HSJ

Health Sciences Journal
EISSN: 2959-2259; PISSN:2959-2240

Vol.3, Issue. 1

Received: 24 OCTOBER 2024 Accepted: 9 DECEMBER 2024 Published: 9 DECEMBER 2024

MDRI

Research Article

Isolation, Biochemical Characterization and Antibiotic Sensitivity of Lactobacilli in Quail Droppings

Hikmat Ullah[1] | Nameera Attiq[1] | Zain ul Zahidin[1] | Anila Khan[2] | Saqib Ali Rustam[1]| Sana Ullah[1] | Aftab Ahmad Jan[3], Atta Ur Rehman[1]*

1Insititute of Microbiology
Faculty of Veterinary and Animal Sciences
Gomal University Dera Ismail Khan
[2]Institute of Biological Sciences
Gomal University Dera Ismail Khan
[3]Department of pharmacy
Qurtuba University of Science and Information Technology
Dera Ismail khan

Correspondence
Atta Ur Rehman
Email: arehman@gu.edu.pk

Citation
Ullah H, Attiq N, Zahidin ZU, Khan A, Rustam SA, Ullah S, Jan AA., Rehman AU. Isolation, biochemical characterization and antibiotic sensitivity of lactobacilli in quail droppings. Health Sciences Journal, 2024;3(1): 61-69

ABSTRACT:

Background: Probiotics are microorganisms which are when given in a specific amount, have favorable effects on the host health. **Objectives:** The current study was conducted on quail's droppings, which were collected from the various sailing points within district Dera Ismail Khan. The core aim of this field study was to isolate the *Lactobacillus* species from the droppings to identify and characterize them. **Methods**: Three species (*lactobacillus brevis*, *lactobacillus salivarius* and *lactobacillus fermentum*) were isolated on the basis of biochemical tests. **Results:** *Lactobacillus salivarius* was found more tolerant at pH 2 as compared to the other two species of *lactobacillus*, likewise at pH 3 *lactobacillus* brevis showed maximum growth, which was significantly higher than other species of *lactobacillus*. Similarly, all the three species of *lactobacillus* showed higher growth on 0.5 % ox gall as compared to the 1 % ox gall. Likewise antibiotic sensitivity testing of *lactobacillus* species shown sensitivity to erythromycin except *L. fermentum* and all three were resistance to tetracycline, kanamycin, ciprofloxacin and gentamycin. **Conclusion:** It is concluded that *lactobacillus* species have huge benefits like their tolerance to low pH and their growth on bile salts. Their use in diets will not only balance the gut microflora but also will inhibit the growth of pathogens in humans, animals and poultry species, as described by different studies which are conducted on above mentioned species.

KEYWORDS:

Lactobacillus, quails droppings, biochemical tests, antibiotic susceptibility

1 | INTRODUCTION

Partially migratory, the Quail (*Coturnixcoturnix*), also known as "Batair" in Urdu, is a member of the *Phasianidae* Family and the Galliformes Order[1].Common Quail is the term used to refer to these medium-sized ground-nesting game birds, which include the American quail (*Coturnixcoturnix*) and Japanese quail (*Coturnixcoturnix japonica*)[2]. They have a rounded body, short legs, and a characteristic male head pattern with three black crown stripes and creamy stripes. They are studied for their migration between Europe and Asia. Males have a buff upper breast with a pale shaft strip, a black chin, and a white throat. Females have heavy black streaking on the upper breast and lack the throat pattern[3]. Agricultural areas with semi-hilly terrain and plains are home to common quail (*Coturnixcoturnix*). The majority migrate throughout provinces, while a smaller portion remains in one area, and the weather has an impact on their seasonal movements. The main diet of common quail mostly consists of grass, chicory and seeds, including fallen cereal grains in stubbles. According to a study on common quail eating habits, 90% of the quails' weight is made up of weed seeds, such as those from grasses and legumes; only 18% is made up of cultivated grains, primarily millet; and the remaining

Health Sciences Journal
EISSN: 2959-2259; PISSN:2959-2240

8% is made up of insects and arachnids [4]. Quail constitutes high protein contents and is very much exposed to gastrointestinal disorders caused due to bacterial pathogens. Lactic acid bacteria (LAB) keep equilibrium between pathogenic bacteria and gut microflora by producing antibacterial compounds, like lactic acid or short chain fatty acid[5]. Lactic Acid Bacteria (LAB) residing in GIT of quails are *Lactobacillus salivarius,* and *Lactobacillus fermentum*, these bacteria abilities to act as probiotic [6]

According to the definition provided by WHO/FAO probiotics are microorganisms which are when given in a specific amount have beneficial effects on host health. Another scientist defines that probiotics are living microorganisms which are better substitute to the antibiotics and their use in poultry rations has favorable effects on health, growth and performance of birds [7]. These are used for different beneficial purposes in human beings and animals. In poultry and other birds its benefits include better FCR, protection from pathogens especially from different bacteria, immune response, weight gain, digestion, and nutrients uptake and strengthening of gut microbiota. The exact mode of action of probiotics is still under investigations, most of the researchers agree that the mechanism include production of antibacterial substances, competing debarment of pathogens, digestive enzymes production and stimulation of immune response [8]

Gram-positive, rod-shaped, non-pathogenic bacteria called *lactobacilli* are thought to be advantageous members of the gastrointestinal microbiota in both humans and animals, including birds. By ecologically interacting with the local flora to preserve the microbial balance around mucous membranes and by positively influencing the immune system through the GALT, they have a significant physiological impact on their host. Additionally, they eliminate harmful substances and enhance nutrient absorption and digestion[9]. *Lactobacilli* are frequently used as probiotics. The European Food Safety Authority (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) of EFSA states that those bacterial strains which are used as feed additives must be tested to determine their susceptibility to the most pertinent antibiotics and chemotherapeutics. Some studies indicate that the strains of *Lactobacilli* which carry genes for resistance of antibiotics can be transferred to other bacteria in gut of host. Therefore, it is important to evaluate the properties of antibiotic resistance in probiotics.

Probiotics are becoming more widely recognized for their potential advantages in poultry nutrition, especially as antibiotic substitutes. Their application is not without limitations; however, the following are the main difficulties in adding probiotics to birds feed. The particular strains utilized can have a major impact on how effective probiotics are. Studies and practical applications may yield conflicting results because different strains may have different effects on the health and performance of chickens.[10] There isn't a probiotic dosage that is considered ideal for every bird. Different inclusion levels can provide different results, and additional variables that can exacerbate this association include the age, breed, and health of the birds. [11] The effectiveness of probiotics can be impacted by the diet's overall composition. Probiotic efficacy can be compromised by feed ingredients, nutrient availability, and the presence of anti-nutritional factors. As a result, it is challenging to forecast probiotic effects across a range of feeding conditions. The effectiveness of probiotics might be greatly impacted by the initial state of the birds' digestive system. Probiotics may not have the same beneficial effects on birds with damaged gut health the same way they do on healthier birds, which makes using them as a regular feed component more difficult [13] Therefore, the aim of our present research was to conduct screening tests for *Lactobacilli* strains from fecal samples of quails and to check their potential use as probiotics. Therefore, we were focused to isolate, identify and characterize strains of *Lactobacillus* from fecal samples of quail and investigate their certain characteristics.

2 | MATERIAL AND METHODS

Different sailing points of quails in local market of Dera Ismail Khan were selected as study area for the sample collection in this study.Fresh samples of droppings from quails (n=50) were randomly collected from different shops of the market of study area. Samples were collected in sterilized containers, transported to microbiology laboratory in Faculty of Veterinary and Animal Sciences, (FVAS) Gomal University D.I.Khan at temperature of 4 °C, and stored at -20 °C until further analysis was done.

2.1 | Isolation of *Lactobacilli*

De Man Rogosa sharpe agar (MRS) was used for isolation of *Lactobacilli* from the samples of droppings after incubation for 48 hours at 37 °C in condition having oxygen. After culturing, *lactobacillus* bacteria produced colonies of different color, shape and size were examined.

Health Sciences Journal
EISSN: 2959-2259; PISSN:2959-2240

2.2 | Physiological and Biochemical Identification

MRS broth containing 5% NaCl concentration was used to assess the NaCl tolerance of isolated *Lactobacillus* species. Fresh culture (50 µl) was introduced and incubated at 37°C for 48 hours. Only the media served as a Negative control. Change in turbidity showed the positive results and the negative control showed no signs of growth.

2.3 | pH Tolerance Test

10N HCl and 1N NaOH were used to modify MRS broth at pH values of 2, 3, and 4. Test tubes containing fresh bacterial cultures were inserted with the appropriate MRS broth and incubated for 48 hours at 37 °C. Only the media served as a negative control. The culture media's turbidity was measured after 24 and 48 hours to determine the results. The negative control showed no signs of growth.

2.4 | Bile Tolerance Test

A 50µl fresh culture of the test isolate was inoculated in MRS broth containing 0.5%, and 1.0% ox-gall and MRS without ox-gall for 24 hours. The absorbance at 600 nm was then monitored after 0 hours and 24 hours to see if bile could suppress or inhibit the growth of specific strains

2.5 | Carbohydrate Fermentation Test

1% (w/v) sugar was added to MRS broth in order to test for sugar fermentation. In this test twelve different sugars were used. The indicator used was phenol red solution. Durham's tube was placed inverted into each test tube, and 10 milliliters of media were delivered. After being injected, fresh culture was cultured for 24 hours at 37°C. Only the media served as a negative control. Results were seen in the production of gas and color changes.

2.6 | Testing for Antibiotic Susceptibility

MRS agar was utilized for the antibiotic resistance profiling. The medium was autoclaved, poured in sterile Petri plates under a Laminar Flow Hood (LFH). Plates were inverted and stored at room temperature after solidification. For every isolate, two agar plates were obtained. To prepare the inoculum, a loopful of bacterial cultures was mixed with 1ml of distilled water, and 50µl was spread on an agar plate. On each agar plate, three antibiotic discs were then positioned. After that, the plates were incubated for 24 hours at 37°C. The sensitivity of the disc may be determined by calculating the diameters of the zones of bacterial growth inhibition, as recommended by the disc manufacturer [12] Six commonly used antibiotics including ciprofloxacin, tetracycline, gentamycin, kanamycin, ampicillin, and erythromycin was utilized for this purpose.

.

3 | RESULTS

3.1 | Isolation of *Lactobacilli*

Fifty (50) fecal samples from quails were cultivated on MRS agar and were used to identify twenty-six distinct microaerophilic colonies. The primary identification of all the isolates was as Lactic acid Bacilli due to their growth-promoting activity on MRS agar. Gram staining, colony morphology, physiological and biochemical behavior were used to confirm further. Each isolate was a short rod that was Gram positive individually and in chain form. Every isolate was found to be catalase and oxidase negative based on physiological and biochemical activity. The selected isolates showed visible growth at 30°C and 45 °C but no growth at 50 ° C. The isolates also indicated growth at 5% NaCl as indicated in Table 1.

Table 1: Lactobacillus isolates representing morphology of colonies

Isolates	Colony Morphology
LB1	Raised, milky white, opaque, circular with entire margin,0.8 mm in diameter
LB2	Milky white, opaque, raised, circular with entire margin,1 mm in diameter
LB3	Milky white, opaque, raised, circular with entire margin,0.9 mm in diameter
LB4	Milky white, opaque, raised, circular with entire margin,2 mm in diameter

Health Sciences Journal
EISSN: 2959-2259; PISSN:2959-2240

LB5	white, opaque, convex, circular with entire margin,1 mm in diameter
LB6	white, opaque, , convex, circular with entire margin,2 mm in diameter
LB7	white, opaque, convex, circular with entire margin,0.8 mm in diameter
LB8	Milky white, opaque, raised, circular with entire margin,0.9 mm in diameter
LB9	Milky white, opaque, flat, circular with entire margin,1.5 mm in diameter,
LB10	white, opaque, raised, circular with entire margin,2 mm in diameter
LB11	Off white, opaque, flat, circular with entire margin,1 mm in diameter
LB12	Milky white, opaque, flat, circular with entire margin,1 mm in diameter
LB13	Milky white, opaque, raised, circular with entire margin,1 mm in diameter
LB14	Off white, opaque, flat, circular with entire margin,1 mm in diameter
LB15	Milky white, opaque, raised, circular with entire margin,1 mm in diameter
LB16	Milky white, opaque, raised, circular with entire margin,0.5mm in diameter
LB17	Off white, opaque, flat, circular with entire margin,0.9mm in diameter
LB18	white, opaque, flat, circular with entire margin,1 mm in diameter
LB19	Milky white, opaque, raised, circular with entire margin,1.8 mm in diameter
LB20	Milky white, opaque, raised, circular with entire margin,0.8 mm in diameter
LB21	Milky white, opaque, flat, circular with entire margin,1.5 mm in diameter
LB22	white, opaque, , flat, circular with entire margin,1 mm in diameter
LB23	Milky white, opaque, raised, circular with entire margin,1.1 mm in diameter
LB24	Milky white, opaque, raised, circular with entire margin.0.8 mm in diameter
LB25	Milky white, opaque, raised, circular with entire margin,1 mm in diameter
LB26	Milky white, opaque, raised, circular with entire margin,1 mm in diameter

Table 2: Microscopy and physiological identification of *Lactobacilli*

S.No	Microscopy and physiological identification	
	Tests	**Isolates (LB1-LB26)**
1	Gram's strain	+
2	Morphology	Shortened Rod
3	Catalase	-
4	Oxidase	-
5	NaCl05%	+
	Growth at	
6	30ºC	+
7	45ºC	+
8	50ºC	-

After confirmation through Gram staining and some physiological analysis, nine (9) isolates exhibiting identical colony morphology were selected for carbohydrate fermentation profiling.

3.2 | Carbohydrate Fermentation

Carbohydrate fermentation (biochemical) test results revealed distinct profile of each Lactobacilli isolates. All isolates (LB2, LB3, LB8, LB11, LB14, LB17, LB23, LB25, and LB26) showed positive reactions for D-glucose, D-maltose, and D-sucrose indicating their ability to ferment these sugars while negative reactions for L- rhamnose, D- sorbitol, D-tagatose, and L- xylose.

Health Sciences Journal
EISSN: 2959-2259; PISSN:2959-2240

Table 3: Biochemical identification of *lactobacilli* by fermentation of different sugars

Sugars	LB2, LB3, LB8	LB11, LB14, LB17	LB23, LB25, LB26
L. arabinose	+	-	+
D. xylose	+	-	-
L. rhamnose	-	-	-
Esculin	-	+	-
D. sorbitol	-	-	-
D. maltose	+	+	+
D. sucrose	+	+	+
D. trehlose	-	+	-
D. tagatose	-	-	-
D. glucose	+	+	+
D. mannose	-	+	-
L. xylose	-	-	-

The carbohydrate fermentation (biochemical) pattern of LB2, LB3, and LB8 revealed that they all were Lactobacillus brevis, LB11, LB14, and LB17 were Lactobacillus salivarius and LB23, LB25, and LB26 were Lactobacillus fermentum. We selected one representative isolate from each specie group for further analysis. Survival at low pHThe response of pH on growth of bacilli after the incubation for 60 minutes (1 hour) is explained in table No 4.4. One Way analysis of variance (ANOVA) showed that at pH 2 maximum growth was observed in the colonies of Lactobacillus salivarius (6.00 ± 0.05log10 CFU/ml) as compared to the Lactobacillus brevis and Lactobacillus fermentum. Similarly at pH 3 Lactobacillus brevis showed a significant increased growth (7.16 ± 0.10log10 CFU/ml) as compared with Lactobacillus salivarius and Lactobacillus fermentum, likewise at pH 4 significantly (0.05) higher growth was observed in Lactobacillus brevis (7.37 ± 0.10log10 CFU/ml) as compared to the other two species of lactobacilli. At pH 2 L. salivarius has maximum survival rate. Over all better survival rates was seen in the colonies cultured at pH 4.

Table 4: pH effect on *Lactobacillus* brevis, *Lactobacillus salivarius*, and *Lactobacillus fermentum* growth (log10 cfu/ml ± Standard deviation)

No	Isolates	pH		
		2	3	4
1	*Lactobacillus brevis*	5.73± 0.06	7.16 ± 0.10	7.37 ± 0.10
2	*Lactobacillussalivarius*	6.00 ± 0.05	6.62± 0.02	6.67 ± 0.02
3	*Lactobacillusfermentum*	5.92 ± 0.02	6.40 ± 0.05	6.71 ± 0.02

The values are mean ± standard deviation of mean

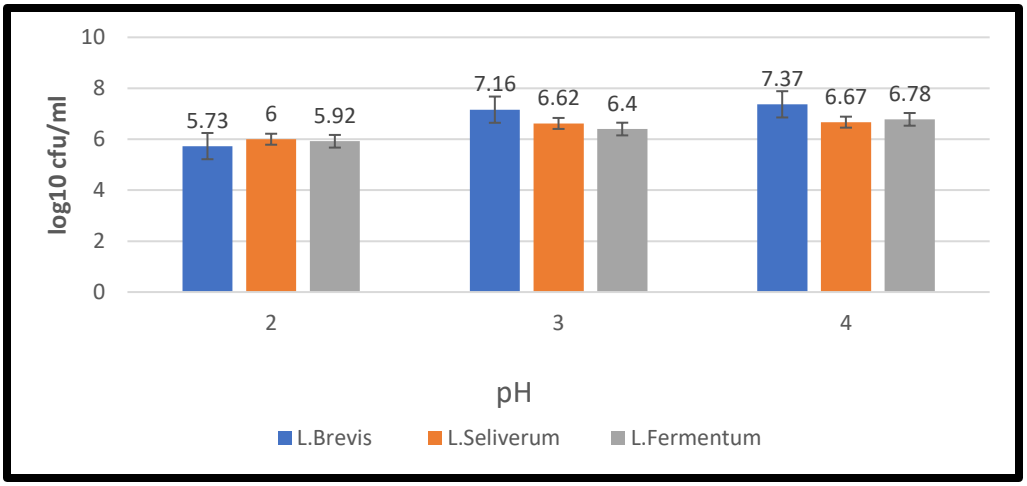

Figure 1: Graph showing growth pattern of lactobacilli on different pH after 60 minutes.

Health Sciences Journal
EISSN: 2959-2259; PISSN:2959-2240

3.3 | Tolerance to Bile

The results of an investigation into the impact of ox gall on the growth of lactic acid bacilli can be seen in Table 5. The findings indicate that Lactobacillus brevis (0.54 ±0.005) had the maximum growth (OD value) at 0.5% ox gall concentration, followed by Lactobacillus salivarius (0.49 ± 0.002) and Lactobacillus fermentum (0.49 ± 0.002), in that sequence. After a twenty-four-hour period of incubation, Lactobacillus brevis (0.45 ±0.006), Lactobacillus salivarius (0.40 ± 0.003), and Lactobacillus fermentum (0.40 ± 0.004) exhibited the highest growth (OD value) at 1% ox gall concentration. Therefore, the growth rate of Lactobacillus brevis, Lactobacillus salivarius, and Lactobacillus fermentum is significantly affected ($P < 0.05$) by ox gall. Additionally, by comparing the means reveal that ox gall of 1% concentration has significantly ($P < 0.05$) reduced the growth of Lactobacillus fermentum, Lactobacillus brevis and Lactobacillus salivarius and followed by 0.50% ox gall. High growth rate was observed in the 0.5% gall as compared with the 1 %, in other words survival rate was higher in the lactobacilli species grown on 0.5 % gall.

Table 5: Effect of ox gall on the growth (OD value ± Standard deviation) of *Lactobacillus brevis*, *Lactobacillus salivarius* and *Lactobacillus fermentum*

S . No	Bile Salts (Ox Gall)	Isolates		
		L. brevis	***L.salivarius***	***L.fermentum***
1	0.5%	0.54 ± 0.005	0.49 ± 0.002	0.49 ± 0.002
2	1%	0.45 ± 0.006	0.40 ± 0.003	0.40 ± 0.004

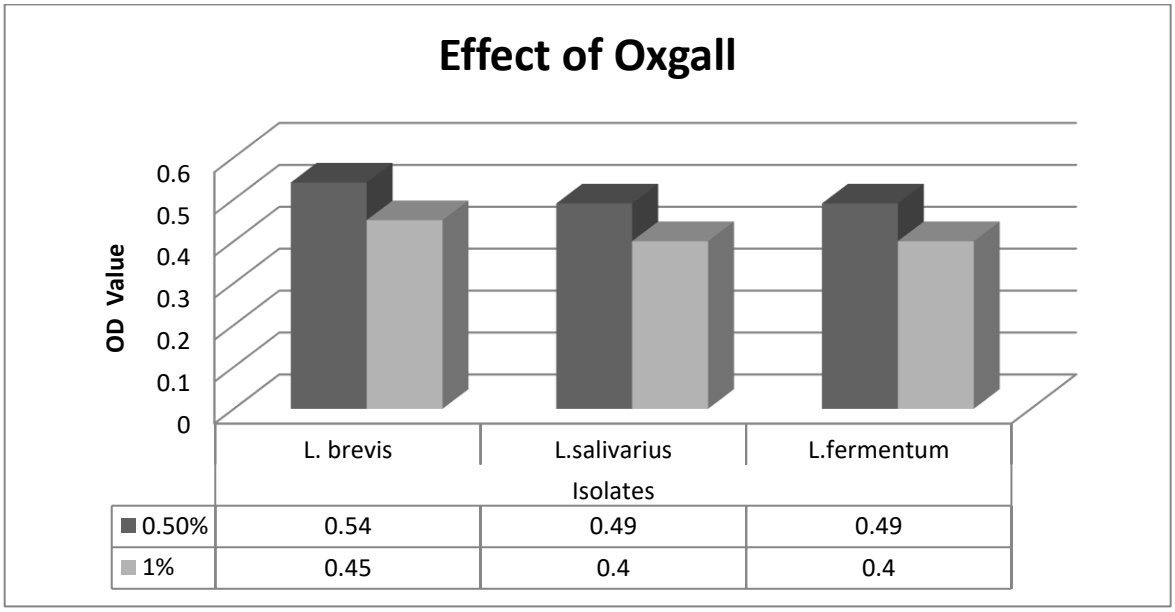

Figure 2: Effect of ox gall on the growth (OD value + bar indicate the standard error) of *Lactobacillus brevis*, *Lactobacillus salivarius* and *Lactobacillus fermentum*

3.4 | Susceptibility to Antibiotic Testing

Three Lactobacillus species; Lactobacillus brevis, Lactobacillus fermentum, and Lactobacillus salivarius have their antibiotic susceptibility profiles shown in the table (6) against six widely used antibiotics: ampicillin, ciprofloxacin, kanamycin, erythromycin, gentamicin, and tetracycline. L. brevis was sensitive to erythromycin and ampicillin and was resistant to kanamycin, tetracycline, gentamycin, and ciprofloxacin. L. fermentum was sensitive to ampicillin and moderately sensitive to gentamycin and tetracycline and was resistant to kanamycin, ciprofloxacin, and erythromycin. L. salivarius was moderately sensitive to erythromycin and was resistant to all other antibiotics. Overall, the antibiotic sensitivity testing of lactobacillus species shown sensitivity to erythromycin except L. fermentum and all three were resistance to tetracycline, kanamycin, ciprofloxacin and gentamycin

Health Sciences Journal
EISSN: 2959-2259; PISSN:2959-2240

Table 6: Antibiotic sensitivity testing of *Lactobacillus brevis*, *Lactobacillus salivarius* and *Lactobacillus fermentum*

S. No	Antibiotics	Concentration	Isolates		
			L. brevis	***L. fermentum***	***L. salivarius***
1	Ciprofloxacin	5 µg	Resistant (R)	Resistant (R)	Resistant (R)
2	Gentamicin	10 µg	Resistant (R)	Moderate sensitive (MS)	Resistant (R)
3	Tetracycline	30 µg	Resistant (R)	Moderate sensitive (MS)	Resistant (R)
4	Ampicillin	10 µg	Sensitive (S)	Sensitive (S)	Resistant (R)
5	Erythromycin	15 µg	Sensitive (S)	Resistance (R)	Moderate sensitive (MS)
6	Kanamycin	30 µg	Resistant (R)	Resistant (R)	Resistant (R)

4 | DISCUSSION

Probiotics are living microorganisms that, which are when consumed in specific quantity, give various benefits to host in terms of health, such as improving gut health, enhancing the immune response, and preventing/treating infectious diseases and cancer 13. Common probiotic strains include Lactobacillus, Bifidobacterium, Lactococcus, and Streptococcus, which mimic the beneficial gut microflora and aid in maintaining overall health. Probiotics act through mechanisms like producing postbiotics, strengthening the gut barrier, competing with pathogens for binding sites, and enhancing adhesion to the intestinal mucosa. They have shown promise in treating various conditions like allergies, gastrointestinal disorders, urogenital infections, obesity, cholesterolaemia, and diarrhea. Additionally, probiotics are being explored as alternatives to antibiotics to combat antimicrobial resistance and restore gut microbiota balance, highlighting their potential in promoting human and animal health. The selection of appropriate probiotic strains is crucial for ensuring efficacy and health benefits, emphasizing the importance of strain specificity in probiotic products [14] Probiotic microorganisms have been the subject of extensive research in the previous few years to become effective treatments for quail intestinal pathogens. By generating lactic acid, which lowers pH, and bacteriocin, an antibacterial compound, lactic acid bacteria have expressed the inhibitory action against the pathogens of intestine. Lactic acid bacteria must meet specific requirements in order to be chosen as a possible probiotic option in quail. The capability of the lactic acid bacteria to endure in the bile salt and gastric juice of the quail's GIT is one of the selection criteria used in vitro[15]. There is bile salt in the upper part of intestine and the pH of the quail intestine fluctuates, staying between 1.5 and 3 In vitro, probiotics are primarily assessed as lactic acid bacteria at pH 3). Therefore, the best way to assess a probiotic's ability to resist acid and bile is to screen lactic acid bacteria at acidic pH i,e 3 and bile salt having concentration of 1.5%

In this work Lactobacilli presented in the dropping of the quails were characterized as L. fermentum, L. brevis, and L. salivarius. These strains showed outstanding tolerance to low pH and bile concentration. These results agreed with previous study. who isolated [12] Lactobacillus strains from Japanese quail[15]. In this current research the selected strains were assessed in our research at pH values of 2, 3, and 4 for over an hour of incubation. After an hour at pH 2, Lactobacillus salivarius (6.00 ± 0.05) exhibited the maximum growth (log10 CFU/ml), followed by Lactobacillus fermentum (5.92 ± 0.02) and Lactobacillus brevis (5.73± 0.06). Lactobacillus salivarius, and Lactobacillus fermentum have demonstrated almost identical responses at pH 3. Our findings match with those of [16] .At pH 4 Lactobacillus brevis showed higher growth rate (7.37 ± 0.10) followed by Lactobacillus fermentum (6.71 ± 0.02) and Lactobacillus salivarius (6.67 ± 0.02) respectively.

The capacity of the probiotic bacteria to withstand the bile salt found in quail guts is another factor in the selection process. Bile salt concentrations range from approximately 0.5 percent on average to 2% in severe cases). The bile salt is antimicrobial because it disrupts the cellular membrane[16.] The normal range of bile salt concentrations for probiotic assessment for bile salt tolerance is 0.1% to 4.0%.We assessed the chosen strain in this study at ox gall concentrations of 0.5% and 1%. After incubation for 24 hours, the OD value was obtained. With their respective OD values of 0.45 ± 0.007, 0.40 ± 0.001, and 0.40 ± 0.003, Lactobacillus brevis, Lactobacillus salivarius, and Lactobacillus fermentum demonstrated almost similar resistance to bile salt at 1% ox gall. The high bile salt resistance of Lactobacillus fermentum and Lactobacillus brevis has also been demonstrated by another study conducted by [17]

Health Sciences Journal
EISSN: 2959-2259; PISSN:2959-2240

Lactobacillus salivarius showed resistance to 1% ox gall, by producing the enzyme bile salt hydrolase, which hydrolyzes bile salt to its inert state, the probiotic bacteria demonstrate their resistance to bile salt Another criterion used to select probiotic bacteria is their capacity to exhibit antibacterial properties against the prevalent enteric infection of quail, after exhibiting resistance to the acidic and bile salt environment of the gastrointestinal tract [18]. In a study L. brevis isolated from the faeces of broiler chicken in Malaysia was resistant to kanamycin, gentamycin, and ciprofloxacin [19] In the current research L. brevis was also resistant to ciprofloxacin, gentamycin, kanamycin, and tetracycline. L. fermentum was sensitive to ampicillin, moderately sensitive to tetracycline and resistant to ciprofloxacin, kanamycin, and erythromycin which were similar. According to previous research by., [21] some strains of L. salivarius have been reported to exhibit high resistance to aminoglycosides such as gentamicin, kanamycin, streptomycin, and neomycin which was in agreement with this study.[22]

5 | CONCLUSION

Probiotics have many health benefits. One of most important function of probiotics is to maintain gut flora and to reduce the population of harmful bacteria which may cause disease if their population exceeds in the enteric or gut environment. Our study also reveals the above said statement as strains of lactobacillus showed their tolerance against low pH, bile salts and resistance against bacteria. It is concluded that three strains of lactobacillus were isolated on the basis of their characteristics. From our study it is shown that these strains i,e., lactobacillus brevis, L. fermentum, and L.salivarius can become beneficial in maintaining the gut microflora and reducing the harmful microbes in the body of the birds . It is recommended that these strains must be further confirmed on molecular level through polymerase chain reaction (PCR) and enzyme linked immunosorbent essay (ELIZA). Further these and other isolated strains of lactobacillus strains should be used in poultry industry to keep them healthy, thus avoiding the use of antibiotics in them for disease prevention which will also helpful in reducing the drug resistance in human beings, which is a very hot issue now a days from public health point of view.

REFERENCES

1. Abd El-Hack ME, El-Saadony MT, Shafi ME, Qattan SY, Batiha GE, Khafaga AF, Abdel-Moneim AM, Alagawany M. Probiotics in poultry feed: A comprehensive review. Journal of animal physiology and animal nutrition. 2020 Nov;104(6):1835-50.
2. Afdora PT, Ardiyati T, Sjofjan O, Kalsum U. Potential antibacterials compounds of lactic acid bacteria (LAB) from quail intestine (Coturnix japonica) in inhibition growth of Escherichia coli and Salmonella typhimurium. Journal of Tropical Life Science. 2010 Nov 16;1(1):28-31.
3. Blajman J, Gaziano C, Zbrun MV, Soto L, Astesana D, Berisvil A, Scharpen AR, Signorini M, Frizzo L. In vitro and in vivo screening of native lactic acid bacteria toward their selection as a probiotic in broiler chickens. Research in Veterinary Science. 2015 Aug 1;101:50-6.
4. Jha R, Das R, Oak S, Mishra P. Probiotics (direct-fed microbials) in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: A systematic review. Animals. 2020 Oct 13;10(10):1863.
5. Jin LZ, Ho YW, Abdullah N, Jalaludin S. Acid and bile tolerance of Lactobacillus isolated from chicken intestine. Letters in applied Microbiology. 1998 Sep 1;27(3):183-5.
6. Kalsum U, Sjofjan O. The Growth of Lactobacillus fermentum Isolated from Quail Intestine on Rice Bran Medium. Journal of Tropical Life Science. 2012 Dec 24;2(3):58-61..
7. Kamel ER, Mohammed LS, Abdelfattah FA. Effect of a diet containing date pits on growth performance, diet digestibility, and economic evaluation of Japanese quail (Coturnix coturnix japonica). Tropical Animal Health and Production. 2020 Jan;52:339-46.
8. Asim M, Rizwan MA, Khan SU, Razzaq N, Haider SS, Qureshi SM, Kaleem M, Zeeshan M, Ahsan T, Ghazali10 MH. Mitigating Zoonotic Transmission: Probiotics and Prebiotics Strategies in Control of Brucellosis.
9. Kizerwetter-Swida M, Binek M. Selection of potentially probiotic Lactobacillus strains towards their inhibitory activity against poultry enteropathogenic bacteria. Polish journal of microbiology. 2005 Jan 1;54(4):287-94.
10. Jacobsen CN, Rosenfeldt Nielsen V, Hayford AE, Møller PL, Michaelsen KF, Paerregaard A, Sandstrom B, Tvede M, Jakobsen M. Screening of probiotic activities of forty-seven strains of Lactobacillus spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. Applied and environmental microbiology. 1999 Nov 1;65(11):4949-56.
11. Jannah SN, Dinoto A, Wiryawan KG, Rusmana I. Characteristics of lactic acid bacteria isolated from gastrointestinal tract of Cemani chicken and their potential use as probiotics. Media Peternakan. 2014 Dec 17;37(3):182-9.

12. Campedelli I, Mathur H, Salvetti E, Clarke S, Rea MC, Torriani S, Ross RP, Hill C, O'Toole PW. Genus-wide assessment of antibiotic resistance in Lactobacillus spp. Applied and environmental microbiology. 2019 Jan 1;85(1):e01738-18.
13. Dec M, Puchalski A, Nowaczek A, Wernicki A. Antimicrobial activity of Lactobacillus strains of chicken origin against bacterial pathogens. Int Microbiol. 2016 Mar 1;19(1):57-67.
14. Ehrmann MA, Kurzak P, Bauer J, Vogel RF. Characterization of lactobacilli towards their use as probiotic adjuncts in poultry. Journal of applied microbiology. 2002 May 1;92(5):966-75.
15. Gill HS, Guarner F. Probiotics and human health: a clinical perspective. Postgraduate Medical Journal. 2004 Sep;80(947):516-26.
16. Gupta M, Raut R, Manandhar S, Chaudhary A, Shrestha U, Dangol S, GC S, Budha KR, Karki G, Díaz-Sánchez S, Gortazar C. Identification and characterization of probiotics isolated from indigenous chicken (Gallus domesticus) of Nepal. PloS one. 2023 Jan 19;18(1):e0280412.
17. Hajar S, Hamid TH. Isolation of lactic acid bacteria strain Staphylococcus piscifermentans from Malaysian traditional fermented shrimp cincaluk. International Food Research Journal. 2013;20(1):125.
18. Lertworapreecha N, Poonsuk K, Chalermchiakit T. Selection of potential Enterococcus faecium isolated from Thai native chicken for probiotic use according to the in vitro properties. Songklanakarin Journal of Science & Technology. 2011 Jan 1;33(1).
19. Mujnisa A, Natsir A. Lactic acid production and the inhibitory capability of lactic acid bacteria isolated from poultry feces. InIOP Conference Series: Earth and Environmental Science 2021 Jun 1 (Vol. 788, No. 1, p. 012190). IOP Publishing.
20. Reda FM, El-Saadony MT, El-Rayes TK, Attia AI, El-Sayed SA, Ahmed SY, Madkour M, Alagawany M. Use of biological nano zinc as a feed additive in quail nutrition: biosynthesis, antimicrobial activity and its effect on growth, feed utilisation, blood metabolites and intestinal microbiota. Italian Journal of Animal Science. 2021 Jan 1;20(1):324-35.
21. Saeed A, Ali H, Yasmin A, Baig M, Ullah A, Kazmi A, Ahmed MA, Albadrani GM, El-Demerdash FM, Bibi M, Abdel-Daim MM. Unveiling the antibiotic susceptibility and antimicrobial potential of bacteria from human breast milk of Pakistani women: an exploratory study. BioMed research international. 2023;2023(1):6399699.