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Antibacterial Activity of Lactobacilli Against *Salmonella typhi*

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Abstract: Salmonellosis is considered one of the most serious bacterial diseases affecting humans worldwide. Several literatures indicated the need for biologically-originated antibiotics to combat this pathogen. In the present study, eight lactobacilli have been isolated from local dairy products. Two isolates designated LAB5 (*Lactobacillus plantarum*) and LAB7 (*Lactobacillus paracasei*) have shown potentialities against *Salmonella typhi.* The efficiency of these two isolates has been tested in mice, where six groups were enrolled in the study. One group served as control and the others were treated either with Lactobacilli alone or in combination with *S. typhi*. The histopathological results obtained indicated that using Lactobacilli with *S. typhi* has a positive effect on liver and intestine. These data has been confirmed with several immune-related parameters such as TNF and IL-1b. We concluded that using Lactobacilli to compact the pathogenicity of *S. typhi* could be considered a good approach to treat this pathogen.

Key words: Lactobacilli · *Salmonella* · *Lactobacillus plantarum* · *Lactobacillus paracasei* · Anti bacterial activity

INTRODUCTION

Nature has been a wellspring of therapeutic agents for a huge number of years [1]. A great number of cutting edge medications have been separated from microorganisms, principally taking into account their utilization in customary solution [2]. In the previous century, in any case, an expanding part has been played by microorganisms in the creation of anti-infection agents and different medications [3].

Among the bacterial strains having antimicrobial movement are Lactic Acid Bacteria (LAB). Lactic Acid Bacteria disconnected from dairy items have gotten expanded consideration as a potential nourishment additive because of their opposing movement against numerous sustenance conceived pathogen, for example, *Lactobacilli* [4-6].

LAB are generally circulated in the nature, they are commonly included in countless unconstrained sustenance maturation and they have been broadly contemplated. Some individuals from LAB produce bacteriocins and bacteriocins-like substances which may repress development of waste and pathogenic microorganisms .

Salmonella is a gram-negative, facultative anaerobic, whipped bacterium [7]. It is the pathogenic specialists of Salmonellosis, a noteworthy reason for enteric disease and typhoid fever, prompting numerous hospitalizations and a couple of uncommon passing if no anti-microbials are managed. Salmonella has been segregated from an extensive variety of creatures and was found in drinking water [8, 9].

Salmonella flare-ups are connected to unhygienic nourishment arrangement, cooking, warming and stockpiling practices [10]. Salmonella was found to bring about mutagenic activities prompting tumor [11, 12]. The bacterium can be separated from crude meat and poultry items and additionally from milk and milk-based items. The identification of Salmonella thusly remains an exceptionally vital issue in microbiological examination for nourishment security and guidelines [13]. The overall aim of the present study was to investigate the role of LAB as a potent antibacterial activity against Salmonella.

MATERIALS AND METHODS

Sample Collection and Processing: The LAB samples were isolated from thirteen Egyptian dairy products. The

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fermented milk samples were incubated at 30°C and 37°C, while, yogurt samples were cultured in sterilized reconstituted skim milk before incubation until coagulation. Coagulated samples were then streaked on De Man Rogosa Sharpe (MRS) agar (Himedia, India). Samples were streaked on MRS agar under anaerobic condition using the gas pack system for 48 hours according to Soda *et al.* [14].

Identification of Isolated Lactic Acid Bacteria

API 50CH System: All the isolates were previously confirmed and identified by the standard bacteriological methods and were further confirmed and identified to species level using API 50CH for LAB isolates according to manufacturer instructions.

16S rRNA Gene Amplification: The isolates whose showed significant inhibitory effect on Salmonella growth were further identified by 16S rRNA sequencing, The fragment of the 16S rRNA was amplified from genomic DNA extract using PAF and 536R primers previously reported by Yeung *et al.* [15].

Sequencing of 16S rRNA Gene: To confirm the identification results obtained by API 50 CHL and PCR, the identities of 6 randomly selected isolates of LAB were rechecked by sequencing of the 16S rRNA genes. Identification of the sequences homology with other sequences in database was performed by blast search (NCBI blast tool).

Experimental Animals: Five weeks old male CD-1 Mice weighting 22-30 g each were purchased from Animal House Colony, Pharmacology & Chemistry Research Centre, 6th October, Egypt. Animals were maintained on standard lab diet (protein: 160.4; fat: 36.3; fiber: 41g/kg of metabolenergy =12.08 MJ) and were housed in filter-top polycarbonate cages in a room free from any source of chemical contamination, artificially illuminated (12h dark / light cycle) and thermally controlled (25± 1°C) at the Animal House Lab., Pharmacology & Chemistry Research Centre. After an acclimatization period of 1 week, the animals were divided into six groups (10 mice/ group). All animals were received human care in compliance with the guidelines of the Animal Care and Use Committee of the Pharmacology & Chemistry Research Centre.

Experimental Design: Animals within different treatment groups were treated daily for eight days as follows:

Group I: Untreated control; Group-II: animals challenged with single inoculation *S. typhi* (200 µl aliquot of 1X 108/ P.O), Group-III: animals treated orally with *L. plantarum* (200µl aliquot of 1X 108/ P.O) for seven days, Group-IV: animals treated orally with *L. paracasei* (200µl aliquot of 1X 108/ P.O) for seven days, Group-V: animals pretreated orally with single inoculation *S. typhi* (200 µl aliquot of 1X 108/ P.O) for one day and treated with *L. plantarum* for 7 days and Group-VI; animals pretreated orally with single inoculation of *S. typhi* (200 µl aliquot of 1X 108/ P.O) for one day and treated with *L. paracasei* for 7 days.

At the end of the treatment period, all animals were fasted for 12 hr and blood samples were collected from tail vein from each animal under ether anesthesia according to the method of Cocchetto and Bjornsson [13]. Blood samples were left to clot and the sera were separated using cooling centrifugation at 3000 rpm for 15 min and stored at -20°C until analysis.

DNA Extraction: One milliliter of MRS broth containing bacteria was transferred into a two ml screw-cap microcentrifuge tube then the sample was centrifuged for 2 min. in a microcentrifuge at 12, 000 rpm, the supernatant was then aspirated and discarded. Then 100 µL of PrepMan Ultra Sample Preparation Reagent was added to cell pellet then the cap was closed tightly. The sample vortex was done for 10 to 30 seconds and the sample was heated for 10 min. at 100°C in a heat block and then cooled to room temperature for 2 min. finally the sample was centrifuged for 2 min. in a microcentrifuge at 12, 000 rpm.

PCR Profile: Primers were used to amplify the 5' region of the 16S rDNA gene. In which a 50-µl reaction mixture was prepared. It consisted of 1× buffer without MgCl2, 1.5 mM MgCl2, 20 µMdNTP, 0.1 µM forward primers PAF, 0.1 µM reverse primers 536R, 1.5 U Taq Polymerase and 3 µl of template. The amplification was programmed as follows: pre-incubation at 94°C for 2 min, followed by 40 cycles at: 94°C for 45 s, 55°C for 45 s and 72°C for 60 s. After these cycles, the reaction was maintained at 72°C for 7 min and then cooled to 4°C. Then 5 µl of the PCR products were visualized after electrophoresis in a 1.5% agarose gel and were subsequently visualized by UV illumination after ethidium bromide staining.

Bacteriocin Production: The bacteriocin-producing strains were grown in MRS broth (pH 5.5) at 37 C for 18-20 h. The lactobacilli culture was centrifuged at 8000-9000 rpm for 5-6 min and then the supernatant was adjusted to pH 6.5-7.0 with 1N NaOH and stored at favorable conditions for further usage.

Immunological Parameters: Tumor necrosis factor α and interleukin 1-β were measured in all samples after being treated. A standard curve is used to determine the amount of both TNF-α and interleukin 1-β in an unknown sample. The standard curve is generated by plotting the average O.D. (at 450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding concentration (pg/mL) on the horizontal (X) axis.

RESULTS

Isolation and Identification of Lactic Acid Bacteria: Eight lactic acid bacteria were isolated from dairy products including *pediococcus pentosaceus*, two isolates of *Enterococcus faecium* and two isolates of *lactococcus lactis*, *Lactobacillus plantarum* and *Lactobacillus paracasei*. Isolates identification were performed using API 50CH system. Also, 16s sequencing were used to confirm the identity of the isolated strains.

API 50CH system showed identity of lactic acid bacteria where it had identification percentage of 70% (8/10) of isolates belong to Lactobacillus delbrueckii subspecies bulgaris, rhamnosus, paracasei, acidophilus and plantarum and 20% (2/10) were identified as Lactobacillus Lactis and 10% (1/10) were identified as Aerococcus viridans as shown in Fig. (1), the isolated LAB identity and their ID % are shown in Table (1).

Identification of Lactobacilli Using 16S Ribosomal RNA: In the present study, we employed 16S rDNA sequencing to identify the LAB isolates, whose cell-free-extract showed significant inhibitory effect on *S. typhi*. The amplification of 16S rDNA fragment against 536R primers showed the expected size resembling the LAB. The PCR produced sequences were identified as *Lactobacillus paracasei* as shown and *Lactobacillus plantarum*. The PCR product was electrophoresed (1.2%) after being stained with ethidium bromide (Figure 2).

The PCR products were sequenced and the blast analysis on the NCBI database showed that the sequences of LAB5 and LAB7 isolates are related to *Lactobacillus plantarum* and *Lactobacillus paracasei* strains, respectively (Figures 3 and 4).

Antimicrobial Activity of LAB: In the present study, we evaluated the antimicrobial activity of the isolated lactic acid strains against many reference strains including *Bacillus subtilis* ATCC *23857, Escherichia coli ATCC 25922, Pseudomonas aeruginosa* ATCC *27853, Staphylococcus aureus* ATCC *25923, Candida albicans ATCC 90028, Micrococcus luteus,* ATCC *21882 and salmonella typhi.* Results obtained showed that LAB5 and LAB7 had a significant antimicrobial activity against the tested strains (Table 2).

Bacteriocin Activity: It is well known that bacteriocin production is a pH-dependent mechanism and it plays a crucial role in bacterial cell proliferation. The bacteriocin activity of the two selected isolates; LAB5 (*Lactobacillus plantarum*) and LAB7 (*Lactobacillus paracasei*), was tested at different stress conditions. The two selected bacterial isolates were exposed to a range of pH values (2, 4, 5, 7 and 9) and the diameter of the inhibition zone was measured accordingly (Figure 6).

Meanwhile, in the current study, the ability of the LAB5 and LAB7 strains to alleviate the risk of infection with *Salmonella typhi* was evaluated using animal model. Six groups of mice were used. Three groups were used as a negative and positive control. The other groups were exposed to infection with *Salmonella typhi* in presence and absence of LAB5 and LAB7. Widal test was used to measure the severity of the infection with *salmonella* by measuring the antibodies titer (Table 3). The results obtained showed no significant amounts of antibodies in groups (III) and (IV), which were treated with LAB5 and LAB7. Group (II) showed a high rate of antibody titer against *Salmonella*.

Levels of Tumor Necrosis Factor Alpha (TNF-α): The obtained data indicated that TNF was significantly increased in the group treated with *S. Typhi* only. However, applying Lab5 and LAB7 resulted in an increase in TNF levels as compared to control. Data presented in Figure (7) and Table (4) show the variable response of different mice groups in terms of TNF levels.

Analysis of Interleukin 1-Beta (IL-1β):
Figure (8) represents the effect of different treatments on IL-1β as revealed *in vivo.* The data indicated that there were significant differences between different treatment groups. In terms of the concentration of IL-1β, the application of LAB5 and LAB7 did not show significant differences as compared to control.

Histopathological Examination
Histopathological Examination of Liver Tissues: The histological examination of liver section of the control group showed normal distribution of central vein and blood sinusoids (Figure 9a). The mice treated with *L. plantarum* showed normal central vein and hepatocytes (Figure 9b). Histological examination of hepatic cell in mice treated with *L. paracasei* displayed

Table 1: The identity of isolated LAB by API CHL V5.1

Sample number	Identity	% ID
LAB 02	*Pediococcus pentosaceus*	64.7
LAB 10	*Enterococcus faecium*	53.8
LAB 06	*Lactococcus lactis*	57.3
LAB 07	*Lactobacillus paracasei*	100
LAB 17	*Lactococcus lactis*	76.8
LAB 35	*Enterococcus faecium*	98.7
LAB 05	*Lactobacillus plantarum*	80.5
LAB 22	*Lactobacillus plantarum*	66.5

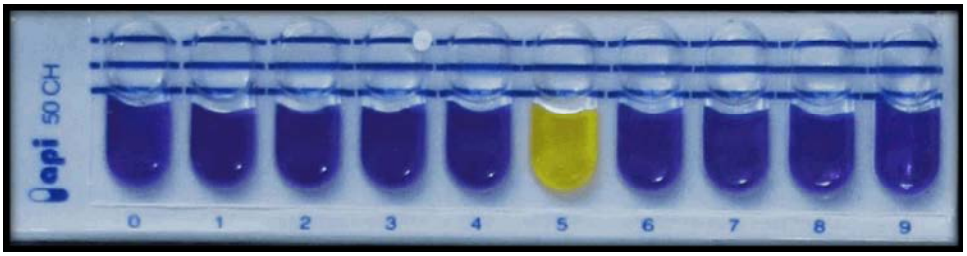

Fig. 1: API identification system of *Lactobacillus paracasei.*

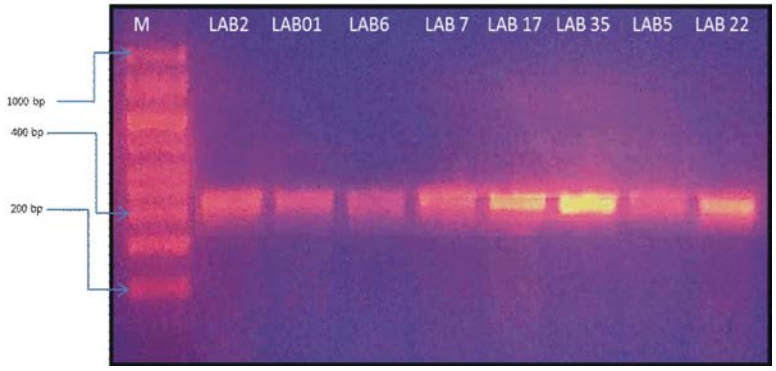

Fig. 2: Electrophoresis of the 16S rRNA amplification product against 536R primers.

Table 2: The antimicrobial activity of crude extract obtained of different isolates

No.	Isolate	*B.subtilis*	*E. coli*	*P.aeruginosa*	*S.aureus*	*C.albicans*	*M.luteus*	*S. typhi*
LAB1	*Pediococcus pentosaceus*	--	--	--	--	--	--	--
LAB2	*Lactobacillus lactis*	--	--	--	--	--	--	--
LAB3	*Lactococcus lactis*	--	--	--	--	--	--	--
LAB4	*Enterococcus faecium*	--	--	--	--	--	--	--
LAB5	*Lactobacillus plantarum*	10mm	10mm	10mm	11mm	7mm	12mm	10mm
LAB6	*Lacobacillus brevius*	--	--	--	--	--	--	--
LAB7	*Lactobacillus paracasei*	11mm	8mm	12mm	11mm	9mm	13mm	12mm
LAB8	*Enterococcus faecium*	--	--	--	--	--	--	--

Table 3: The antibody titer against *Salmonella typhi* in the different experimental groups.

Groups	Group type	Average of Antibody titer
Group I	Negative control	< 40
Group II	Treated with *Salmonella*	160
Group III	control LAB7	<40
Group IV	Control LAB 5	<40
Group V	Treated with LAB5 and *Salmonella*	< 40
Group VI	Treated with LAB7 and *Salmonella*	<40

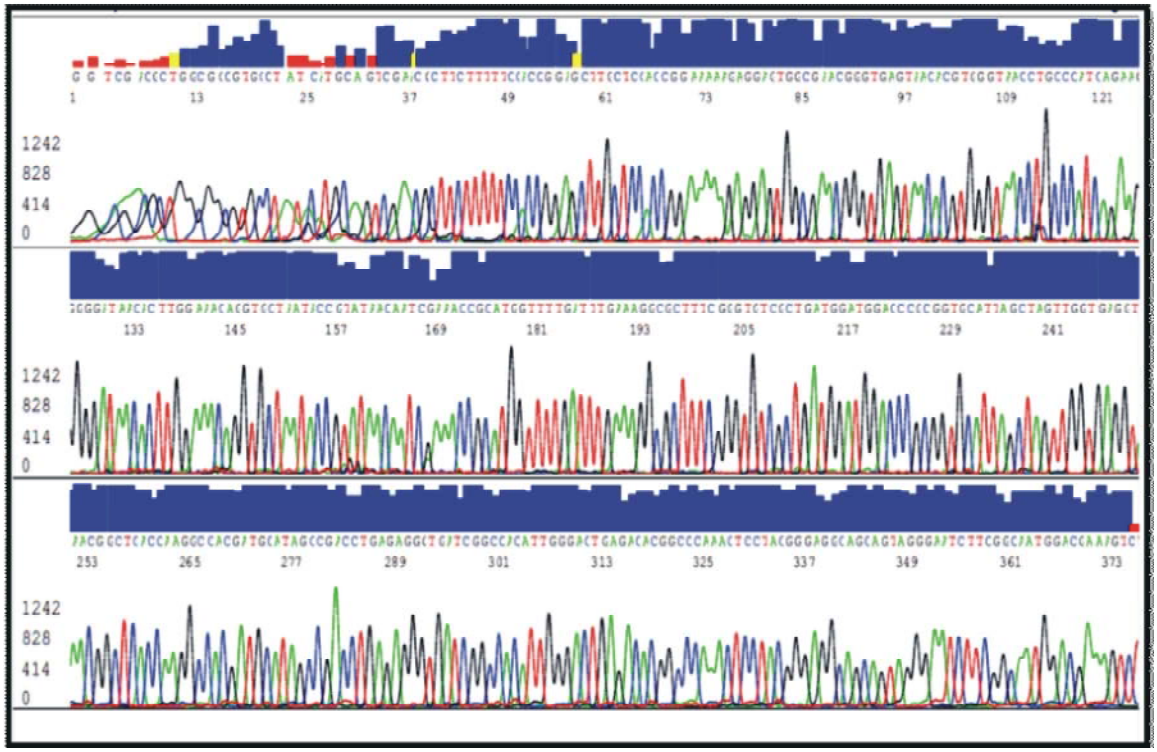

Fig. 3: Chromatogram of the 16srRNA sequence of *Lactobacillus plantarum*.

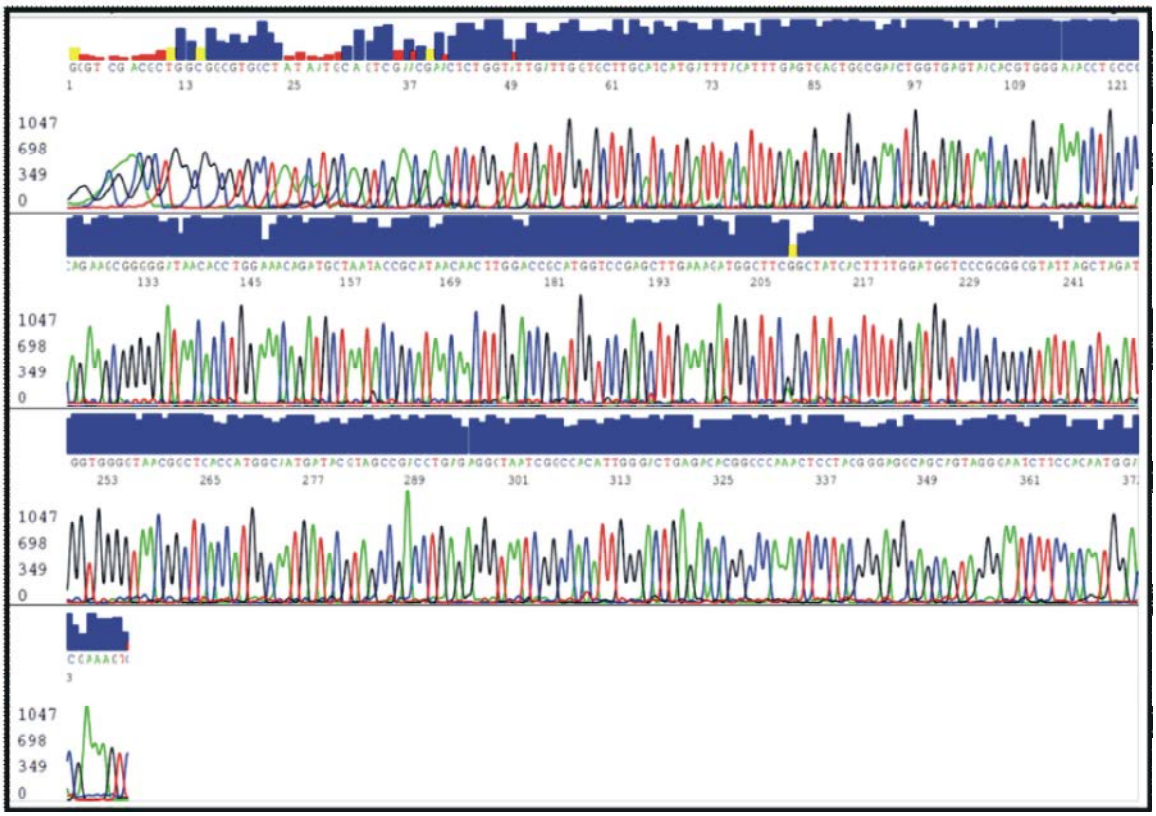

Fig. 4: Chromatogram of the 16srRNA sequence of *Lactobacillus paracasei*.

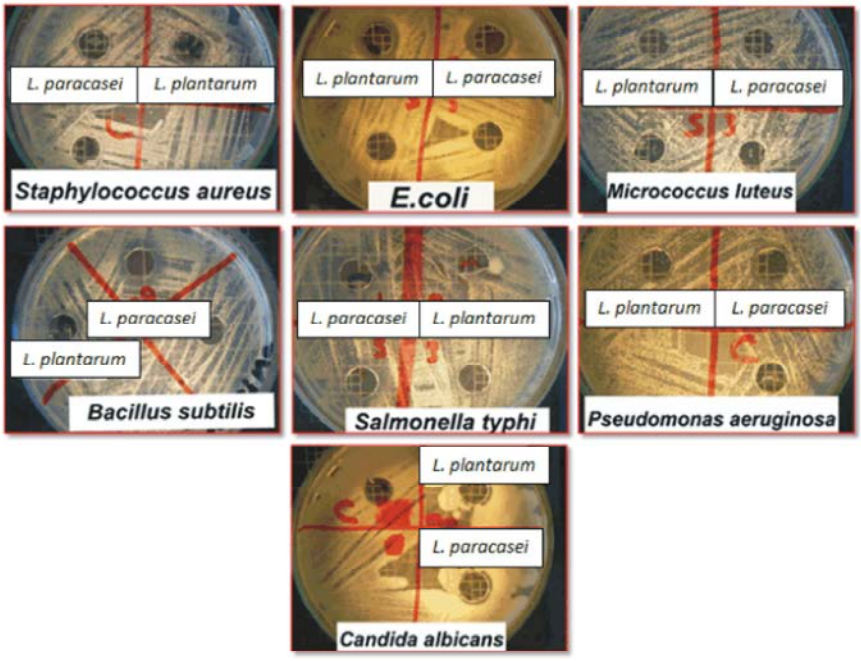

Fig. 5: Inhibitory effect of *L. plantarum* and *L. paracasei.*

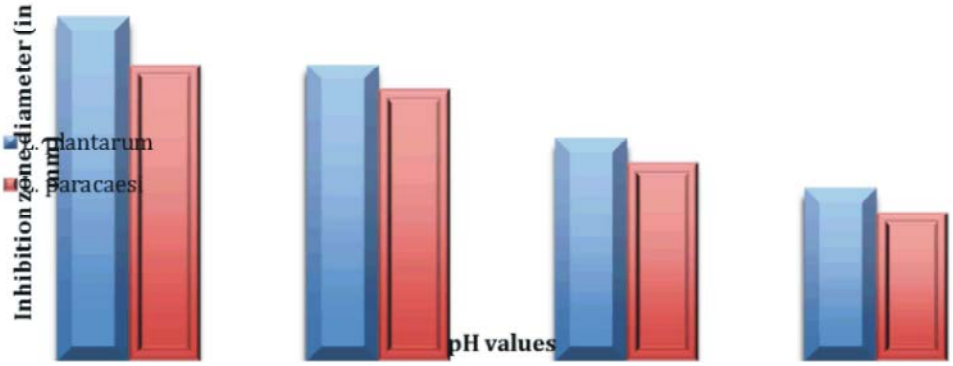

Fig. 6: The effect of different pH values on the antimicrobial activity of *L. plantarum* and *L. paracasei* on *Salmonella typhi*.

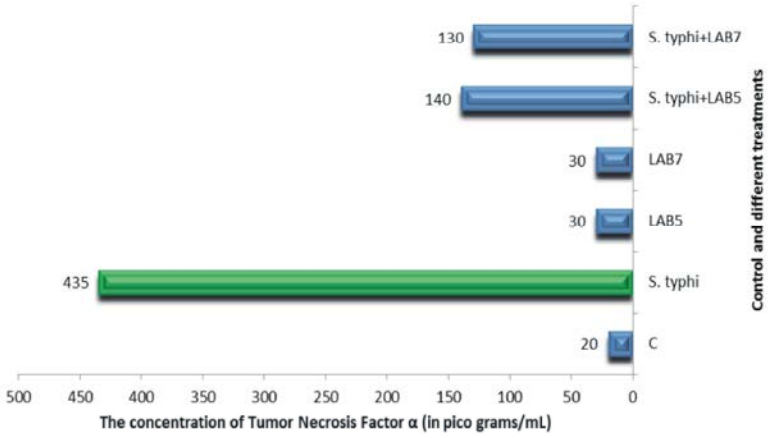

Fig. 7: Effects of LAB5 and LAB7 on TNF-α level in mice exposed to *S. Typhi*.

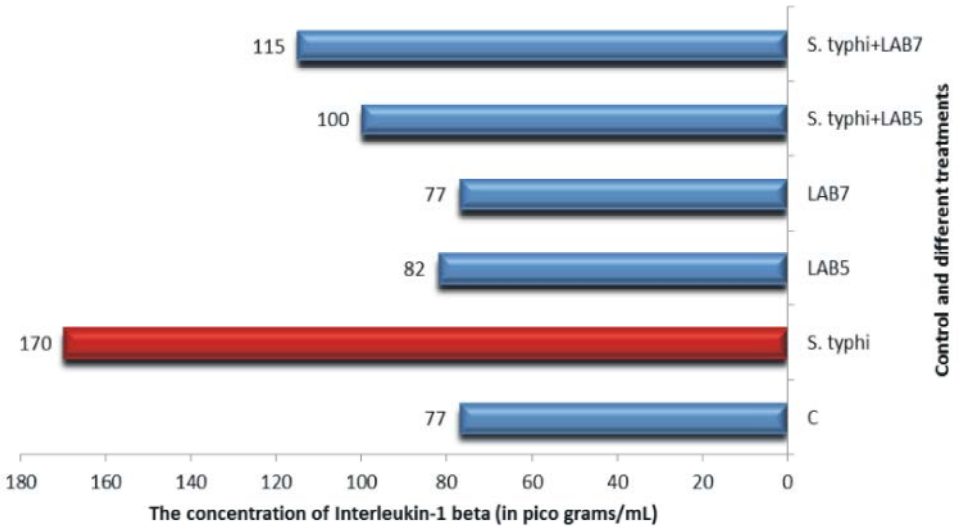

Fig. 8: Effects of LAB5 and LAB7 on IL-1β level in mice challenged with *S. typhi.*

Table 4: Analysis of Variance for the differences in TNF- α between different treatment groups (P<0.05)

S.O.V	Df	MS
Between groups	5	140804.29*
Within groups	24	3108.83
Total	29	

S.O.V: Source of variation
Df: degrees of freedoms,
MS: means square

Table 5: Analysis of Variance for the differences in IL-1β between different treatment groups (P<0.05)

S.O.V	Df	MS
Between groups	5	5587.295*
Within groups	24	103.775
Total	29	

S.O.V: Source of variation
Df: degrees of freedoms,
MS: means square

normal distribution of hepatocytes (Figure 9c). The liver section of animals exposed to *S. typhi* infection showed complete distortion of the lobules and replacement by variable aggregation of the inflammatory cells around the portal tracts, multifocal necrosis and with fibrous tissue (Figure 9d). Photomicrograph of liver section of infected mice with *S. typhi* and treated with *L. plantarum* showed marked decrease in inflammatory cells and restore the portal tract size, as well as few small nodules of inflammatory cells (Figure 9e). Hepatic tissues of group treated with *L. paracasei* and challenged with *S. typhi* infection displayed marked improvement in hepatocytes and the central vein. Restore the portal tract structure and decrease in the inflammatory cells and fibrous tissues (Figure 9f).

The histological examination of the intestine section of the control group showed normal mucosa is thrown into finger like villi. The crypts of Lieberkuhn appear extending down to the muscularis mucosa (Figure 10a). The mice treated with *L. plantarum L. plantarum* showed normal tall finger like intestine villi while some enterocytes nuclei were pyknotic (Figure 10b). Histological examination of intestine cells in mice treated with *L. paracasei* displayed normal tall finger like intestine villi, some crypts are damaged with congested blood capillaries (Figure 10c). The intestine section of animals challenged with *S. typhi* infection show shortening of the villi with bunt, broad apical surfaces, destruction and exfoliation in the epithelial lining of villi which was replaced by pyknotic nuclei. The core of the villi shows cellular infiltration and empty intercellular spaces which may be due to edema. It also shows congested blood capillaries in the lamina propria as compared with control group (Figure 10d). Photomicrograph of intestine section of infected mice with *S. typhi* and treated with *L. plantarum,* showed the surface columnar cells of some villi contain vesicular nuclei and regular brush border (Figure 10e). Magnification of a section in the intestine of salmonella and *L. paracasei* treated mouse, showed multiple crypt lined by normal enterocytes between the bases of the villi (Figure 10f).

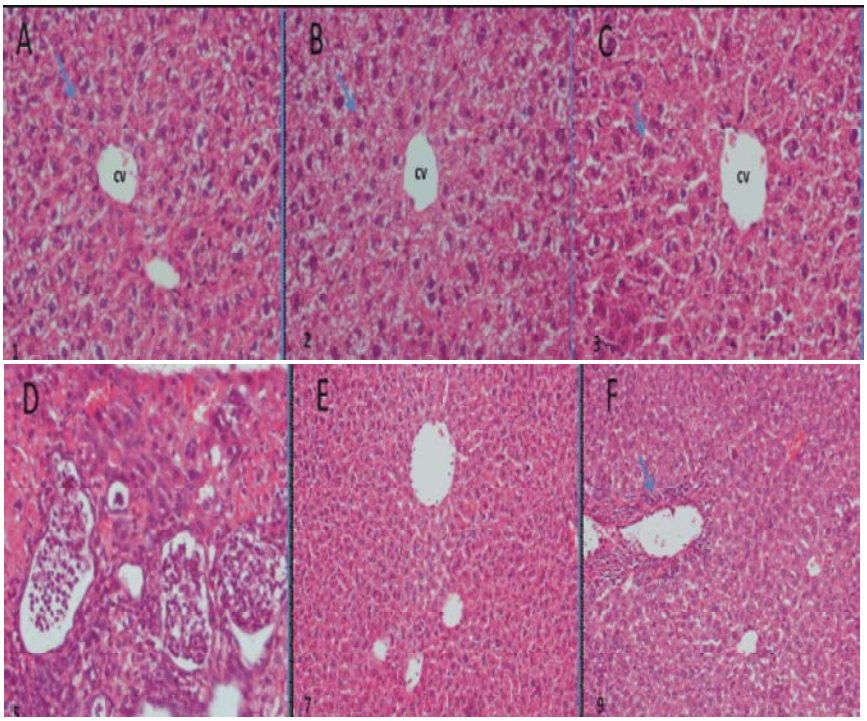

Fig. 9: A photomicrograph of a section of the liver. a: the control group, b mice treated with *L. plantarum*, c: mice treated with *L. paracasei*, d: mice treated with *S. typhi*, e: mice treated with *S. typhi* + *L. plantarum*, and f: mice treated with *S. typhi* + *L. paracasei*.

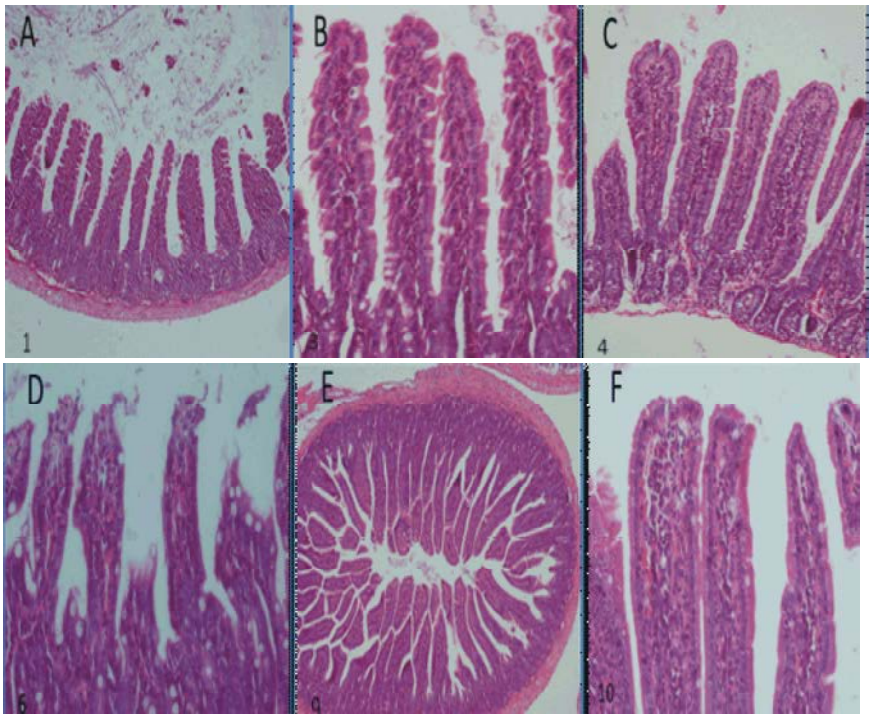

Fig. 10: A photomicrograph of a section of the intestine. a: the control group, b mice treated with *L. plantarum*, c: mice treated with *L. paracasei*, d: mice treated with *S. typhi*, e: mice treated with *S. typhi* + *L. plantarum*, and f: mice treated with *S. typhi* + *L. paracasei*.

DISCUSSION

The human intestinal tract is inhabited by different species of microorganisms; some of which are responsible for the microbial balance in the normal flora of healthy hosts, as the balance of intestinal system flora is dependent on the interactions between the beneficial and harmful microorganisms [16]. Many probiotic effects are mediated through immune regulation, particularly through balance control of pro-inflammatory and anti-inflammatory cytokines [17].

In the present study, eight lactic acid bacteria were isolated from dairy products, including *pediococcus pentosaceus*, two isolates of *Enterococcus faecium* and two isolates of *lactococcus lactis*, *Lactobacillus paracaesei Lactobacillus plantarum* and .

Biochemical identification of the isolates was performed using API 50CH system. Also, 16s rRNA sequencing was used to confirm the identity of the isolated strains. The amplified bands were separated on agarose gel electrophoresis (1.2%) and migration profile could differentiate between *Lactobacillus plantarum* and *Lactobacillus paracasei* strains. Studying the antimicrobial activity of the isolated lactic acid strains was performed against many reference strains including *Bacillus subtilis* ATCC 23857, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 90028, *Micrococcus luteus* ATCC 21882 and *Salmonella typhi*. *Lactobacillus plantarum* and *Lactobacillus paracasei* strains had a significant antimicrobial activity against the tested strains. Furthermore, we tested both strains for their ability to produce bacteriocins and we also studied their effect on mice infected with Salmonella. Meanwhile, we also compared the immunological properties of the two strains in conjunction with Salmonella Typhi, or individually through testing TNF- α and IL-1β levels in mice blood.

The largest group of lactic acid bacteria belong to the genus of Lactobacillus that comprises more than 50 different species [18, 19]. The lactobacilli constitute a major group of the Lactic Acid Bacteria (LAB) [20].

In vitro survival of bacterial strains in low pH is a more accurate indication of the ability of strains to survive passage through the stomach. The organisms taken orally have to face stresses from the host which begin in the stomach, with pH between 1.5 and 3.0 [21]. Many scientific reviews demonstrated the capacity of *L. plantarum* to tolerate gastric acidic conditions [22]. Our data demonstrated that an inhibition zone of both *L. plantarum* and *L. paracaesei* was up to pH 2, which was consistent with the previously published data. Many studies reported that tolerance to acid and other gastrointestinal stresses is strain specific [23, 24]. For strains to survive and colonize the gastrointestinal tract, microorganisms should express tolerance to acid and bile [25]. It has been suggested that food intake could protect bacteria during gastric passage [26]. The pH, physical and chemical characteristics of a food carrier in which potential probiotics are relayed into the gut may have a buffering effect and significantly influence survival of the microorganisms [27].

In the present study, API 50 CHL systems was used to identify lactobacillus strains. The isolates were identified with 99.9% confidence *as L. paracaesei* and *L. plantarum*, based on the fermentation patterns.

Molecular identification methods had great significance where high heterogeneity between isolated stains was present [28]. Phylogenetic analysis using 16srDNA showed 100% idenetity for *L.plantarum*. 16srDNA was used in the present study to prove both lactobacillus strains, *L. plantarum* and L.*paracasei* identity in concordance with previous studies.

Antibiotic resistance of microorganisms used as probiotic agents is an area of growing concern. It is believed that antibiotic used for food-producing animals can promote the emergence of antibiotic resistance in bacteria present in the intestinal microflora. Then, the antibiotic-resistant bacteria can transfer the resistance factor to other pathogenic bacteria through the exchange of genetic material [29]. A recent study reported that the lactobacilli isolated from commercial products in Europe comprised strains resistant to tetracycline (29.5%), chloramphenicol (8.5%) and erythromycin (12%) and overall, more than 68% of the isolates exhibited resistance to two or more antibiotics [30]. It was reported that the most resistant species to the tested antibiotics were *L. plantarum* and *P.pentosaceus*. It was demonstrated that Lactobacilus strains showed high minimum inhibitory concentration (MIC) values to Ciprofloxacin (MIC of 50 to 64 µg/ml) [31]. Similar results were previously reported [32].

An antagonistic effect of the Lactobacillus strains was proved against many pathogenic strains such as *L. casei, L. acidophilus* HM1*, L. fermentum* HM2*, L. fermentum* HM3*, L. buchneri* FG1*, L. buchneri* FD1*, L. buchneri* FD2*, L. casei* BF1*, L. casei* BF2 *and L. casei* BF3, but the degrees of antagonism varied among the Lactobacillus strains [33].

Antimicrobial Assay of the Bacteriocin, a product of lactobacillus acidophilus, was performed to test the spectrum of inhibition of lactic acid bacteria products against *S. typhi*, *M. luteus*, *S. faecalis* and *B*. subtillis, *S. aureous*, *P. aeruginosa*, *S. albus* and *E. coli* and resulted in an inhibition zone with different diameters.

Lactobacillus plantarum showed bactericidal action against many reference microorganisms , These data were consistent with our results of spectrum of inhibition using agar well diffusion test where the product of *L. Plantarum* was effective on *P. aeruginosa* with inhibition zone of 10 mm, *S.aureus* with inhibition zone of 11 mm, *M. luteus* with inhibition zone of 12 mm, moreover our test showed that the inhibition zone for *Salmonella typhi* was 10 mm.

Metabolites of *L. acidophilus* significantly inhibited the growth of both pathogenic bacteria used and can be used as potential antibiotic or probiotic agents [34].

Moreover it could be interpreted that *L. paracasei* possessed bactericidal properties by producing some inhibitory substances, for its ability to inhibit *Bacillus subtilis*, *Pseudomonas auerginosa*, *Staphylococcus aureus* [35].

Mixture of four Lactobacillus or four Bifidobacterium species reduced DSS-induced weight loss, colonic damage, inflammatory cytokine expression in colon, through reduced mRNA expression of TNF-alpha and TGF-beta1and down-regulation of the production of TNF-alpha from distal colon explants [36]. *F. prausnitzii* induced antin Flammatory effect in TNBS induced colitis in mice; through reduction of colonic inflammatory cytokines as it was able to block NF-kappa B activation and IL-8 production on Caco-2 cells [37].

Lactobacillus plantarum has been found to produce bacteriocins that were found to confer anti-bacterial and antifungal activity against many pathogens including potential pathogens and food spoiling bacteria [38]. Several *L.plantarum* strains have been screened for medical use *in vivo* and *in vitro* for example, *L. plantarum* exhibited anti-Salmonella mechanisms through secretion of antimicrobial compounds, adhesion ability and competitive adhesion to mucin and HT-29 cell line [39]. In vitro evidence suggested that *Lactobacillus paracasei* also called ST11 is a potent strain with immune modulation properties [40]. *Lactobacillus paracasei* ST11 was previously shown to adhere to intestinal epithelial cell line and have antimicrobial activity *in vitro* [41].

Salmonellosis are considered to be the major bacterial disease in the poultry industry world-wide [42]. Several potential virulence factors of *Salmonella enteritidis* may contribute to infection and intestinal mucosal damage [43].

Agglutination is a classic serologic reaction that results in clumping of a cell suspension by a specific antibody, directed against a specific antigen [44]. Widal agglutination test was used for TNF-alpha's administration 24 h before the administration of bacteria reduced the establishment of intracellular infection in the intestinal epithelial cells and development of bacteremia in mice models [45]. TNF-a is crucial to host resistance to Salmonella spp., being constantly required for the suppression of bacterial growth in the RES (plateau phase) [46, 47]. The in vasive wild type Salmonella strains were observed in the laminae propriae of the intestinal villi [44]. In the present study, the intestine villi of Salmonella treated rats showed congestion, degeneration of some cryptal cells and interstitial connective tissues. Also, mice liver sections showed inflammatory in some areas of necrosis in the positive control group. Also, the number of infiltrating cells was greatly reduced in the probiotic, *E. coli* and gentamicin groups compared with the positive control group, suggesting that oral administration of probiotics, nonpathogenic *E. coli*, or gentamicin could attenuate liver injury.

CONCLUSIONS

We can conclude that Lactobacilli are promising candidate in terms of protection and eradication of a wide range of bacterial infection due to its anti-microbial, anti-inflammatory and immunodulatory activities.

REFERENCES

1. Kumar, M., R. Nagpal, V. Verma, A. Kumar, N. Kaur, R.Hemalatha, S. K. Gautam and B. Singh, 2013. Probiotic metabolites as epigenetic targets in the prevention of colon cancer. Nutr. Rev., 71: 23-34.
2. Bisanz, J.E., M.K. Enos, J.R. Mwanga, J. Changalucha, J.P. Burton, G.B. Gloor and G. Reid, 2014 . Randomized open-label pilot study of the influence of probiotics and the gut microbiome on toxic metal levels in Tanzanian pregnant women and school children. MBio, 5, e01580-14.
3. Brandsma, J.L., M. Harigopal, N.B. Kiviat, Y. Sun, Y. Deng, D. Zelterman, P.M. Lizardi, V.S. Shabanova, A.T. Levi. T. Yaping, X. Hu and Q. Feng, 2014. Methylation of twelve CpGs in human papillomavirus type 16 (HPV16) as an informative biomarker for the triage of women positive for HPV16 infection. Cancer Prev. Res. Phila, 7: 526-33.

4. Casey, E., J. Mahony, H. Neve, J.P. Noben, F. Dal Bello and D. Van Sinderen, 2015. Novel phage group infecting Lactobacillus delbrueckii subsp. lactis, as revealed by genomic and proteomic analysis of bacteriophage Ldl1. Appl. Environ, Microbiol., 81: 1319-26.
5. Desguin, B., P. Goffin, N. Bakouche, A. Diman, E. Viaene, D. Dandoy, L. Fontaine, B. Hallet and P. Hols, 2015. Enantioselective regulation of lactate racemization by LarR in Lactobacillus plantarum. J. Bacteriol., 197: 219-30.
6. Mezaini, A., N.E. Chihib, A. Dilmi Bouras, N. Nedjar-Arroume and J.P. Hornez, 2009. Antibacterial Activity of Some Lactic Acid Bacteria Isolated from an Algerian Dairy Product. J. Environ Public Health, pp: 6.
7. Garg, R., A.S. Akhade, J. Yadav and A. Qadri, 2015. MyD88-dependent pro-inflammatory activity in Vi polysaccharide vaccine against typhoid promotes Ab switching to IgG. Innate Immun., pp: 30-77.
8. Hendriksen, R.S., P. Leekitcharoenphon, M. Mikoleit, J.D.R.S. Jensen, R.S. Kaas, L. Roer, H.B. Joshi, S. Pornruangmong, C. Pulsrikarn, G.D. Gonzalez-Aviles, E.A. Reuland, N. Al Naiemi, A.L. Wester, F.M. Aarestrup and H. Hasman, 2015. Genomic dissection of travel- associated extended- spectrum- beta-lactamase- producing Salmonella enterica serovar typhi isolates originating from the Philippines: a one-off occurrence or a threat to effective treatment of typhoid fever? J. Clin Microbiol., 53: 677-80.
9. Parry, C.M., N.T. Thieu, C. Dolecek, A. Karkey, R. Gupta, P. Turner, D. Dance, R.R. Maude, V. Tran, C.N. Thi, P.L. Be, B.P. Phi, L.T. Ngoc, R.N. Ghose, A. Dongol, S. Campbell, J.I. Thanh, D.P. Thanh, T.H. Moore, C.E. Sona, S. Gaind, R. Deb, M. Anh, R. Phetsouvanh, P. Newton, B. Basnyat, J.J. Farrar and S. Baker, 2015. Clinically and microbiologically derived azithromycin susceptibility breakpoints for Salmonella enterica serovars Typhi and Paratyphi A. Antimicrob Agents Chemother, 59: 2756-64.
10. Kortmann, J., S.W. Brubaker and D.M. Monack, 2015. Cutting Edge: Inflammasome Activation in Primary Human Macrophages Is Dependent on Flagellin. J. Immunol., 195: 815-9.
11. Shruti Sharmaa, M. Allison, P. Camp, C. Jennie, A. Stefan Schattgena, Gregory M. Orlowskic, Ribhu Nayarc, Annie H. Huylera, Kerstin Nündeld, Chandra Mohane, Leslie J. Bergc, Mark J. Shlomchikf, Ann Marshak-Rothsteind and Katherine A. Fitzgeralda, 2015. Suppression of systemic autoimmunity by the innate immune adaptor STING 34-78.
12. Andreas, V., L. Victoria, M. Demetriou, 2009. Molecular beacon-based real-time PCR detection of primary isolates of Salmonella Typhimurium and Salmonella Enteritidis in Environmental and Clinical Samples, pp: 512-980.
13. Cocchetto, D.M. and T.D. Bjornsson, 1983. Methods for vascular access and collection of body fluids from the laboratory rat. J. Pharm. Sci., 72(5): 465-492.
14. Soda, M.E., N. Ahmed, N. Omran, G. Osman and A. Morsi, 2003. Isolation, identification and selection of lactic acid bacteria cultures for Cheesemaking, 61: 867-74.
15. Yeung, P.S., M.E. Sanders, C.L. Kitts, R. Cano and P.S. Tong, 2002. Species-specific identification of commercial probiotic strains. J. Dairy Sci., 85: 1039-51.
16. Fujimura, K.E. and N.A. Slusher, 2010. Role of the gut microbiota in defining human health. Expert Review of Anti-infective Therapy, 8(4): 435-454.
17. Isolauri, E. and Y. Sütas 2001. Probiotics: effects on immunity. The American Journal of Clinical Nutrition, 73: 444s-450s.
18. Stiles, M.E. and W.H. Holzapfel, 1997. Lactic acid bacteria of foods and their current taxonomy. Int J. Food Microbiol., 36(1): 1-29.
19. Tannock, G.W., 2004. A Special Fondness for Lactobacilli. Applied and Environmental Microbiology, 70: 3189-3194.
20. Avonts, L. and L. De Vuyst, 2001. Antimicrobial potential of probiotic lactic acid bacteria. Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet., 66(3b): 543-550.
21. Corzo, G. and S.E. Gilliland, 1999. Measurement of bile salt hydrolase activity from Lactobacillus acidophilus based on disappearance of conjugated bile salts. J. Dairy Sci., 82: 466-471.
22. Kalui, C.M., M.J.M.P.M. Kutima, C. Kiyukia and L.E. Wongo, 2009. Functional characteristics of Lactobacillus plantarum and Lactobacillus rhamnosus from ikii, a Kenyan traditonal fermented maize porridge. African Journal of Biotechnology, 8: 4363-4373.
23. Morelli, L., 2000. In vitro selection of probiotic lactobacilli: a critical appraisal. Curr Issues Intest Microbiol., 1: 59-67.
24. Huang, Y. and M.C. Adams, 2004. *In vitro* assessment of the upper gastrointestinal tolerance of potential probiotic dairy propionibacteria. Int J. Food Microbiol., 91: 253-260.
25. Gibson, G.R., 1999. Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. J. Nutr., 129(7 Suppl): 1438S-1441S.

26. Charalampopoulos, D. and S.S. Pandiella, 2003. Evaluation of the effect of malt, wheat and barley extracts on the viability of potentially probiotic lactic acid bacteria under acidic conditions. Int J. Food Microbiol., 82: 133-141.
27. Patel, H.M. and S.S. Pandiella, 2004. Influence of malt, wheat and barley extracts on the bile tolerance of selected strains of lactobacilli. Food Microbiology, 21(1): 83-89.
28. Terzic-vidojevic, A., M. Likolic, J. Lozo, J. Begovic, A.A.K. Sahib, G.O. Gulahmadov and M. Dalgalarrondo, 2009. Phenotypic and genotypic characterization of lactic acid bacteria isolated from Azerbaijani traditional dairy products. African Journal of Biotechnology, 8: 2576-2588.
29. Mathur, S. and R. Singh 2005. Antibiotic resistance in food lactic acid bacteria--a review. Int J Food Microbiol., 105(3): 281-295.
30. Temmerman, R. and B. Pot, 2003. Identification and antibiotic susceptibility of bacterial isolates from probiotic products. Int J. Food Microbiol., 81(1): 1-10.
31. Rojo-Bezares, B. and Y. Saenz 2006. Assessment of antibiotic susceptibility within lactic acid bacteria strains isolated from wine. Int J. Food Microbiol., 111(3): 234-240.
32. Elkins, C.A. and L.B. Mullis, 2004. Bile-mediated aminoglycoside sensitivity in Lactobacillus species likely results from increased membrane permeability attributable to cholic acid. Appl. Environ. Microbiol., 70(12): 7200-7209.
33. Shokryazdan, P. and C.C. Sieo, 2014. Probiotic Potential of Lactobacillus Strains with Antimicrobial Activity against Some Human Pathogenic Strains. BioMed Research International 2014:
34. Assan, P., 2013. Characteristics and Antibacterial Activity of Metabolites from Lactobacillus acidophilus Strains Produced from Novel Culture Media. International Journal of Pharmacology, 9: 92-97.
35. Tariful Islam, F.S., E.M. Billah, K.M. Didarul Islam 2012. Analysis of Antimicrobial Activity of Lactobacillus paracasei ssp. paracasei-1 Isolated from Regional Yogurt, 1(4): 74-104.
36. Avonts, L. and L. De Vuyst, 2001. Antimicrobial potential of probiotic lactic acid bacteria. Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol. Wet., 66(3b): 543-550.
37. Sokol, H. and B. Pigneur, 2008. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proceedings of the National Academy of Sciences.
38. Grosu-Tudor, S.S. and M.M. Stancu, 2014. Characterization of some bacteriocins produced by lactic acid bacteria isolated from fermented foods. World J. Microbiol. Biotechnol., 30(9): 2459-2469.
39. Uraipan, S. and P. Brigidi, 2014. Antagonistic mechanisms of synbiosis between Lactobacillus plantarum CIF17AN2 and green banana starch in the proximal colon model challenged with Salmonella Typhimurium. Anaerobe, 28(0): 44-53.
40. Bernet, M.F. and D. Brassart, 1994. Lactobacillus acidophilus LA 1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. Gut., 35(4): 483-489.
41. Kabir, S.M.L., 2010. Avian Colibacillosis and Salmonellosis: A Closer Look at Epidemiology, Pathogenesis, Diagnosis, Control and Public Health Concerns." Int J Environ Res Public Health, 7(1): 89-114.
42. Awad, W.A. and J.R. Aschenbach, 2012. Intestinal epithelial responses to Salmonella enterica serovar Enteritidis: Effects on intestinal permeability and ion transport. Poultry Science, 91(11): 2949-2957.
43. Olopoenia, L.A. and A.L. King, 2000. Widal agglutination test - 100 years later: still plagued by controversy. Postgrad Med. J., 76(892): 80-84.
44. Degre, M. and G. Bukholm, 1990. Effect of tumor necrosis factor-alpha on infection with Salmonella typhimurium in a mouse model. J. Biol., Regul Homeost Agents, 4(4): 157-161.
45. Mastroeni, P. and A. Arena, 1991. Serum TNF alpha in mouse typhoid and enhancement of a Salmonella infection by anti-TNF alpha antibodies. Microb Pathog, 11(1): 33-38.
46. Mastroeni, P. and B. Villarreal-Ramos, 1993. Effect of late administration of anti-TNF alpha antibodies on a Salmonella infection in the mouse model. Microb. Pathog, 14(6): 473-480.
47. Nakoneczna, I. and H.S. Hsu, 1980. The comparative histopathology of primary and secondary lesions in murine salmonellosis. British Journal of Experimental Pathology, 61(1): 76-84.