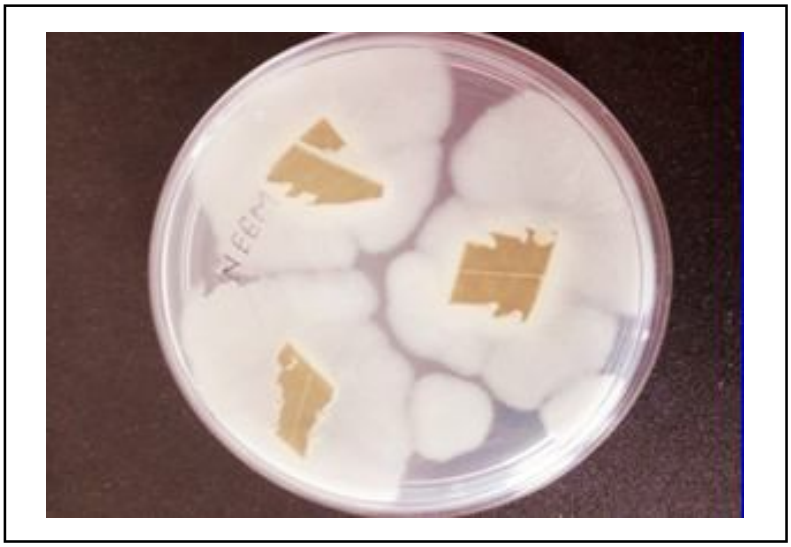

Figure 1: Growth of endophytic bacteria from leaves of *A. indica* on kings B media.

3.1.2 Cultivation morphology of endophytic bacteria on sheep blood enriched medium

The bacteria, originally streaked onto King's B medium, were swabbed onto a different, more selective agar containing ovine blood. These plates were incubated for a full 24 h at 37°C temperature in incubator. The growth characteristics of bacteria from *A. indica* were examined thereafter (Figure 2). The bacteria exhibited various forms, elevations, margins, surfaces, opacities, and chromogenesis. Most of the bacteria had an irregular form with raised elevation, undulated margins, smooth surfaces, and opaque opacity, and do not produce chromogenesis. A few bacteria displayed a circular form with similar raised elevation, undulated margins, and smooth surfaces, with one showed a glistening surface. Some are notable for having a flat elevation and an entire margin with a rough surface. Notably, one strain was the only one exhibited green chromogenesis, while the rest do not show any chromogenesis. This variation highlights the diversed morphological and growth patterns of endophytic bacteria from *A. indica* on sheep blood agar.

Conclusively, the results showed that 76% of the growth was irregularly shaped whilst 34% were circular. In the case of elevation on petri plates 84% had raised elevation, while 16% had flat elevation. Regarding colony margins, 88% had undulated margins, while 12% of aforementioned showed entire margins. Growth surface texture was smooth in 92% and rough in 8%. Additionally, 96% of isolates that showed opaque growth. None of the isolated strain exhibited hemolysis or chromogenic activity.

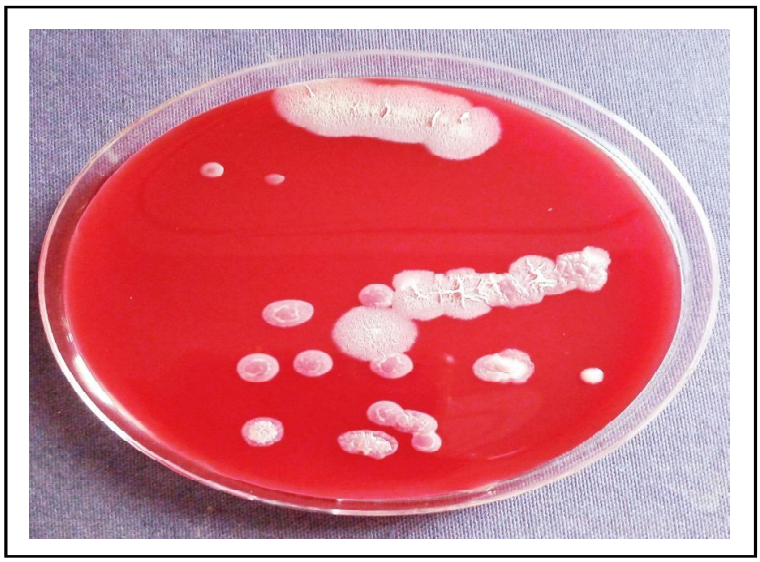

Figure 2: Growth of endophytic bacteria from leaves of *A. indica* on 5 per cent sheep blood agar.

3.1.3 The growth characteristics of endophytic bacteria in brain-heart infusion broth.

Endophytic bacteria were cultivated on blood agar plates. The resulting colonies were transferred to sterile brain-heart infusion (BHI) broth. These samples were incubated for a day at room temperature (37°C) to promote bacterial growth. All bacteria exhibited turbidity, indicating growth throughout the broth. Most bacteria also showed flocculant presence and pellicle formation but no sediment or ring formation. However, some bacteria did exhibit ring formation. Additionally, certain bacteria formed sediment at the bottom of the broth. This data highlighted the varied growth patterns and characteristics of endophytic bacteria in BHI broth, showcasing differences in physical manifestations such as turbidity, flocculant presence, pellicle formation, sediment, and ring formation.

Conclusively, all of the isolates showed 100% turbidity in broth culture. Flocculant growth was observed in 92%; however, all of the isolates (100%) formed a pellicle on the surface of broth. While 88% isolates did not show any sediment formation, 52% showed ring formation.

3.1.4 Examination of endophytic organism in microscope

Gram staining was used to study the endophytic bacteria under a microscopic regime (Figure 3). Most of the bacteria were Gram-positive bacilli, indicating they were rod-shaped and reacted positively to Gram staining. A few exceptions included some Gram-negative bacilli and cocci, which were spherical in shape. The majority of the bacteria were categorized as "<1", indicating fewer specific traits, while a few were marked as "1", indicating the presence of certain traits. Notably, there were a few Gram-negative cocci and bacilli among the primarily Gram-positive bacilli population. This diversity highlighted the variation in cell wall structure and morphology among the isolated endophytic bacteria from neem.

The results indicated that the majority of isolates were Gram-positive, accounting for 88%, while only a few represented Gram-negative, accounting for 12%. In relation to the cell shape, it was depicted that rod-shaped bacterium dominated, accounting for 88% of the endophytes while the cocci represented 12%. From the microscopic study, it was shown that most of the isolated isolates 84% contained different types of endophytic bacteria.

3.2 Body weight and feed consumption

The broilers' body weight and feed consumption with respect to different treatments are shown in Table 1 and Figure 4. There was progressive increase in body weight from the 1st to the 4th week.

However, only groups probiotics treated birds (T2) and endophytic bacteria treated birds (T3) showed significant (p<0.05) weight gains in the 3rd week; in the other weeks, no significant differences were noted compared among all groups. Feed consumption also showed a progressive increase with the experiment. In the 2nd and 3rd weeks, the increases in feed intake were significant (p<0.05) in both groups T2 and T3. Feed intake during the 1st and 4th weeks did not show any significant differences.

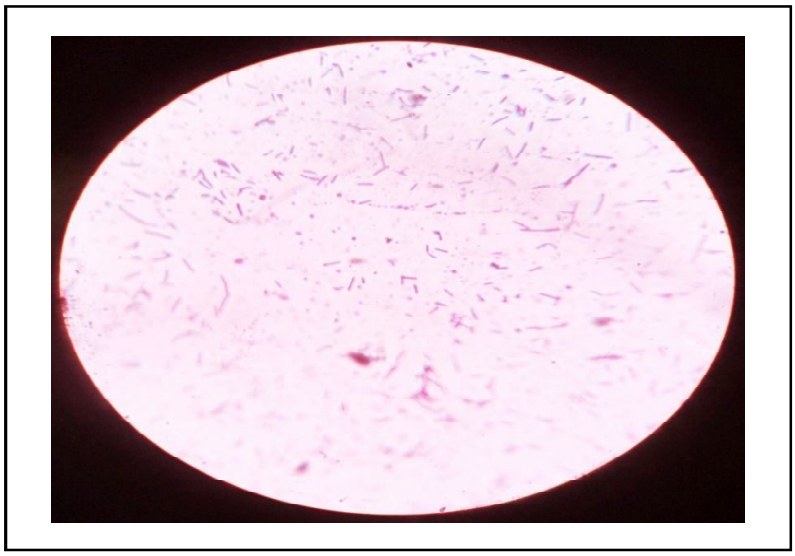

Figure 3: Grams staining of endophytic bacteria from leaves of *A. indica* on 5 per cent sheep blood agar.

Table 1: Effect of endophytic bacteria from *A. indica* on weight gain g/bird of birds

Week	Control (T-1)	Probiotics (T-2)	Endophytic bacteria (T-3)
1st	99 ± 4.04	98 ± 1.86	99 ± 2.96
2nd	275 ± 5.69	281 ± 8.19	287 ± 2.52
3rd	437[b] ± 6.44	468[a] ± 10.99	459[a] ± 7.57
4th	490 ± 1.86	491 ± 3.61	498 ± 5.51
Total	1301 ± 3.12	1338 ± 4.26	1343 ± 6.89

Values (Mean ± SE) differ in superscripts indicate significant (p<0.05). Read data row wise.

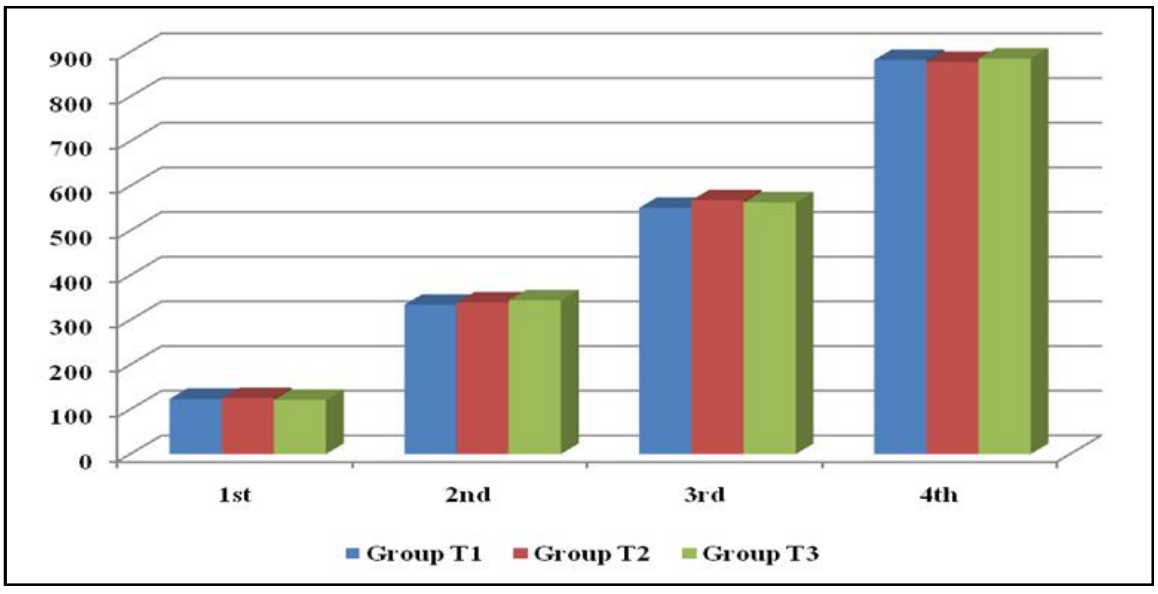

Figure 4: Feed intake (g/day) in different groups of birds.

3.3 Feed conversion ratio (FCR)

Result for FCR among different broilers group showed (Figure 5) that during the initial weeks, FCR values were not showing any significant differences amongst groups. However, in the 2nd and 3rd weeks, probiotics treated birds, T2 and T3 (endophytes bacteria treated) birds revealed enhanced feed conversion efficiency compared to the control group. FCR values increased in all of the groups in the 4th week. In general, these groups with treatments of probiotics and bacteria endophyte manifested better feed conversion efficiency than the control group, especially during the middle weeks of the experiment.

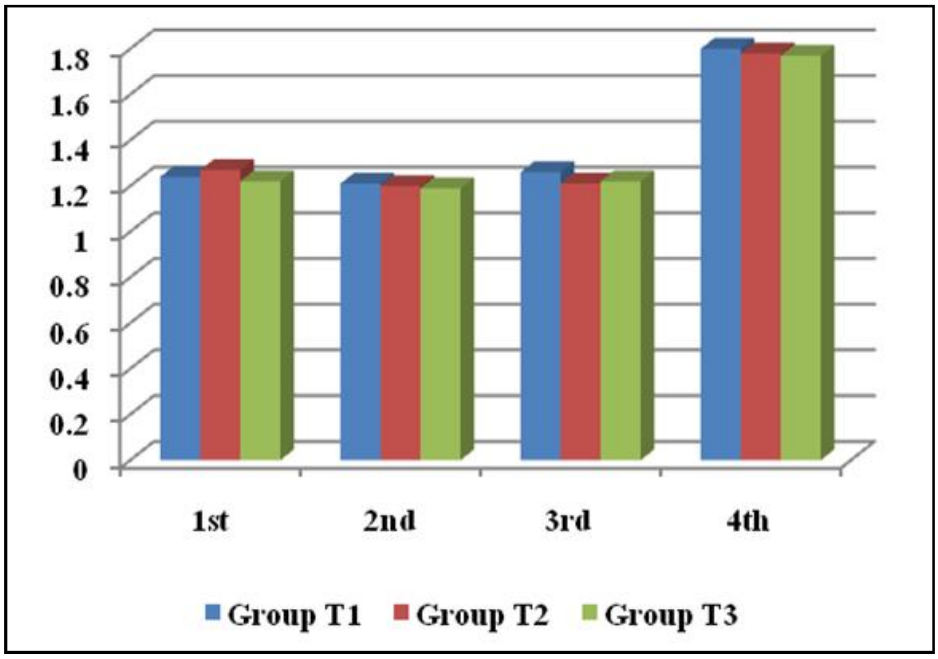

Figure 5: Feed conversion ratio of experimental birds of different groups.

3.4 Total viable count

Fecal samples from broiler birds were collected throughout the experimental period at the commencement of 0, 7, 14, 21, and 28 days of age for total viable counts (Figure 6). The result shown indicated that the total viable count decreased on a weekly basis in both group T2 and group T3. The control group showed no notable changes in fecal bacteria levels throughout the study. In the first two weeks, all groups had similar fecal counts. However, by the third and fourth weeks, the groups receiving probiotic treatments showed a significant reduction in fecal bacteria compared to the control group (Table 2).

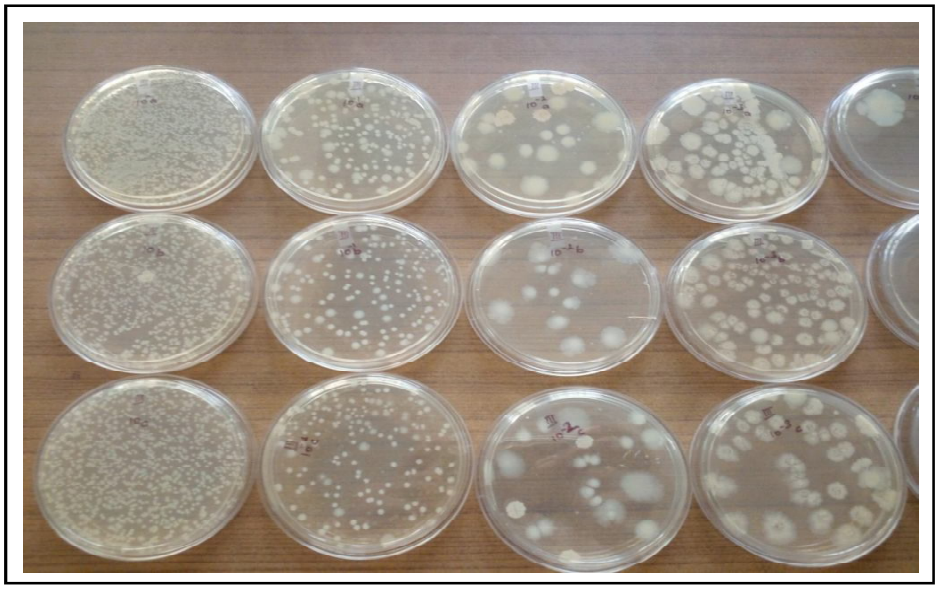

Figure 6: Total viable count of bird fecal sample.

Table 2: Effect of endophytic bacteria from *A. indica* on total viable counts in birds faeces (cfu/g)

Day	Group T1	Group T2	Group T3
0	$0.66 \pm 0.04 \times 10^7$	$0.65 \pm 0.04 \times 10^7$	$0.65 \pm 0.02 \times 10^7$
7	$0.71 \pm 0.07 \times 10^7$	$0.68 \pm 0.02 \times 10^7$	$0.7 \pm 0.01 \times 10^7$
14	$0.74 \pm 0.03 \times 10^7$	$0.69 \pm 0.02 \times 10^7$	$0.67 \pm 0.02 \times 10^7$
21	$0.73^b \pm 0.03 \times 10^7$	$0.64^a \pm 0.02 \times 10^7$	$0.62^a \pm 0.01 \times 10^7$
28	$0.70^b \pm 0.02 \times 10^7$	$0.64^b \pm 0.01 \times 10^7$	$0.63^a \pm 0.01 \times 10^7$

Compare values raw wise, data with different superscripts differ significantly ($p<0.05$).

3.5 *In vivo* antibacterial activity effect of endophytic bacteria on broiler birds

On the 28th day of experiment, 8 birds each were randomly selected from group T1, group T2, and group T3. *S. Typhimurium* infection was made orally in these broiler birds. The birds were kept for observation for a week for the symptoms of *Salmonella* infection and any type of mortality. In group T1, 6 birds out of 8 showed signs of salmonellosis infection; three birds died due to this infection. In group T2, 3 birds out of 8, worked in showing signs of salmonellosis infection with no mortality recorded. The same case was replicated in group T3, whereby from the 8 birds, 4 showed signs of salmonellosis infection with no mortality recorded.

4. Discussion

The morphology observed in many endophytic bacteria cultured on Kings B medium underlines the richness of the microbial community diversity inside *A. indica* leaves. The dominance of irregular forms with undulated margins could be indicative of adjustments to the internal environment of the plant. This diversity described different colony morphologies among endophytes isolated from plants with medicinal properties (Ek-Ramos *et al*., 2019). Growth of endophytic bacteria isolated from *A. indica* leaves was found to be good in King's B medium, a medium generally used for the cultivation of *Pseudomonas* species, which are common endophytes (Lugtenberg and Kamilova, 2009). Growth of these *A. indica* leaf endophytes in this medium may indicate that they could be primarily from the genus *Pseudomonas* or other related genera of Gram-negative bacteria. Characteristics of growth, regarding colony shape and color or production of pigments, were the initial indications of taxonomic identity.

Endophytic bacterial isolates from *A. indica* leaves exhibited variations in their growth on 5% blood agar of sheep. Most of the isolates showed nonhemolytic, opaque, and smooth surface colonies. This also confirms that most such endophytes are not pathogenic in nature since hemolytic activity can often be implicated as one of the virulence factors for bacteria (Bhakdi and Tranum-Jensen, 1991). The fact that most of the isolates did not depict hemolysis is favorable for potential probiotic applications, considering the safety criteria set forth in recent guidelines for probiotics (Binda *et al*., 2020). Lack of chromogenic activity further suggests that these endophytes do not produce any pigments that may stain or discolor the growth medium. These observations are typical of the normally innocuous nature of endophytic bacteria, generally not causing harm to host.

Growth features of *A. indica* leaf endophytes on BHI broth are like turbidity, flocculation, pellicle formation, and sediment production, which reflect their physiology and metabolic features; for example, turbidity may stand in place of intense cell proliferation, while pellicle may suggest the ability to form surface-attached biofilms (Costerton *et al*., 1995). Such growth patterns are common to a diversity of bacterial genera and can be further explored in understanding the ecological roles and potential applications of these endophytes. The dominance of Gram-positive bacilli among the isolates concurs with recent studies on plant endophytes. Usually, Gram-positive bacteria, in particular *Bacillus* and such related genera are reportedly common endophytes in many plant species (Reinhold-Hurek and Hurek, 2011; Frank *et al*., 2017). The occurrence of some Gram-negative isolates and the presence of cocci form further corroborate that there is diversity in the bacterial community inhabiting *A. indica* leaves. This diversity might be useful in contributing towards the medicinal properties displayed by this plant and provides a variety of potential probiotic candidates (Singh *et al*., 2017; Deka *et al.,* 2021).

After Gram's staining, microscopic examination revealed that most of the isolated endophytes were Gram-positive, rod-shaped bacteria. This is in agreement with the general trend of *Bacillus* and related spore-forming genera as common endophytes in plants (Hardoim *et al*., 2015). The presence of a small proportion of Gram-negative, coccoid endophytes suggests diversity in the bacterial community within *A. indica* leaves. Further taxonomic identification by molecular techniques would be required to precisely define the endophytic bacterial species.

In the third week of the experiment, chickens given either standard probiotics (T2) or *A. indica* leaf (T3) endophytic bacteria supplements showed greater increases in body weight and consumed more feed than the control group (T1). This difference was statistically significant. The results thus obtained proved that applied probiotic treatments, including endophytic bacteria, had positive influence on the growth performance of broiler chickens (Gaggìa *et al*., 2010; Olnood *et al*., 2021). Specifically, the apparently equal performance of the endophytic bacteria to the standard probiotics is very promising and warrants their potential as novel, plant-derived probiotics for poultry. The improved feed conversion ratio among the T2 and T3 groups similarly proves the positive effect of supplementation with the probiotics. Improved FCR in T2 and T3, particularly during the third week, indicates that both standard probiotics and *A. indica* endophytes improve feed efficiency. This perhaps is due to an improvement in digestion of nutrients and their absorption-possibly from beneficent modulation of gut microbiota. Similar improvements in FCR with probiotic supplementation in broiler chickens had been reported (Jha *et al.,* 2020). Similar findings on increased body weight from neem leaf use were reported (Abujradah *et al.,* 2018; Kanwal *et al.,* 2022). The weight gain in groups T2 and T3 may be due to the reduction in pathogenic organisms, as indicated by total viable count. Inhibiting pathogenic

microbes enhances nutrient availability, and endophytic bacteria may function as probiotics. Probiotics can alter intestinal microbiota and the immune system, reducing pathogen colonization. These endophytic bacteria are promising antibiotic alternatives as the pressure to eliminate growth-promoting antibiotics rises. The intestinal microbiota, epithelium, and immune system collectively resist enteric pathogens, a phenomenon referred to as bacterial antagonism, bacterial interference, barrier effect, colonization resistance, or competitive exclusion (Shabani *et al.,* 2012).

What is noteworthy in this case is that the protective effect of *S. typhimurium* infection by endophytic bacteria was identically efficient as that exerted by standard probiotic treatments. Birds from groups T2 showed milder clinical symptoms and did not register mortality until the end of the experiment, thus supporting the assumption that either these endophytic bacteria may have antimicrobial properties or they enhance the immune response of the bird. Some probiotic strains have been shown to protect poultry against *Salmonella* infection through competitive exclusion and immune modulation (Wang *et al*., 2020). This protective effect was already seen with the *A. indica* endophytes, hence suggesting comparable antimicrobial or immunomodulatory properties that give rise to new avenues as natural antibiotic alternatives in poultry.

These results suggest that endophytic bacteria, similar to conventional probiotics, have the potential to affect broiler resistance to Salmonella infection by competitive exclusion, production of antimicrobial compounds, and modulation of the host response to infection (Cutting, 2011; Lutful Kabir, 2009). Concretely, this study also provides results showing that *A. indica* leaf endophytes could be important alternative probiotic candidates in poultry production.

5. Conclusion

Present study suggests that endophytic bacterial isolates from *A. indica* leaves may be potential probiotics for broiler chickens. These bacteria also improved growth performance, feed efficiency and resistance to *S. typhimurium* infection in the current study. These benefits were similar to those provided by standard probiotics, leading to speculate that the endophytic bacteria might one day be used as a natural alternative for antibiotic growth promoters in poultry industry. Interesting findings, suggested that the possible utilization of *A. indica* derived endophytic bacteria as a naive approach to address antibiotic resistance issues in poultry sector yet their complete potential and applications need further exploration.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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