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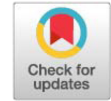
Probiotic and Antimicrobial Potential of *Lactobacillus acidophilus* Isolated from the Gut of Domestic Chicken



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ABSTRACT

Probiotics are living microbes, beneficial to host when administered in sufficient amounts, known for their characteristics, such as no pathogenicity, tolerance to diverse environments, ability to colonise on mucosa of gastrointestinal tract and act as resident microflora. Samples (n=20) were collected from domestic chicken gut (35 days old) and spread over DeMan Rogosa Sharpe (MRS) agar plates and incubated at 37°C for 24-48h anaerobically. The desired isolates were preliminary selected based on colony characteristics, morphology, and catalase reaction, and finally confirmed using API CHL50 kits. Potential probiotic isolate *Lactobacillus acidophilus* was analysed for its antimicrobial potential against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli O157:H7*, *Mucor mucedo* and *Aspergillus niger*. Inhibition zones (17-20mm) were recorded against *S. aureus*, followed by *Salmonella typhi* (10-16mm) and *E. coli O157:H7*(14-19mm), while inhibition potential was observed against the target fungal species. The *Lactobacillus acidophilus* was exposed to diverse conditions (ranges of pH, NaCl, bile salt). *Lactobacillus acidophilus* showed good growth at pH 2, 3 and 5, with limited growth under an alkaline condition (pH8). Retains good growth at 2-6% NaCl, and unable to grow in 8-10%, showed tolerance to 0.3, 1, and 2% bile salt, with growth at 10, 25 and 30℃, but no growth at 44℃. Growth at diversified temperatures showed, its suitability to be used as feed supplement, replacing antibiotics. Good hydrophobicity shows its ability for better colonization in gut. The organism was positive for protease production with no lipase and phytase production. *L. acidophilus* showed good DPPH (61±0.02 and 62±0.02 %), FRAP (44±0.02 and 68±0.17%) activity, and total phenolic contents (34 and 38μg/mL equivalent to gallic acid) for old and fresh cultures with slight variations. The isolate (*L. acidophilus*) lacks the ability to produce biogenic amines histamine and tyrosine in decarboxylase medium, thus making it a potential candidate to be used in food industry. It retained viability (90%) in stimulated gastric juice with varying pH (2,3,4), whereas reduced growth was observed after extended (3h) incubation in alkaline pH. The bacteriocin production was confirmed by LC-MS with molecular weight (5.34kDa). *L. acidophilus* was found potent to be used as a potential probiotic in food safety system and for humans and animals.

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Key words
Bacteriocin, Antimicrobials, Chicken gut, Bile salt, Phytase, Lipase

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INTRODUCTION

The relationship between diet and health advantages has been a subject of exploration for quite some duration. Recent times have witnessed a surge in dynamic exploration concerning probiotics, owing to the escalating commercial attention towards probiotic-infused food products. This surge has significantly contributed to the comprehension and distinctive profiling of particular probiotic microorganisms and their associated health advantages. This surge in research is primarily driven by the recognition of the intestinal microbiota as a significant reservoir of bacteria within animal systems (Wei *et al*., 2013). When administered in sufficient amounts, these beneficial microbial strains, collectively referred to as probiotic, confer health benefits on the host (Hill *et al*., 2014).

A crucial aspect in choosing a probiotic strain

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involves ensuring it lacks antibiotic resistance genes that might transfer to pathogenic microbes, prioritizing safety considerations (Imperial and Ibana, 2016). On the other hand, when antibiotics are used as medicinal products for animal health, probiotic microbes may be exposed to antibiotics in the animal gut. As a result, for probiotics to be effective, they must have non-transferable resistance that aids *in vivo* survival (Shakoor *et al*., 2017). Utilizing native lactic acid bacteria (LAB) strains as probiotics for animals offers a highly promising alternative for managing and preventing diseases in animal populations (Tavakoli *et al*., 2017). In the quest for potential probiotic strains, survival within the gastrointestinal tract (GIT) and adherence to the intestinal wall stand as primary criteria. Moreover, sought-after probiotics exhibit advantageous bioactivities, including antimicrobial effects against pathogens and enhancement of digestive functions. Thus, the profound significance of probiotics drives continuous endeavours to unveil potent strains capable of addressing specific challenges, such as countering diseases like coccidiosis in poultry.

Lactobacilli are the significant part of chicken intestinal micro flora and their relationship with health is under serious investigation. The genus *Lactobacillus* have great economic importance due to their use in fermented food. *L. acidophilus* is non-pathogenic and part of the normal flora of chicken intestine, poses well investigated antagonistic effect against the pathogenic bacteria and yeast in small intestine (Akbar *et al*., 2016). They are widely used in commercial fermented milk products as dietary adjuncts where they produce lactase which is responsible for the digestion of milk and thus help lactose intolerant individuals thus confer beneficial effect on human health (Akbar *et al*., 2022).

The pioneering work by Barefoot *et al*. (1994) marked the first study delving into *L. acidophilus* bacteriocin production. They unveiled Lactacin B as the bacteriocin produced by this strain. Through meticulous conditions excluding the impact of organic acids and hydrogen peroxide, they verified that the antibacterial effect solely originated from the bacteriocin itself. This research notably positioned *L. acidophilus* as a bacteriocin producer within the Lactobacilliaceae family. Furthermore, contrary to previous reports, *L. acidophilus* failed to display broad-spectrum inhibitory activity when hydrogen peroxide and organic acids were removed, revealed close association of these agents with antagonistic ability of isolates. Lactacin B production exhibited pH dependence, displaying peak activity in broth cultures sustained at pH 6 (Barefoot *et al*., 1994). This study aimed to assess the probiotic attributes and ascertain its antifungal and antibacterial capabilities. The goal was to evaluate its potential as an antimicrobial agent for incorporation into poultry feeds.

MATERIALS AND METHODS

Isolation and identification of potential probiotic bacteria

For sample collection and isolation was done following Shakoor *et al*. (2017) with slight modifications. A total of 20 samples were collected from 35-45 days old healthy chicken from the local market of Quetta. The whole intestines of each chicken were kept individually in a sealed plastic bag placed in ice. The intestinal contents of each chicken were scrapped, weighed for 1.0g, and vortexed with 9mL of 0.9% NaCl (w/v) for 30 seconds and then serially diluted 10 folds up to 10^{-6}, and subsequently spread over sterile MRS (Thermo Fisher Scientific) agar plates supplemented with 0.02% bromocresol blue and incubated at 37°C for 24-48 h under anaerobic condition. The isolates were purified by subculturing. For preliminary conformation, along with colony morphology observation the isolates were processed for gram staining and catalase test (Torshizi *et al*., 2008) followed by the confirmatory test using API-50 CHL Medium (BioMérieux, Marcy l'Étoile, France) and stored in 20% glycerol in -80° C freezer for future uses.

Determination of antibacterial activity

The antibacterial activity was determined following methods previously adopted by Akbar and Anal (2014). *Staphylococcus aureus, Salmonella typhi* and *E. coli. O157: H7* were used as target species, whereas agar well diffusion and spot on lawn methods were applied.

Agar well diffusion

Cell free supernatant was prepared by inoculated fresh LAB culture into MRS broth and incubated at 37 °C for 24h. The LAB cultures were centrifuged at 6000rpm for 15 min. The supernatant was separated and filtered sterilized before further use. Pre-inoculated Mueller Hinton agar medium (Oxoid, UK) was poured into sterile Petri dishes and allowed to solidify. Four wells of 6mm diameter were bored in the agar medium using sterile borer. An amount of 50-100 µL of pre-sterilized cell free supernatant were poured into each well, the inoculated plates were kept 1h at room temperature and then incubated at 37°C for 16-24h. Inoculated and inoculated petri plates were used as positive and negative controls. After incubation the zones of inhibition around each well were measured in mm and the results were recoded.

Spot on lawn methods

In spot on lawn method, about 20µL of 24h fresh culture of LAB bacteria was spotted on the surface of MRS agar plate and incubated under aerobic condition at 37 °C for 24h. Fresh culture of indicator bacteria were inoculated

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into 7ml of soft nutrient agar medium (*Himedia, India*) with 0.8% agar. The inoculated soft agar was overlaid onto MRS plates with test bacteria. The antagonism was detected by formation of inhibitory zones around test isolate.

Antifungal activity of lactic acid bacteria

The antifungal activity of lactic acid bacteria was determined on solid media by using spot on lawn method as adopted by (Arasu *et al*., 2014). Common mold species (*Mucor mucedo* and *Aspergillus niger*) previously isolated and stored at Food Microbiology and Bioprocess Technology Lab, Department of Microbiology, University of Balochistan, Quetta were used in this study as a target spoilage fungus. The organisms were grown in potato dextrose broth and agar (Oxide UK).

Lactic acid bacteria were spotted on the surface of MRS agar and incubated at 37 °C for 24 to get visible colonies. Following the incubation, a semisolid SDA media was inoculated with 10^6 spores of *Mucor mucedo* and *Aspergillus niger* and spread over the surface of petri dishes containing visible lactic acid bacterial colonies. The plates were kept at 35°C and were incubated for 4 days. The plates were observed for the presence of a clear zone against the target fungi.

Probiotics characteristics

The LAB with good antibacterial activity were further subjected to probiotics characterization following method as described by Ibourahema *et al*. (2008). Such as pH, Salt and Bile salt tolerance and Growth at various temperature**.** For pH tolerance studies 2 µL fresh cultured of isolated LAB (10^6-10^7 CFU/mL) was inoculated in MRS broth with varying pH 2, 3, 5, and 8, incubated under anaerobic condition at 37℃ for 24-48h and growth were observed. For NaCl tolerance, 2 µL fresh prepared cultured of LAB were inoculated in MRS broth supplemented with NaCl at various concentration 4, 6, and 10% incubated under anaerobic condition at 37℃ for 24 to 48h and growth were observed. The bile salt tolerance of isolate was determined by adding 0.3% and 2% of bile salt (Oxgall) in MRS broth incubated under anaerobic condition at 37℃ for 24-48h and growth were determined by using spectrophotometer at 620nm. For growth at varying temperature the LABs isolates were inoculated into MRS broth and incubated at different temperature range 10, 25, 35 and 44℃ for 24 h to observe visible growth.

Hydrophobicity determination

For hydrophobicity method previously adopted by (Akbar *et al*., 2014) was applied. Fresh (24h) and a old (48h) culture of LAB bacteria was centrifuged for 20 min at 8000 rpm and washed twice with normal saline. The suspension of cell was measured for optical density (OD_{600}) (A_0). About 3mL of cell suspension was mixed with 1mL toluene, blended for 2 min and allowed to stand for 15-20 min to get dissociated into two separate phases. The optical density (OD_{600}) of (A_0) lower phase was measured. For cell surface hydrophobicity calculation (% H) the following equation was applied (Blajman *et al*., 2015).

$$H (\%) = (A_0 - A) / A_0 \times 100$$

Total phenolic content

For determination of total phenol contents methodology of (Akbar *et al*., 2014) was followed. About 0.5 ml of LAB culture was mixed with 0.2ml of Folin-Ciocalteu and after 10 min (0.6ml) of 20% sodium carbonates was added, incubated at 40°C for 30 mints completely protected from available light by covering with aluminium foil. Optical density of phenol contents was determined by absorbance at 765 nm.

DPPH radical-scavenging activity determination

The antioxidant activity of LAB was determined following Akbar *et al*. (2014) with slight modifications, investigated against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) by mixing DPPH methanol solution (0.025g DPPH/500 ml methanol) with 0.25 ml supernatant of 24h old culture and was placed in dark fully protected from sunlight for 30 min at room temperature. Absorbance at 517nm was measured. For control DPPH methanol solution without sample was used, methanol as a blank 1. The following formula was used for scavenging activity calculation.

Scavenging activity (%) = [1-(absorbance of sample/ absorbance of control)] x 100

Ferric reducing antioxidant power (FRAP) test.

The FRAP assay was done following Luo *et al*. (2012). Supernatant (5µL) from fresh bacterial culture were mixed with 180µL of FRAP working solution and incubated for 5 min at 37°C. The absorbance of sample was measured at 593 nm. The standard curve of $FeSO_4$ was prepared over a concentration range from 0.15 to 1.5 mM.

Screening for enzymes production (proteases, lipases, amylases and phytase)

Screening of proteases, lipases, amylases and phytase enzymes were revealed by supplementing agar with specific substrate by following the methodology of Akbar *et al*. (2014).

For protease enzyme MRS agar was supplemented with (Skim milk 10%, Glucose 1%, Yeast extract 0.5%, Agar 1.5% pH 6.0) inoculated with LAB and were observed for appearance of clear zones around the bacterial colonies

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after incubation at 37°C for 24-48 h.

Lipase screening was carried out by Tween agar (peptone 10 g, calcium chloride hydrate 0.1 g, sodium chloride 5 g, tween-80 10 g, agar 15 g in 1-liter distilled water) pH was set between 7.0-7.4 pH and Tributyrin agar base (Himedia, India) provided with 1% tributyrin was used for lipases screening. The agars were inoculated and incubated at 37°C for 24-72 h to observe clear zone around the colonies.

Starch agar was selected for detecting amylase enzyme. About 1% of potato starch was added to MRS agar isolated lactic acid bacteria were inoculating to the Petri dishes and incubated at 37°C for 24-48 h. The plates were then stained with iodine and were observed for clear zones around the colonies.

For phytase enzyme phytase screening medium (PSM) supplemented with following agents (Glucose 1.5%, $(NH_4)_2SO_4$ 0.5%, KCl 0.05%, $MgSO_4.7H_2O$ 0.01%, NaCl 0.01%, $CaCl_2$. $2H_2O$ 0.01%, $FeSO_4$ 0.001%, $MnSO_4$ 0.001%, sodium phytate 0.5%, agar 1.5%, pH 6.5) was used. The bacteria were introduced into the plates and incubated for 24-72h at 37 °C. Phytase production was signaled by appearance of clear zone around colonies (Akbar *et al*., 2014).

Biogenic amines production

The ability of the lactobacillus acidophilus to produce histamine was analysed following Mah *et al*. (2003) by introducing the probiotic strain to synthetic media well provided with (Tryptone yeast extract, NaCl glucose, tween80, $MgSO_4$. $7H_2O$, $CaCO_3$, bromocresol purple, $MnSO_4$. $4H_2O$, $FeSO_4$. $7H_2O$, agar, precursor amino acids) pH was set to 5.3 and autoclaved at 121 °C for 10 min and the colour changes were observed.

Lactobacillus *viability in simulated gastric fluids (SGF)*

Viability of isolate in gastric fluids was analysed adopting methodology of Zárate *et al*. (2004). Gastric fluids were prepared (125 mM NaCl, 7mM KCl, 45mM $NaHCO_3$, 3g/L pepsin) HCl for adjustment of pH to 2, 3 and 4 pH and sterilized by filtration. Intestinal fluid was prepared by mixing following percentage, pancreatin (w/v) = 0.1% and bile salt (w/v) = 0.15% while pH was adjusted at 8. Freshly prepared LAB culture (10^6-10^7 CFU/mL) in MRS broth were centrifuged for 15 min at 5000 rpm. The cells were washed twice in sterile normal saline and introduced to gastric fluid (GF), incubated at 37°C on shaking water bath. *Lactobacillus* viability was observed for three hours in GF at specific time interval by using standard plate count (SPC). The SPC were done at 0, 30, 60, and 180 min. The GF were centrifuged after 180 min of incubation and then the cells were re-inoculated in intestinal fluid and incubated again. SPC were done at 0, 1 and 3 h.

Partial purification of bacteriocin and LCMS analysis

Isolated LAB was cultivated in 1000 mL MRS broth at 37°C for 24 h. The broth was then centrifuged at 8000 rpm for 15 min, followed by supernatant precipitation using 80% ammonium sulphate following method of (Parente and Ricciardi, 1999; Maldonado-Barragán *et al*., 2016). After centrifugation at 12,000 rpm for 40 min at 4°C, the precipitate was reconstituted in potassium phosphate buffer (50 mmol/L, pH 7.0). This solution underwent overnight extensive dialysis through a 1000 Da molecular weight-cut-off dialysis membrane (Spectra/Por 7 dialysis tubing, 1 K MWCO, 38 mm flat width) against the same buffer. The final solution was filtered through a 0.2 µm pore diameter filter. Confirmation of antibacterial activity was performed by spotting 20 µL onto nutrient agar seeded with target bacteria, incubating at 37°C for 16–24 h. The final purified sample of bacteriocin containing solution was sent to external lab for LCMS (Liquid chromatography-mass spectrometry) analysis.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a 16.5% and 10% gel (Akbar *et al*., 2019) low molecular weight peptide markers (BioRad, USA) served as standards for molecular mass comparison with the bacteriocin. The gel was stained using Coomassie brilliant blue R-250 (1 g/L) in a solution of 50% methanol and 10% acetic acid.

Statistical analysis

All the results were analysed for mean and standard deviation along with one-way (ANOVA) test to determine significant differences ($p < 0.05$) between samples using excel and SPSS.

RESULTS

A total of 84 colonies were initially selected for processing and identification based on their morphology (small, pinpointed round and creamy white colonies) appeared on MRS agar plates. Out of the 84 isolates, 47 were catalase negative and the remaining 37 of the isolates were gram positive. The catalase negative isolates were tested for its antimicrobial potential and finally only 3 isolates were selected for morphological and biochemical profiling. Out of which two isolates were conformed to be *Lactobacillus acidophilus*, was further evaluated for probiotic potential.

Antagonistic activity against targeted microbes

The *L. acidophilus* showed a broad spectrum of antimicrobial properties against *Staphylococcus aureus,*

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Salmonella typhi and *E. coli. O157: H7*. The inhibitory zone against *S. aureus* was 17-20 and 7-12mm by agar well diffusion and spot on lawn method respectively, while 10-16 and 8-10mm against *S typhi*, whereas 14-19 and 2-4mm against *E. coli. O157: H7* as showed in (Table I). *L. acidophilus* was found active against fungal species (*Aspergillus niger* and *Mucor mucedo*) in both liquid and solid media methods with average zone of inhibitions 13-17mm (Fig. 1).

Table I. Antagonistic activity of *Lactobacillus acidophilus* against targeted bacteria.

Method	*Staphylococcus aureus*	*Salmonella typhi*	*E. coli O157: H7*
Agar well diffusion method	17-20mm	10-16mm	14-19mm
Spot on lawn method	7-12mm	8-10mm	2-4mm

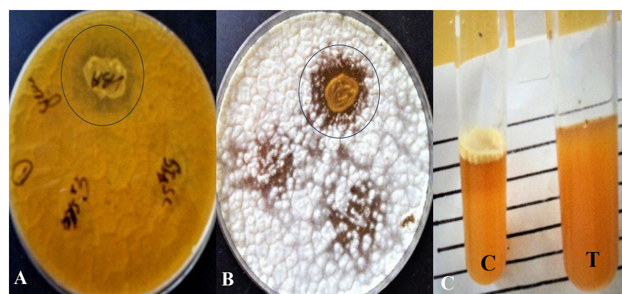

Fig. 1. Antagonistic activity of *Lactobacillus acidophilus* against target fungi. (**A**) and (**B**) clear zone surrounded by the circle indicating the antifungal activity of the *Lactobacillus acidophilus*, whereas (**C**) the test tube labelled as C, indicate the positive control showing the fungal growth at the top, while T indicate the test with no fungal growth.

Probiotics characteristics of the LAB isolates

Probiotic properties of *L. acidophilus* were conducted by exposing to different ranges of pH and temperature, varying concentration of salt and bile salt and its percentage hydrophobicity mentioned in (Table II). *L. acidophilus* exhibits stable growth at *p*H 2.0, 3, 0 and 5.0 but failed to grow at *p*H 8.0 this declares that Lab strain was most stable in acidic environment than in basic this characteristic makes them ideal probiotic to be used for poultry chicken instead of antibiotic. *L. acidophilus* well tolerated NaCl concentration from 4% to 6% but failed to tolerate high salt concentration. The isolate well tolerated the bile salt percentage expected to be present in chicken GIT. Showed stable growth at varying temperature 10, 25, 30°C but failed to grow at higher temperature 44°C.

Table II. Probiotic characteristic of *Lactobacillus acidophilus.*

Characteristics	Results
Growth on temperature (°C)	
10	+ve
25	+ve
30	+ve
44	-ve
Salt (NaCl tolerance) (%)	
4	+ve
6	+ve
8	-ve
10	-ve
Bile salt tolerance (%)	
0.3	+ve
0.5	+ve
1.0	+ve
2.0	+ve
pH tolerance	
2	+ve
3	+ve
6	+ve
8	±ve
Enzymes production	
Protease	+ve
Amylase	-ve
Lipase	-ve
Phytase	-ve
Hydrophobicity	
% hydrophobicity (old culture)	44.4 ± 0.2%
% hydrophobicity (fresh culture)	39.30 ± 0.3%

Hydrophobicity

The cell surface hydrophobicity of *Lactobacillus* result indicates that *L. acidophilus* showed higher surface hydrophobicity irrespective to culturing hours, from the result of our investigation both fresh and old culture exhibited satisfactory hydrophobicity, with slight variation observed between fresh and old culture. *L. acidophilus* fresh culture revealed to have 39.30 ± 0.3% hydrophobicity compared to old culture 44.4 ± 0.2%. The incubation period has no influence on hydrophobicity of *L. acidophilus* thus retained their characteristic surface hydrophobicity.

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Antioxidant activity of the LAB isolates

In the present study, the isolated *L. acidophilus* showed DPPH activity of 61±0.02 and 62±0.02 % and FRAP activity was 44±0.02 and 68±0.17% for old and fresh culture and total phenolic components were calculated to be 34 for old culture and 38 µg/mL equivalent to gallic acid in fresh culture of the isolate. These antioxidant activities make *L. acidophilus* a good agent to be used as health promoting probiotic.

Enzyme production

The LAB strain was also investigated for enzyme production. The strain harbors the ability to produce protease while was negative and lacked the ability to produce lipase, phytase and amylase.

Biogenic amines production

No biogenic amine productions activity has been observed for *L. acidophilus*, which is a value addition for its uses as a probiotic in different food safety systems.

Activity in simulated gastric juice

Hydrochloric acid of human and chicken gastrointestinal tract degrades all sorts of macromolecules' proteins, lipid etc. including bacteria both beneficial and pathogenic. The tolerance of *L. acidophilus* to artificial gastric juice and intestinal fluids were tested. The tested bacteria showed more than 90% survival rate and there were no significant differences under acidic conditions, In contrast significant differences were observed under basic conditions The data showed that the survival rates of *L. acidophilus* against stimulated gastric juice with pH 2.5-4 pH was high irrespective to incubation period and bacterial isolate retained its viability but the situation was quite different under basic condition where isolate showed minimal growth after 1 h of incubation, then again decrease in viability was observed after incubation period was stretched to 3h. In intestinal fluid reduction in bacterial growth occurred at pH 8 with time interval however viability was retained. Isolate shows good growth under acidic environment but the conditioned didn't remained same under alkaline condition with pH 8 where *L. acidophilus* showed moderate growth (Fig. 2).

Bacteriocin LC/MS

Bacteriocin was found to be little heterogenic in its composition due to multiple peaks produced at retention time of 29.1-33.1 min (Fig. 3). M/Z = 534 .2534 which correspond to $(M+10^{10})^{10}$. Therefore, the calculated molecular mass of the bacteriocin was 5.34 kDa. A single band with molecular weight of 5.6KDa was displayed on SDS PAGE by active fraction. The calculated mass exactly in accordance with the molecular weight determined by SDS PAGE.

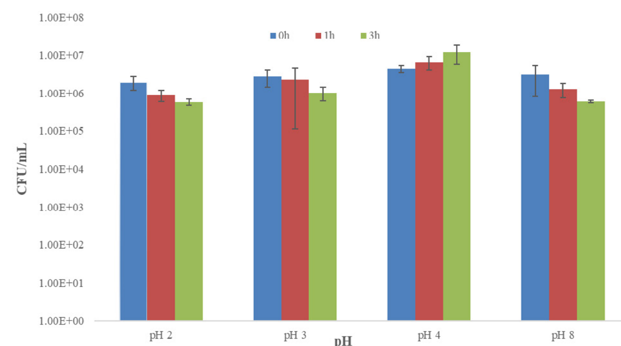

Fig. 2. Growth of *Lactobacillus acidophilus* in stimulated gastric juice at varying pH.

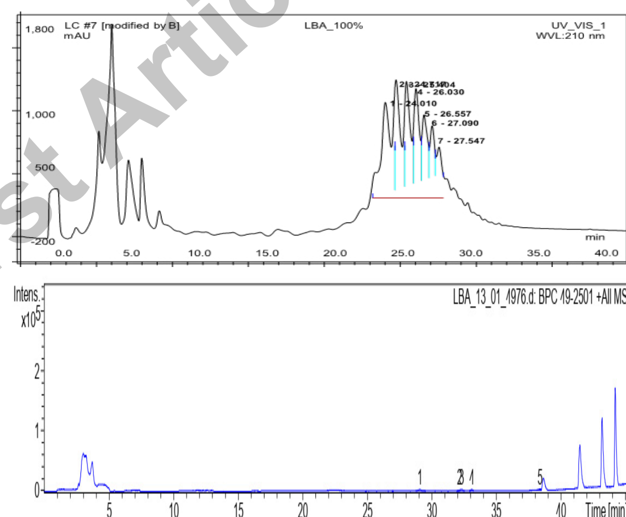

Fig. 3. LC-MS peaks of the bacteriocin.

DISCUSSION

L. acidophilus was isolated from GIT of domestic chicken and identification and conformation was done with the help of gram staining, catalase, and carbohydrate fermentation, subjected to API 50 CHL kit. The antagonistic activity was checked against targeted organism *Staphylococcus aureus, Salmonella typhi* and *E. coli. O157: H7* by two methods, spot-on lawn, and agar well diffusion method. In both cases well defined zone of inhibition was observed that means it can be suitable alternative to antibiotics. The antagonistic action of the *Lactobacillus* species against pathogenic bacteria is attributed to its capability to secrets a variety of compounds including organic acids, hydrogen peroxides and bacteriocin

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(Rodgers, 2003). Rodríguez *et al*. (2012) reported that almost 30% of LAB isolates from infant faeces exhibited 10 mm inhibitory zone against *Escherichia coli* and *Listeria innacuo*. Malini and Savitha (2012) revealed that majority of LAB from food sources including cheese, sausages, belonged to the groups *Lactobacillus, Lactococcus, Pediococcus* and *Bifidobacteria*, and among them 75% displayed satisfactory antibacterial activity. Apart from the ability of potential probiotics to tolerate bile salts, which is considered to be important characteristic of good probiotic (Rodríguez *et al*., 2012). it is also expected that probiotics should be able to tolerate the acidic environment of the GIT as they pass through to colonize the gut of their host. The secretion of gastric juice with pH 2 causes the death of microbes when ingested into the gastrointestinal tract. In our study it was demonstrated that isolated probiotic retained its viability under acidic condition where pH 2.3 and 4 were applied but under alkaline conditions exhibited moderate stability. Pan and Zhang (2008) reported the growth stability of *L. lactis* subsp. lactis at low pH (2.5 and 4.5) thus *L. acidophilus* can withstand acidic environment of both stomach and intestine (Tang *et al*., 2016). The isolated LAB strains showed good tolerance to 4- 6 % NaCl concentration but at 8 to 10% NaCl weak growth of the strains was recorded. The ability of *Lactobacillus* bacteria to tolerate varying concentration of NaCl depends on the habitat of the probiotic isolates from where it has been collected. The NaCl tolerance of probiotic LAB is a value addition, as it enhances the release of important metabolites. The tolerance to high NaCl concentrations could help the isolate to survive in the gastrointestinal tract of the animal, this ability is of prime importance for probiotic bacteria to maintain their osmotic balance to survive and grow in the gastrointestinal tract having osmolarity equivalent to 0.3 mol/L (Kobierecka *et al*., 2017). Bile salts usually damages the membrane structure of the bacterial cells. It has been reported, that resistance to bile salt is an important characteristic of bacterial isolates to be used as probiotics and harbour the small intestine of the host to provide health enhancing effects (Argyri *et al*., 2013). Only strain that can resist bile percentage of 0.1-0.3 and 0.5% can be applied as probiotic strain for chicken, as it resembles the chicken intestinal bile salt condition (Erkkilä *et al*., 2000). In our finding we found that *L. acidophilus* isolate survived and retained its viability at 0.1, 0.3 and 0.5% bile salt concentration after even 6h of incubation. Effect of temperature on growth of LAB was demonstrated by growing of isolates at varying temperature 10, 25, 30 and 44 °C. Optimal temperature for cultivation was 37°C. Under this condition maximal density was reached after 24 h of incubation. *L. acidophilus* showed good growth at 10, 25 and 37°C, limited growth activity and viability was observed at 44 °C. We found in our study that our isolated strain optimally grows at 37 °C. These finding are in agreement with (Karnwal and Malik, 2023). The *L. acidophilus* retained its growth in both simulated gastric juice and intestinal fluids with acidic and alkaline pH. *L. acidophilus* showed strong hydrophobicity, thus can strongly colonies the gut if given to chicken in feed additive to exert its health promoting benefits. Research have proven that some lactobacilli strains have antioxidant activity that have health promoting potential, once they colonized and proliferates in the host gastrointestinal tract thus same has been proved and *L. acidophilus* harbour good antioxidant activity (Kanno *et al*., 2012). In the present study the *L. acidophilus* exhibits strong antioxidant activity.

L. acidophilus produced no biogenic amine (histamine and tyrosine) in decarboxylase medium in our study thus it cannot cause any allergy if used as probiotic in both human and animals. It has been investigated that production of biogenic amine is basically strain dependent rather specie dependent our study result are in agreement with he studies of (Deepika and Rakshit, 2011), Bacteriocin are heterogeneous group and can be either small peptides consisting of 19 to 37 amino acids with molecular weights <5 to 10 kDa or larger peptides with molecular weights up to 90 kDa Bacteriocin production has been suggested as a key probiotic trait and *L acidophilus* is known to secrete a diverse array of bacteriocin, such as lactation B, F, and acedoxin (Chumchalova *et al*., 2004, Souza *et al*., 2005). *L. acidophilus* produces a small bacteriocin, with molecular mass of 5.34 kDa that is considered to be acidophilic, with an estimated molecular mass of less than 6.5 kDa. (Chumchalova *et al*., 2004) isolated bacteriocin form *L. acidophilus* with molecular with more than 5 kDa. The isolated *L. acidophilus* produced no detectable biogenic amine thus makes it good probiotic agent as it cannot exert any allergy in host.

CONCLUSION

The *L. acidophilus* bacteria were isolated from the chicken gut, which showed good growth activities at acidic and alkaline pH, with tolerance to bile salt and NaCl concentrations. It exhibits good antibacterial and antifungal potential against commonly known undesirable microorganisms, with a potential to produce potent bacteriocin. All these finding showed that *L. acidophilus* is a suitable probiotic, which can be used for the growth enhancement of chicken, as it is not only antimicrobial, but also withstand the diverse growth conditions resembling to human and chicken gastrointestinal tract.

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DECLARATIONS

Funding

The study did not receive any external funding.

Ethical statement and IRB approval

This research study was approved by the ASRB, UoB (No. UoB/Reg:/GSO/464) and IRB, UoB (UoB/ORIC/24/123).

Statement of conflict of interest

The authors have declared no conflict of interest.

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