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Research Article

Isolation of *Lactobacillus* sp. from Indigenous poultry (*Gallus gallus*) and their antagonistic activity against bacterial pathogens

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Abstract

Probiotics are extensively utilized in the poultry and livestock industry due to their advantageous properties, including their ability to enhance growth performance. These probiotics have a positive impact on the physiology of the host animals by modulating their immune system, averting pathogens, and regulating micro-ecological imbalances. The effectiveness of *Lactobacillus* sp. as an antibacterial agent in anaerobic conditions, specifically within the gut, was the focus of this study. In this study, fifteen samples were collected from the caecum of indigenous poultry, and *Lactobacillus* strains were isolated using selective agar. The isolates (n=10) were confirmed as *L. brevis* (2), *L. coleohominis* (1), *L. mucosae* (1), *L. reuteri* (4), and *L. vaginalis* (2) using both genotypic and phenotypic methods. All isolates were mesophilic in nature except two which were able to grow at 15°C. *Lactobacillus* isolates demonstrated a remarkable ability to suppress the growth of seven distinct bacterial communities, indicating their potential as antagonistic agents. This suggests that specific strains of *Lactobacillus*, such as those identified in the study, could be beneficial as probiotics.

Keywords: Antagonistic Activity, *Lactobacillus*, Probiotics, Sugar fermentation patterns

Highlights

- *Lactobacillus* spp. isolated from poultry caecum have potential probiotic properties.
- These bacteria (mutualistic *Lactobacillus* spp.) exert all beneficial properties in *in-vitro* study.
- They checked the growth of other pathogenic flora in an *in-vitro* study.

INTRODUCTION

The word "Lactobacilli" refers to members of the LAB, a diverse group of Gram-positive coccobacilli, or rod-shaped, catalase-negative, Gram positive, non-spore-forming, non-motile, anaerobic, or aerotolerant bacteria that are distinguished by the production of lactic acid as the primary byproduct of their fermentation of carbohydrates (Raheem *et al*., 2021; Al-yami *et al*., 2022). The current taxonomic hierarchy for *Lactobacillus* is as follows: Domain: Bacteria, Phylum: Firmicutes, Class: Bacilli, Order: Lactobacillales, Family: Lactobacillaceae, Genus: *Lactobacillus* (Al-yami *et al*., 2022). The lactobacilli are commonly isolated from animal feed, soil, and vertebrate and invertebrate creatures (Raheem *et al*., 2021). They normally reside in the urinary system (Raheem *et al*., 2021), genitalia, oral cavity, and digestive tract of mammals (Al-yami *et al*., 2022). The Food and Drug Administration (FDA) classifies lactobacilli as generally recognized as safe (GRAS) as beneficial bacteria are found in large quantities in foods like cheese and yogurt and do not make people sick. *Lactobacillus* has been extensively studied for its potential use as probiotics in various animal species, including broiler chickens. Few lactobacilli, such as *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus plantarum*, where glucose metabolism is known as homo-fermentative, lactic acid is the principal byproduct and makes up at least 85% of the final metabolic products. However, in other species, such as *Lactobacillus brevis* and *Lactobacillus fermentum*, the metabolism of glucose is hetero-fermentative; lactic acid makes up about half of the metabolic byproducts, with ethanol, acetic acid, and carbon dioxide making up the other half (Abedi and Hashemi, 2020). Some hetero-fermentative *Lactobacillus* spp. need to utilise other forms of organic molecules, including galactose, malate, or fructose, for

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energy since they are somewhat inefficient at metabolising glucose (Wang *et al*., 2021). Probiotics are live bacteria that provide health advantages to the host when administered in adequate amounts (FAO, WHO, 2001). In broiler chickens, probiotics have been reported to improve growth performance, immune function (Sumarsih *et al*., 2014; Singh *et al*., 2017; Ye *et al*., 2021), and gut health, while also reducing the occurrence of pathogens (Dhama *et al*., 2011; Saiz *et al*., 2019). *Lactobacillus*, being naturally present in the intestinal tract of broiler chickens (Dec *et al*., 2014), is considered a promising candidate as a probiotic supplement (Abd *et al*., 2020; Zhang *et al*., 2021). However, not all strains of *Lactobacillus* have probiotic potential, and there is a need for characterization and evaluation of potential probiotic strains. This has led to increased research on the isolation, characterization, and evaluation of *Lactobacillus* Spp. as a potential probiotic in broiler chickens (Li *et al*., 2020). Broiler chickens play a major role in meeting the ever-increasing demand for poultry meat worldwide. However, the intensive production practices of broiler chickens have led to various health and welfare problems, including infectious diseases, poor feed utilization, and high mortality rates (Rajoka *et al*., 2018). The use of antibiotics has been a common practice to combat these issues, but concerns regarding the emergence of antibiotic-resistant bacteria have led to stringent regulations and restrictions on their use (Raja *et al*., 2009; Jothi *et al*., 2012). Therefore, there has been a growing interest in alternative approaches, such as the use of probiotics, to promote the health and productivity of broiler chickens. Several initiatives have been made in recent years to identify specific microbial combinations that could protect chickens from *E. coli*, *Salmonella enteritidis*, *Klebsiella oxytoca*, *Serratia* sp., *Citrobacter* sp., *Staphylococcus aureus*, and *Bacillus cereus* infection (Yu *et al*., 2011; Singh *et al*., 2017; Rezaei *et al*., 2021). Probiotics like *Lactobacillus* spp. have the potential to cease the mitigation of several pathogenic microflora within the GI tract (Jothi *et al*., 2012). Thus, the introduction of such beneficial flora can limit the pathogenicity of virulent bacteria and reduce the production cost as the use of antimicrobial substances becomes negligible. Moreover, the use of probiotics usually improves the gut health in poultry, which also reduces the production cost (Raheem *et al*., 2021). The present study was conducted to isolate and confirm *Lactobacillus* strains from local indigenous poultry and to detect their antagonistic activities against the panel of bacterial pathogens.

MATERIALS AND METHODS

Isolation of lactobacilli: Fresh caecum samples from each of fifteen apparently healthy backyard chicken birds (*Gallus gallus*) from various locations were aseptically collected in order to isolate *Lactobacillus* Spp. One gram of caecal content collected from each bird was homogenized in sterile normal saline [1:9 (w/v)]. 1 mL of this mixture was serially diluted, and appropriate dilution was plated into de Man, Rogosa and Sharpe (MRS) agar (HiMedia, India) and incubated at 37°C for 48 hours. The white, creamy, round, 0.5-2.5 mm sized, smooth and submerged colonies were randomly picked up (about 3-4 colonies/sample) and transferred into sterile MRS broth and incubated for 24 hours at 37°C and further sub-culturing was done until pure colonies were obtained. Tentatively identified pure colonies were stored in glycerol broth for future confirmation (Dumitru *et al*., 2023; Keresztény *et al*., 2023).

Morphological examination by negative staining and Gram staining: Each pure isolate was examined under the microscope by negative staining and Gram's staining for cell morphology. Gram positive rods were selected for further processing (Reuben *et al*., 2019).

Catalase activity: The fresh isolates were grown in MRS broth for 24 h, and 4-5 drops of culture were mixed with 4- 5 drops of 3% hydrogen peroxide (SRL India) solution. The slide was observed for the detection of effervescence production due to the liberation (Alrubaye *et al*., 2018).

Growth at different temperatures: The isolates were tested for their ability to grow in MRS broth at 15°C, 37°C and 45°C by incubating for 1 to 5 days. For this, 5 mL of MRS broth tubes were inoculated with 1% of lactobacilli isolate cultures. The development of turbidity in culture tubes was recorded as the ability of isolates to grow at 15°C, 37°C and 45°C and results were noted as positive or negative (Reuben *et al*., 2019; Mudawaroch *et al*., 2020).

Salt tolerance: A fresh culture of *Lactobacillus* isolates was inoculated in MRS broth with different NaCl concentrations (4.0% and 6.5%) and incubated at 37°C for 48 hours. The culture tubes were evaluated on visual inspection of turbidity for the presence or absence of growth, and results were noted as positive or negative (Vineetha *et al*., 2016; Ahmed *et al*., 2019).

Homo/hetero fermentation: Overnight grown test

cultures were inoculated at 5.5% (w/v) in MRS broth containing inverted Durham's tubes and incubated at 37°C. The gas production from glucose to detect homo or hetero fermentation nature was observed after 48 hours (Kavitha *et al*., 2016).

Arginine hydrolysis: For arginine hydrolysis, the culture was inoculated into arginine media (HiMedia, India) and incubated for 48 hours. The cultured media was mixed with 4-5 drops of Nessler reagent. The production of reddish-brown colour indicates positive for arginine hydrolysis (Arena *et al*., 1999).

Molecular identification of *Lactobacillus* genus: PCR amplification was carried out to confirm the isolates in a thermal cycler (Eppendorf, USA). The reaction mixture (20 µL) contained 25 pmol of each primer [(CTC AAA ACT AAA CAA AGT TTC (LbLMA1-rev) and CTT GTA CAC ACC GCC CGT CA (R16-1)], 0.2 mM of each deoxyribonucleotide triphosphate (GCC Biotechnology Limited, India), 1U PCR buffer without $MgCl_2$, 1.5, 2.0, 2.5 or 3.0 mM $MgCl_2$ depending on the experiment, 50-100 ng of bacterial DNA and 2.5 U of Taq DNA Polymerase (GCC Biotechnology Limited, India) (Dubernet *et al*., 2002).

Sugar fermentation patterns: A series of tests was deployed using CHL media as basal medium with bromocresol purple (BCP) to detect sugar fermentation ability of *Lactobacillus* spp. Four mL of the sterile medium and one sugar disc were taken in each tube aseptically. 0.1 mL of bacterial culture was added separately on each tube and incubated at 37°C fo 48 h, and the colour changes from purple to yellow were noted as positive, while constant colouration indicated negative results. However, in the case of Esculin sugar moiety, the colour change was purple to black for positive results, while no change in colour indicates negative as well. The isolates exert the biochemical property of hetero-fermentative lactobacilli and were subjected to a battery of specific sugar test for these group according to Bergey's Manual of Systematic Bacteriology, Vol. 2, 2007, (Facultative hetero-fermentative- Amygdalin, Gluconate, Raffinose, Xylose, Arabinose, Mannitol, Ribose, Cellobiose, Melezitose, Sorbitol, Esculin, Melibiose, Sucrose; Obligatory hetero-fermentative- Arabinose, Maltose, Raffinose, Xylose, Cellobiose, Mannose, Ribose, Esculin, Melezitose, Sucrose, Galactose, Melibiose, Trehalose) (Kavitha *et al*., 2016).

Antagonistic activity: For determination of inhibitory activity of *Lactobacillus* isolates on pathogenic bacterial species, the well diffusion assay was applied. Plates containing solidified nutrient agar were overlaid with soft nutrient agar (0.7% agar in nutrient broth) which was mixed with overnight fresh culture of (5 mL nutrient broth culture) individual bacteria 10 µL each (*E. coli*, *Salmonella enteritidis*, *Klebsiella oxytoca*, *Serratia marcescens*, *Citrobacter freundii*, *Staphylococcus aureus* and *Bacillus cereus*) separately, then it was spread by spread plate technique, allowed it to solidify. Then, wells were cut at a considerable distance (2 cm) of the agar plate measuring 6 mm in diameter, followed by 100 µL of the active culture of each *Lactobacillu*s poured into each well. The plates were incubated for 8-10 h at 37°C and examined for the inhibition zone. The diameter (mm) of clear inhibition zone including the wells was measured. The size of the clearing zone was assumed to be directly proportionate to the isolate's antagonistic activity (Yu *et al*., 2011; Singh *et al*., 2017; Rezaei *et al*., 2021). The antagonistic activity was evaluated against several pathogenic strains which were collected from different sources (*E.coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Klebsiella oxytoca* isolated from duck, cow, poultry and milk sample, respectively and obtained from the Department of Veterinary Microbiology, Belgachia; *Serratia marcescens*, *Citrobacter freundii* isolated from dairy products and obtained from the Department of Dairy Microbiology, Mohanpur, West Bengal University of Animal and Fishery Sciences).

RESULTS

Isolation of *Lactobacillus* spp.: Fifty-six bacteria were isolated from 15 caecal samples (n=15) obtained from healthy backyard poultry birds using a selective agar media plate (MRS-Agar). After negative staining and morphological, biochemical and microscopic analysis, 51 isolates appeared to be gram positive bacilli. The isolates number were reduced to 26 during sub-culturing and isolation of pure colony.

Gram staining property: Among the 26 presumptive pure isolates, only 10 were Gram-positive, i.e. violet in color, and catalase negative, evidenced by absence of bubbles during the catalase test. They were classified as bacilli because of their gram-positive characteristics, such as clusters or singles, indicating

Table 1. Details of 10 LAB isolates from backyard poultry caecum and their morphological and biochemical

Isolate no.	Isolate name	Source	Location	District	Colony morphology			Cell morphology by negative staining	Gram's staining	Catalase activity
					Shape	Colour	Size (mm)			
1	DH92	Indigenous hen	Chandan Nagar	Hooghly	Round	Creamy	1.5	Diplo, medium chain, thick rod	+ve	-ve
2	DC31	Indigenous cock	Bandel	Hooghly	Round	White	0.5	Single, diplo chain, thin, small rod	+ve	-ve
3	DH34	Indigenous Hen	Bandel	Hooghly	Round	Creamy	2.5	Single, diplo rod	+ve	-ve
4	DH33	Indigenous hen	Bandel	Hooghly	Round	White	0.5	Medium chain, small rod	+ve	-ve
5	DH171	Indigenous hen	Kanchrapara	Nadia	Round	Creamy	1	Single, diplo rod	+ve	-ve
6	DH182	Indigenous hen	Kanchrapara	Nadia	Round	White	1	Single, diplo, thin rod	+ve	-ve
7	DC91	Indigenous cock	Mohanpur	Nadia	Round	Creamy	1.5	Single, diplo, thick rod	+ve	-ve
8	DH173	Indigenous hen	Kanchrapara	Nadia	Round	Creamy	2.5	Single, diplo, medium chain, thin, small rod	+ve	-ve
9	DH163	Indigenous hen	Jaguli	Nadia	Round	Creamy	1.5	Single, diplo, thick rod	+ve	-ve
10	DH102	Indigenous hen	Mohanpur	Nadia	Round	White	1	Very small, thin, square rod	+ve	-ve

they were LABs (Table 1).

***In-vitro* probiotic property:** Then, 10 identified isolates were further screened for *in-vitro* probiotic properties. Despite the fact that all of these ten (10 nos.) *Lactobacillus* isolates had the ability to grow at a variety of temperatures (15°C, 37°C, 45°C), in this study they proliferated optimally at 37°C and also grew normally at 45°C, but at 15°C the isolates did not grow except for two (DC91, DH163) (Table 2). The results of this study indicated that the isolates were mesophilic. While undergoing a salt tolerance test, only two isolates (DH34, DH182) were found to be 6.5% NaCl tolerant; the rest of the isolates could withstand levels containing 0% and 4% salt. DH34 and DH182 isolates were capable of tolerating 4% and 0% salt concentrations during experimentation (Table 2). The Arginine hydrolysis test yielded that most of the isolates 70% (7/10) were positive, which can actively break arginine and produce post-action by-products. Only three (30%; DH92, DH171, DH102) of them were unable to yield arginine (Fig. 1) (Table 2). In homo-/hetero-fermentation, it was observed that all the *Lactobacillus* isolates were heterofermentative in

Table 2. Survival rate in different temperatures, salt concentrations, capability of Arginine hydrolysis and homo-/hetero- fermentation pattern of LAB isolates

Isolate no.	Isolate name	Temperature profiling			Salt tolerance			Arginine hydrolysis	Homo-/ Hetero-fermentation
		15°C	37°C	45°C	0%	4%	6.50%		
1	DH92	-	+	+	+	+	-	-	Hetero fermentative
2	DC31	-	+	+	+	+	-	+	Hetero fermentative
3	DH34	-	+	+	+	+	+	+	Hetero fermentative
4	DH33	-	+	+	+	+	-	+	Hetero fermentative
5	DH171	-	+	+	+	+	-	-	Hetero fermentative
6	DH182	-	+	+	+	+	+	+	Hetero fermentative
7	DC91	+	+	-	+	+	-	+	Hetero fermentative
8	DH173	-	+	+	+	+	-	+	Hetero fermentative
9	DH163	+	+	-	+	+	-	+	Hetero fermentative
10	DH102	-	+	+	+	+	-	-	Hetero fermentative

nature (Fig. 2) (Table 2).

Molecular identification of *Lactobacillus* Spp.: All the presumptive *Lactobacillus* isolates, which were morphologically and biochemically confirmed as *Lactobacillus* (10 nos.), were confirmed by PCR targeting *Lactobacillus* specific rRNA genes. PCR results revealed that all isolates yielded positive PCR amplicons of 200-250 bp product (Fig. 3).

Sugar fermentation property: Sugar fermentation ability of different species of *Lactobacillus* revealed species level identification and was useful to characterize the different strains, and all these selected presumptive *Lactobacillus* isolates were found to be obligatory heterofermentative according to their sugar fermentation capability (Bergey's Manual of Systematic Bacteriology, Vol.2, 2007). In this study sugar fermentation test showed five different *Lactobacillus* Spp. which includes *Lactobacillus brevis* (DH163, DC91), *Lactobacillus coleohominis* (DH171), *Lactobacillus mucosae* (DC31), *Lactobacillus reuteri* (DH173, DH33, DH34, DH182) and *Lactobacillus vaginalis* (DH102, DH92) (Table 3).

Antagonistic activity of *Lactobacillus* strains: The isolates of *Lactobacillus* strains exhibited antagonistic activity against multiple bacterial strains such as *Escherichia coli*, *Salmonella enteritidis*, *Klebsiella oxytoca*, *Serratia marcescens*, *Citrobacter freundii*, *Bacillus cereus* and *Staphylococcus aureus*, as well as the ATCC *E. coli* 25922 strain. The inhibitory zone demonstrated promising antagonistic activity against both pathogenic bacteria and the *E. coli* ATCC 25922 strain. The sizes of the inhibitory zones varied among different bacterial strains, ranging from 12-15 mm for *Escherichia coli*, *Salmonella enteritidis*, *Citrobacter freundii* and *Klebsiella oxytoca*, while 12-18 mm for *Serratia marcescens*, 10-12 mm for *Bacillus cereus* and 11-15 mm for *Staphylococcus aureus*, respectively (Fig. 4 and Table 4).

Table 3. Results of Carbohydrate Fermentation for *Lactobacillus* Spp.

Isolate no.	Isolate name	Growth 15°C	Growth 45°C	Arg (R)	Ar	Ce	Es	Ga	Ma	Mn	Mez	Mb	Rf	Rb	Su	Te	Xy	Species
1	DH163	+	-	+	+	-	+	+	+	-	-	+	+	+	+	-	+	*L. brevis*
2	DC91	+	-	+	+	-	+	+	+	-	-	+	+	+	+	-	+	*L. brevis*
3	DH171	-	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	*L. coleohominis*
4	DC31	-	+	+	+	-	+	+	+	-	-	+	+	+	+	-	+	*L. mucosae*
5	DH173	-	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	*L. reuteri*
6	DH33	-	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	*L. reuteri*
7	DH34	-	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	*L. reuteri*
8	DH182	-	+	+	+	-	-	+	+	-	-	+	+	+	+	-	-	*L. reuteri*
9	DH102	-	+	-	-	-	+	+	+	+	-	+	+	+	+	-	-	*L. vaginalis*
10	DH92	-	+	-	-	-	+	+	+	+	-	+	+	+	+	-	-	*L. vaginalis*

Arg, Arginine; Ar, Arabinose; Ce, Cellobiose; Es, Esculin; Ga, Galactose; Ma, Maltose; Mn, Mannose; Mez, Melezitose; Mb, Melibiose; Rf, Raffinose; Rb, Ribose; Su, Sucrose; Te, Treahalose; Xy, Xylose. Symbols and abbreviations: +, 90% or more of strains are positive; -, 90% or more are negative. Biochemical classification adopted here from Bergey's Manual of Systematic Bacteriology, Vol. 2, 2007.

Table 4. Antagonistic activity (diameter of inhibition zone; mm) of selected LAB isolates against pathogenic bacteria (at 37°C for 24 h)

Isolate no.	Isolate name	Diameter of inhibition zone (mm)						
		E. coli	*Salmonella enteritidis*	*Klebsiella oxytoca*	*Serratia marcescens*	*Citrobacter freundii*	*Staphylococcus aureus*	*Bacillus cereus*
1	DH92	15	14	14	16	14	12	11
2	DC31	15	15	15	17	13	12	12
3	DH34	14	15	15	18	15	13	12
4	DH33	12	12	13	14	13	15	11
5	DH171	13	12	13	13	14	13	10
6	DH182	12	12	12	13	12	12	11
7	DC91	12	12	12	12	12	12	11
8	DH173	12	13	12	12	12	12	11
9	DH163	12	12	12	13	13	11	10
10	DH102	13	14	13	13	14	13	11

Antagonistic activity of *Lactobacillus* against bacterial pathogens

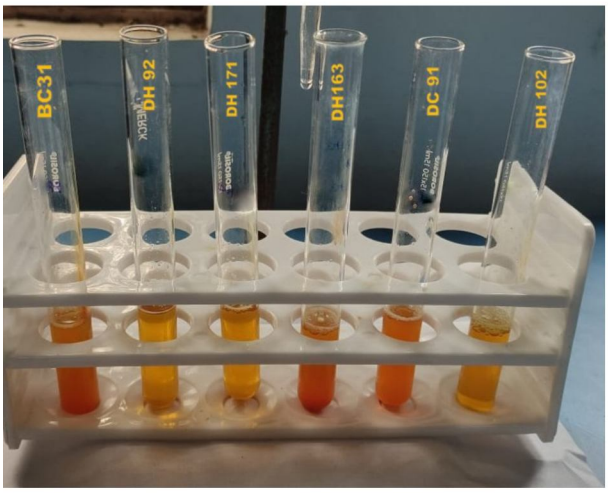

Fig. 1. Arginine hydrolysis (Adding Nessler reagent) of different *Lactobacillus* strains. Brick red colour = +ve, Yellow colour = -ve

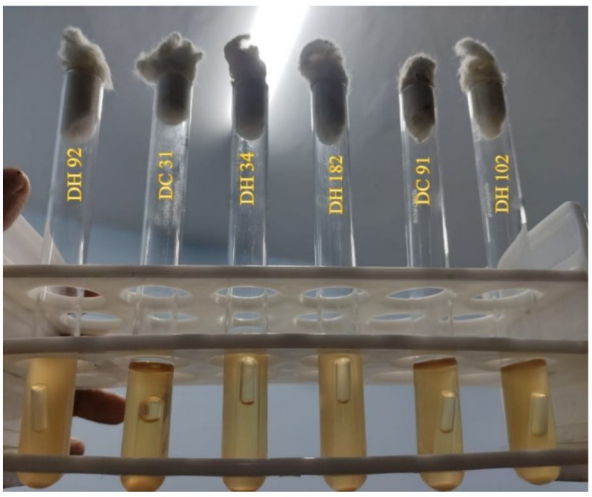

Fig. 2. Homo-/Hetero-fermentation test of *Lactobacillus* strains

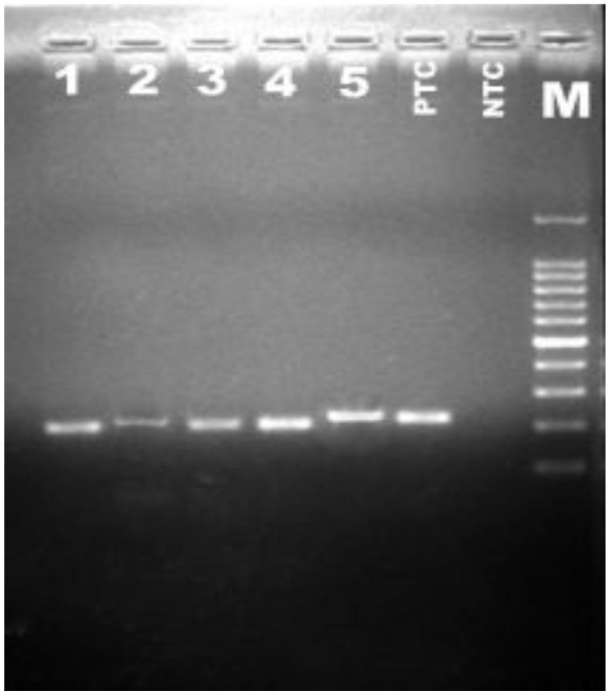

Fig. 3. 1-5 = Amplicon of *Lactobacillus* specific gene, 1= DC91, 2= DH171, 3= DC31, 4= DH33, 5= DH92; 6= Positive template control; NTC= Negative template control; M= 100 BP DNA Ladder

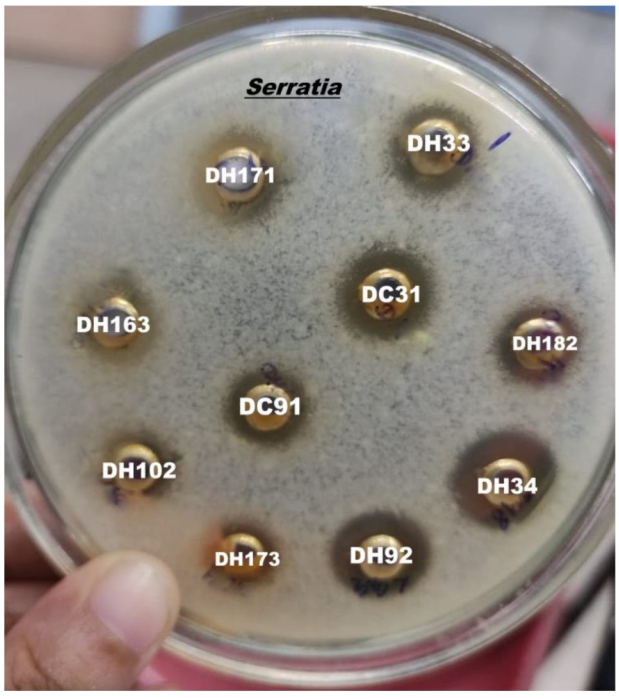

Fig. 4. Antagonistic activity of different *Lactobacillus* strains against *Serratia marcescens* in NA agar plate

DISCUSSION

The presence and persistence of *Lactobacillus* spp. in the gut of poultry have been widely documented in numerous studies (Santhosh *et al*., 2018; Reuben *et al*., 2019; Gupta *et al*., 2023). As a prevalent phenomenon within the gastrointestinal tract of these animals, *Lactobacillus* spp. play a crucial role in promoting the overall health and well-being of their host organism. One key aspect in which these bacteria prove beneficial is through their positive impact on the hosts' immune system, bolstering their ability to fend off various pathogenic threats. Moreover, they contribute to fostering optimal growth rates over time among poultry populations (Vineetha *et al*., 2016). Recognizing their value within this context, the poultry industry has enthusiastically embraced the use of *Lactobacillus* spp. as probiotics, incorporating them into various feeds and supplements for birds such as chickens, turkeys and ducks (Heilig *et al*., 2002; Heravi *et al*., 2011; Fesseha *et al*., 2021). This strategic integration highlights the growing appreciation for the critical functions that these naturally occurring bacteria serve in maintaining the health and vitality of poultry stocks around the world.

The salt tolerance capability of *Lactobacillus* Spp. plays a crucial role in their ability to thrive in the host's gut microenvironment, subsequently providing numerous benefits to their host, particularly in poultry (Vineetha *et al*., 2016; Reuben *et al*., 2019). One of the primary ways these *Lactobacillus* Spp. contribute to the well-being of their host is through synthesizing various essential vitamins and bacteriocins (Dowarah *et al*., 2018; Pei *et al*., 2021; Gupta *et al*., 2023). The production of such substances not only helps *Lactobacillus* Spp. to grow rapidly within the gut environment but also facilitates the control and suppression of pathogenic bacterial strains. This dual-purpose functionality of *Lactobacillus* bacteria plays a critical role in maintaining a healthy balance within the gut microbiome, thereby directly benefiting the overall health and well-being of their poultry hosts. Ultimately, understanding and harnessing this salt tolerance capability may lead to improved probiotic formulations and nutritional strategies for poultry farming efforts.

In this study, we observed that the isolates we obtained exhibited inhibitory effects on the growth of various pathogenic strains such as *Escherichia coli*, *Salmonella enteritidis*, *Klebsiella oxytoca*, *Serratia marcescens*, *Citrobacter freundii*, *Bacillus cereus* and *Staphylococcus aureus*. This significant finding emphasizes the possible use of *Lactobacillus* based probiotics in animal husbandry practices – particularly within the realm of poultry farming – as a viable strategy to alleviate the antimicrobial pressure currently faced by the livestock industry. These findings are consistent with prior research (Dhama *et al*., 2011; Dowarah *et al*., 2018; Noohi *et al*., 2021), thereby reinforcing the potential benefits of incorporating these probiotics in animal rearing methods. The antagonistic activity demonstrated by our isolates not only establishes promising prospects for further exploration of probiotics application in promoting overall animal health, but also advocates for a substantial reduction in antibiotic dependency within the industry (Dowarah *et al*., 2018; Li *et al*., 2020). This extensive avenue has the potential to revolutionize livestock management techniques and foster sustainable farming practices around the globe.

The focus of this study is to highlight the essential role of beneficial microbial populations, specifically the probiotic strain *Lactobacillus* spp., in maintaining a healthy gastrointestinal tract in poultry. This study emphasizes how these probiotics contribute to the overall well-being of the gut by effectively suppressing harmful pathogenic bacteria. Notably, the isolated culture we examined exhibited the ability to flourish in a wide range of pH levels, ensuring its adaptability within the intestinal environment. Additionally, this culture exhibited the capacity to inhibit the growth of common harmful organisms and displayed favourable auto-aggregation and cell surface hydrophobicity properties, particularly in relation to *Escherichia coli*, *Salmonella enteritidis*, *Klebsiella oxytoca*, *Serratia marcescens*, *Citrobacter freundii*, *Bacillus cereus*, and *Staphylococcus aureus.* Given these remarkable attributes, these isolates hold great potential for future applications as probiotics.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author's contributions: GPM, RG: Conceptualization; SM, IS, RG: Methodology; RG, TM, DB, SS: Formal analysis and investigation; RG, DB: Writing original draft preparation; RG, IS, SM: Writing review and editing; GPM, SM, IS: Funding acquisition and supervision.

Data availability statement: This article includes all data generated or analyzed during this study. The corresponding author is willing to provide the raw data upon reasonable request.

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