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Research Article

Cholesterol-lowering Effects and Safety Assessment of *Lactobacillus* spp. *In Vivo* and *In Vitro* Testing for Human Use as Probiotic from Dairy Product in Egypt

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Abstract

The toxicity profile may be strain dependent and among *Lactobacilli*, one of the most important genus of LAB (Lactic acid bacteria) Further *in vivo* studies are necessary, and this study focused on the safety, health and growth performance of mice receiving these two strains. The aim of the present investigation is to characterize a number of metabolic properties of *Lactobacillus* and enhance the understanding of its influence on bacterial metabolism and gut function beside the ability to hydrolyze bile salts Using primer for bile salt hydrolysis gene to confirm probiotic character of superior isolates , the present study showed the isolates meet several function features to be considered as suitable probiotic for application in food fermentation and the isolated bacteria able to tolerate acidic medium and bile salt a favorable enzymatic movement and no hemolytic movement adjacent to has been included within the determination criteria for probiotic strains with cholesterol-lowering impacts, so we consider it awesome potential probiotic character and secure for human utilize.

Keywords: Safety Assessment; *Lactobacillus*; Enzymatic Activity; Probiotic

Introduction

The Greeks and Romans stated that human consumption of fermented foods as beginning of the history of probiotics [38]. Metchnikoff hypothesized that, a healthy and long-lived individual utilized matured dehydrated items that incorporate rod-shaped microbes (*Lactobacillus spp*.). Therefore, these bacteria positively affect the gut microflora and decrease toxic microbial activity [11,13,38]. LAB (*Lactobacillus*) is a nonpathogenic, source of organic nutrient, a type of microbe that get utilized as component for generating numerous mature sustainable items. LAB transforms glucose into lactic acid and corrosive acid, ethanol and CO_2, which adds to the quality, surface and smell of aged items. LAB additionally deliver bacteriocins and different aggravates that hinder the development of [17] microbial cells normally absorb metal particles due to their ability to create cell layers [8]. LAB has recently been applied during metal particle restriction, in spite of the results of reviews that investigated the coupling capacity of metal particles of different microorganisms. Wang and Chen [64] have performed a broad review of past studies on the biosorption limits of individual clusters of microorganisms.

Safety aspects of probiotics

Aged foods are recognized as the essential specialty of LAB action in spite of the fact that Lactic acid growing microbes (LAB) are identified in foods, the environment and the human intestine [30], Nowadays, there's prove that probiotic strains are utilized as commercial microscopic organisms secure for utilize the security of probiotic items is evaluated based on the phenotypic and geno-

typic characteristics as well as measurements of the microbe [11]. Michiel Kleerebezem., *et al*. [46] by preventing the outgrowth of pathogenic and spoiled organisms' role of lactococci in these fermentations, acidification is dependent on efficient conversion of the milk-sugar lactose to lactic acid, which contributes to the extended shelf life of fermented milk products by preventing the outgrowth of pathogenic and spoilage organisms. Security viewpoints of probiotic microbes incorporate the following prerequisites:

- Strains for human are isolated from the healthy human gastrointestinal tract.
- Strains for human are non-pathogenic.
- Strains for human have no history of a relationship with illnesses such as infective endocarditis or gastrointestinal tract disarrange.
- Strains for human do not deconjugate bile salts.
- Strains for human ought to not carry transmissible anti-microbial resistance genes.

Enzymatic activity of *Lactobacillus*

Supplementation of *Lactobacillus* with dairy products exerts a significant influence on microbial metabolism in the colon by reducing the activities of fecal p-glucuronidase and nitroreductase which are related to the release and formation of toxic compounds in the colon [5]. However, the biochemical characteristics of *Lactobacillus* are currently not well understood. Clarification of the bacterial enzymes of *Lactobacillus* may facilitate its identification, aid in its taxonomic placement, increase its utilization in the dairy industry, and enhance the understanding of its influence on bacterial metabolism and gut function. The purpose of the present study is to characterize a number of metabolic characters of *Lactobacillus*. The API ZYM kit was used to detect 19 different hydrolases from *Lactobacillus* and other *lactobacilli* as well as bacterial isolates. This rapid and simple method might be useful for the classification of probiotic bacteria [42].

Anti-microbial resistance in lactic acid microbes separated from food Lactic corrosive microscopic organisms are broadly utilized as probiotics or in starter cultures and have the potential to serve as a haven of anti-microbial resistance genes; hence utilize of LAB increments the chance of exchanging antibiotic resistance qualities to lactic acid microscopic organisms and other pathogenic microbes. In later a long time, there has been expanded center on nourishment as a vehicle of anti-microbial resistance genes [32,41,53]. [16] Report overview of techniques available to study transfer of mobile DNA in microbial communities.

However, there have been very few systematic studies on LAB acquired antibiotic resistance from food. Most data are from opportunistic pathogenic enterococci, while there are few reports on lactococci and lactobacilli. Via the food chain Vancomycin-resistant enterococci (VRE) (which has cross-resistance to vancomycin) are spread, which have emerged in the last decade as a frequent cause of nosocomial infections [37,40,62,63]. using phenotypic analysis for their resistances to a broad range of different antibiotics Enterococcal food isolates (mainly *Enterococcus faecalis and E. faecium*) were, both in raw meat [37,40,53,57] and dairy product and meat [5,31,37,60]. [10] Studied the *in vitro* susceptibility and resistance of *E. faecium* strains separated from crude poultry meat, cheese, crude pork, and arrangements of cheese and crude pork to growth-promoting against bacterial utilized in creatures and antimicrobials utilized remedially in patients. In another study, enterococci are generally regarded as being intrinsically resistant to low levels of gentamicin, *enterococci* isolated from (milk and cheese) were screened for gentamicin resistance [44]. A high-level gentamicin resistance has been detected in many dairy isolates.

[26] assessed the molecular components of gentamicin resistance in Enterococcus separates from creatures, foods and patients. It has been proposed that there are likenesses in gentamicin resistance in enterococci separated from humans, retail food, and cultivate creatures from geologically different ranges as well as prove of the spread of gentamicin-resistant enterococci from creatures to people through the food supply [39]. Tetracycline resistance could be linked to the presence of tet (M) genes in Enterococcal isolates.

Many studies have detailed a generally vulnerability to antimicrobial agents (with the special case of inborn resistance) of strains utilized as meat starter cultures [34,54] or dairy product [40,56]. Information of inherently encoded resistance of LAB to common anti-microbials is fundamental to recognize obtained resistance characteristics.

For a number of lactobacilli, exceptionally tall frequencies of unconstrained transformations in reaction to nitrofurazone (10^{-5}), kanamycin and streptomycin have been found [39]. From this information, it is obvious that intergenus and interspecies contrasts exist, and thus, recognizable proof at the species level is required to translate the phenotypic vulnerability information. Another study was performed to establish the levels of susceptibility of *Lactobacillus spp.* to various antimicrobial agents [18], and this study revealed a species dependence.

In a report performed by [61], The resistance spectrum of *Bifidobacterium* was described by [12]. The investigated (probiotics) *bifidobacteria* were susceptible to many of antibiotic. Resistance, some of which was most likely intrinsic, was found.

Strains of *Lactococcus lactis* were sensitive to amikacin, ampicillin, 1st generation cephalosporin, and man antibiotics [21]. A slightly reduced susceptibility also was observed. Numerous medicate efflux proteins were found in *L. lactis subsp. lactis* [62], one of which was an ABC transporter and the other of which was a proton motive force-dependent drug transporter. The natural substrates of these proteins are not known.

Resistance against vancomycin is due to the nearness of d-alanine: d-alanine ligase-related proteins [29]. Fifteen strains of Streptococcus thermophilus from yoghurt cultures showed varying resistance levels to different antibiotic [60].

In a report done by [4], *S. thermophile* strains were examined to determine their antibiotic resistance patterns and plasmid carriage. Most strains of *S. thermophilus* were resistant to gentamicin and susceptible to others; however, no correlation was observed between the resistance to antibiotics and occurrence of plasmids in some strains. [15] studied the antibiotic resistance and incidence of *Enterococcus species* in white cheese.

The opinion of the Scientific Committee on Animal Nutrition (SCAN) on the criteria that should be used to assess the safety of microorganisms that are resistant to antibiotics of human clinical and veterinary importance was 2002 (European Commission 2002). Agreeing to [59], all bacterial items aiming for utilize as bolster added substances must be inspected to set up the defenselessness of the component strain(s) to a significant extend of antimicrobial. Such tests must be performed in a steady way utilizing globally recognized and standardized strategies.

Effect of *Lactobacillus* species on weight gain

As numerous probiotic strains of *Lactobacillus* and Bifidobacterium are promoted in items for human utilization [33], we hypothesize that broad ingestion of probiotics may advance corpulence by changing the intestinal greenery [56]. However, this hypothesis remains questionable [25]. By contrast, manipulation of the gut microbiota by probiotics has been used to promote growth in farm animals for at least 30 years [34]. Indeed, *Lactobacillus* acidophilus, are often commonly used *Lactobacillus spp.* in agriculture [3].

All these information unequivocally propose that *Lactobacillus* containing probiotics (LCP) may affect the weight control in people and creatures. Numerous considers have detailed the impacts of *Lactobacillus*-containing probiotics (LCP) on weight, but concurring to later information [47], this impact is, at slightest, species subordinate. In this way, we pooled information from creature and human considers getting adequate control to identify a noteworthy impact at the species level.

Methods

Probiotic characterization by molecular tools

Using primer for bile salt hydrolysis gene to confirm probiotic character of superior isolates, giving positive result indicating that isolates able to hydrolyze bile salt as a probiotic character as [66] who reported that characterization and identification of native isolates using molecular tools.

Bile salt hydrolase bsh primer

F 5′ GGATTGTGTATTGCGGGATT 3′ 422

R 5′ AGTCCGCCCATTCCTCTACT 3′

With thermal profile

95°C 4′; 35 cycles of 94°C 1′; 55°C 40 s; 72°C 2′; final one cycle of 72°C 10′ [66].

Safety assessment of *Lactobacillus* isolates

Study enzyme activity of *Lactobacillus* isolates

The API ZYM pack (bio-Mérieux, France) was utilized to consider chemical action generated by confines. Each recognized isolate was developed overnight at 37°C on MRS broth, silt from centrifuged culture broth was utilized to plan a suspension at 10^5

CFU/ml, this suspension used to inoculate the API ZYM kit cupules, hatched for 4 h at 37°C, at that point a surface-active operator (ZYM A reagent) included within the cupules to encourage solubilization of the ZYM B reagent within the medium, color was permitted to create for at slightest 5 min, and values extending from 0-5 were allotted comparing to the colors created, the inexact number of free nmole of hydrolyzed substrate was decided based on the color reinforce negative response; 1: 5 nmol; 2: 10 nmol; 3: 20 nmol; 4: 30 nmol; 5: 40 nmol or higher [58].

Antibiotics susceptibility of *Lactobacillus* isolates

In vitro screening of antimicrobial susceptibility for *lactobacilli* isolates was carried out against 15 antibiotics (Ampicillin/sulbactam, amoxicillin/clavulinic acid, Clarithromycin, erythromycin, naldixic acid, trimethoprime/sulphamethoxazole, ciprofloxacin, tetracycline, vancomycin, rifampicin, nitrofuruntoin, chloramphenicol, tenadazole), by the Kirby-Bauer disk diffusion method [56-60], according to clinical laboratory standards institute (USA), document M100-S18, Using pure culture as inoculum, a few colonies (4 to 5) of the organism to be tested were picked with a wire loop and introduced into a test tube containing 5 ml of MRS broth, these tubes were incubated at 35°C for 2 to 5 hours till light to moderate turbidity equivalent to 0.5 McFarland standard develops. The suspension is then diluted (1:10), if necessary, with water or saline to yield a uniform bacterial suspension containing 10^7 cells/ml, Muller Hinton agar plates was inoculated by bacterial suspension using sterile cotton swab, the discs were firmly applied to the surface of the agar plate using aseptic techniques with centers at least 24 mm apart, The plates incubated immediately at 35°C and examined after 16-18 hours.

Test for hemolytic activity of *Lactobacillus* isolates

Lactobacillus separates were refined on blood agar plates containing 5% sheep blood and brooded at 37°C for 48 hours; hemolytic action was recognized as the nearness of a clear zone around bacterial colonies [49].

In vivo studies of *Lactobacillus* isolates

Lactobacillus isolates

3 probiotic LAB isolates used in animal feed and identified as *L. casei, L. lactis and L. acidophilus* were used in this study.

Strain	Preparation used	Cfu/ml
L. acidophilus grown on MRS	Water form	10^8
L. casei grown on MRS	Water form	10^8
L. lactis grown on MRS	Water form	10^8
Control preparations	Water form	0

Table a

Cultures were concentrated by centrifugation and the cell pellets were suspended in solution after three washes and added into drinking water to give a final concentration of 108 cfu/ml, these preparations were added to the drinking water given to the mice at final concentrations of 20% (v/v).

Animals and diets

Mice, 13 - 17g weight, were housed in groups of 10 (5 males and 5 females) per cage. A normal light-dark cycle and a controlled atmosphere (Temperature 22 C; humidity 55%) were maintained throughout the study. The animals were allowed free access to feed, either a basal diet consisting of barley or, a conventional enriched feed (Mice were provided with drinking water).

Experimental design

The mice were acclimatized for 24 h to the experimental conditions. Supplemented drinking water and feed were changed daily. The treatments for the toxicological study lasted for 4 weeks while the growth-promoting treatment lasted 17 days. Hair luster was observed at the end of the treatment period whereas bodyweight of each mouse was recorded daily. On the final day of the test, all the animals were murdered by cervical separation.

Evaluation of growth performance

Individual bodyweight was recorded daily using a balance for mice (Sartorius.) The weight gain (WG) was expressed as the mean of final weight minus initial weight of each mouse. The specific growth rate (SGR) was expressed as the daily weight gain. Feed Intake (FI) and water intake were monitored daily for each cage and expressed per animal for the total period, dividing feed or water consumption by the number of animals. The Consumption Index (CI) was calculated as the ratio FI/WG [7]. Hemoglobin and liver enzyme as parameter for side effect assessment were performed.

Result and Discussion

Safety assessment of probiotic *Lactobacillus* spp

Characterization of probiotics by molecular tools

Using gene specific primer for bile salt hydrolysis and probiotic character of superior isolates, positive result indicated that isolates were able to hydrolyze bile salt as a probiotic character [66], Ziagova., *et al*. reported that characterization and identification of native isolates using molecular tools.

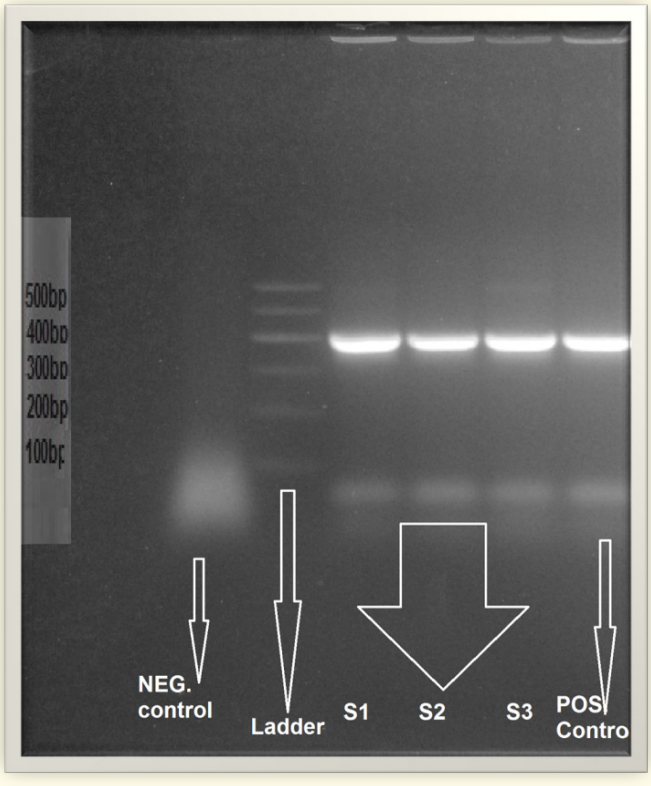

Figure 1: The Gel electrophoresis for Bile salt hydrolysis gene.

Investigation of gene substance of strains yielded as well as numerous strain-specific genes, and the information in this manner had to be combined with other information to associate the watched phenotype with a genotype were expressed in this intestinal compartment. Genes revealed that genes involved in basic cellular anabolic activities like protein, Bile salt hydrolysis.

A bile salt hydrolase gene from *Lactobacillus* plantarum was utilized as a potential food-grade determination marker to develop a novel vector for lactic acid microbes [65].

Cholesterol-lowering impacts of lactic acid microbes may be due in portion to the deconjugation of bile salts by strains that create BSH. As of late, the capacity to hydrolyze bile salts has been included within the determination criteria for probiotic strains with cholesterol-lowering impacts, as numerous non-deconjugating strains were not able to evacuate cholesterol from the culture medium [50]. Numerous reports on hypocholesterolemic impacts *in vivo* by BSH-producing lactic corrosive microbes have driven to expanded consideration in keeping up cholesterol levels in ordinary individuals or the conceivable applications for hypercholesterolemic people in this case, verbal organization of BSH-positive bacterial cells to control serum cholesterol levels appears promising. In any case, other considers moreover appeared that probiotics had immaterial impacts on cholesterol-lowering impacts, hence debating the hypocholesterolemic claim.

Enzymatic Activities of *Lactobacillus* isolates

As a function of time the different endogenous enzymes will become gradually activated and inactivated [45]. All isolates were screened to enzymatic action to distinguish any unfavorable protein just like the carcinogenic enzyme, B-glucuronidase "and presence 'of beneficial enzymes. Enzyme Generation by isolates was an imperative measure in its determination, since carcinogenic Enzymes such as B-glucuronidase can be delivered by microorganisms. When carcinogenic substances such as benzo(a) pyrene enter the human body, their harmful impacts are checked since of conjugation with glucuronic corrosive within the liver. In the event that this conjugated item is excreted with bile corrosive within the digestive tract, cleavage by B-glucuronidase can free these substances to ended up harmful once again [43]. Invertase loses its activity upon fermentation, due to the increasing temperature. The glycosidases α-arabinosidase and β-galactosidase, optimally active under acidic conditions (pH 4.0-4.5), Result recorded in table 8 shown that all isolates did not create the carcinogenic protein, B-glucuronidase, though advantageous was delivered, which is useful for lactose intolerance as shown in (Table 1) These enzymes include leucinearylamidase, crystinearylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, a-galactosidase, P-galactosidase, a-glucosidase, P-glucosidase, and N-acetyl-P-glucosamidase. These results were in understanding with [14]. Similarly, B-galactosidase was found in *Lactobacillus* isolated from fermented oil as reported by [1].

Exchange of resistance to antimicrobial substances is a basic component in LAB in case they are to adjust and survive in par-

Enzyme	Substrate	ph	+ve
Control			
Alkaline phosphatase	2-naphthyl phosphate	8.5	Violet
Esterase	2-naphthyl butyrate	6.5	Violet
Esterase lipase	2-naphthyl caprylate	7.5	Violet
Lipase	2-naphthyl myristate	7.5	Orange
Leucinearylamidase	L-lucyl-2-naphthyl naphthylamide	7.5	Orange
Valinearylamidase	L-valyl-2- naphthylamide	7.5	Orange
Cystinearylamidase	L-CYSTYL-2- naphthyl-amide	7.5	Orange
Trypsin	n-benzoyl-dl-arginin-2-naphthylamide	8.5	Orange
a-Chymotrypsin	n-glutaryl-phenylala-nine-2-naphylamide	7.5	Violet
Acid phosphatase	2-naphthyl phosphate	5.4	Blue
Naphthol-AS-BI-phosphohydrolase	Naphthol-AS-BI-phosphate	5.4	Violet
a-Galactosidase	6-br-2-naphthyl-aD galactopyranoside	5.4	Violet
B-Galactosidase	2-naphyl-BD-galactopyranoside	5.4	Blue
B-Glucuronidase	Naphthol-as-bi~glucopyranoside	5.4	Violet
a-Glucosidase	2-naphyl-Ad	5.4	Violet
B-Glucosidase	6-br-2-naphthyl-aD galactopyranoside5.	5.4	Brown
N-Acetyl-P-glucosaminidase	1 -naphthyl-n-acetyl-aD-glucosaminide	5.4	Violet
a-Mannosidase	6-br-2-naphthyl-aD-aD-mannopyraosid	5.4	Violet
a-Fucosidase	2-naphmyl-aL-fucopyranoside	5.4	Violet

Table 1a: Enzyme activity of *Lactobacillus* isolates using the API ZYM kit.

0, 0 nmol; 1, 5 nmol; 2, 10 nmol; 3, 20 nmol; 4, 30 nmol; 5, ≥ 40 nmol.

ticular environments. Among the resistance components in utilize, protein inactivation of the anti-microbials confined consequence of anti-microbials, dynamic trade of anti-microbials or target alteration may be highlighted [19]. *Lactobacilli* are for the most part resistant to aminoglycosides [20]. Vancomycin resistance is thought to be natural, since about all the strains are constitutively safe to low levels of the antibiotic [41].

Enzyme isolates no	SI	S2	S3	S4	S5	S6	S7	B.M	S8
Control	0	0	0	0	0	0	0	0	0
Alkaline phosphatase	0	0	0	0	0	0	0	0	0
Esterase	0	0	0	0	0	0	0	0	0
Esterase lipase	0	0	0	0	0	0	0	0	0
Lipase	0	0	0	0	0	0	0	0	0
Leucinearylamidase	2	2	2	2	2	2	2	3	2
Valinearylamidase	0	0	0	0	0	0	0	0	0
Cystinearylamidase	1	1	1	1	2	1	1	2	1
Trypsin	0	0	0	0	0	0	0	0	0
a-Chymotrypsin	0	0	0	0	0	0	0	0	0
Acid phosphatase	2	2	2	2	2	2	2	2	2
Naphthol-AS-BI-phosphohydrolase	5	5	5	5	5	5	5	5	5
a-Galactosidase	2	2	2	2	2	2	2	2	2
B-Galactosidase	5	5	5	5	5	5	5	5	5
B-Glucuronidase	0	0	0	0	0	0	0	0	0
a-Glucosidase	2	2	2	2	2	2	1	2	1
P-Glucosidase	2	2	2	2	2	2	2	2	2
N-Acetyl-P-glucosaminidase	3	3	3	3	3	3	3	3	3
a-Mannosidase	0	0	0	0	0	0	0	0	0
a-Fucosidase	0	0	0	0	0	0	0	0	0

Table 1b: Enzyme activity of isolated *Lactobacillus spp.*

Score 0 = 0 nmol, Score 1= 5 nmol, Score 2= 10 nmol, Score 3= 20 nmol, Score 4= 30 nmol, Score 5 ≥ 40 nmol.

Hemolytic activity of *Lactobacillus* spp

All isolate were tried for hemolytic action and gave negative result with this test affirming that LAB are secure for human utilize (12940). This result agree with [58] who detailed that none of probiotics isolates was found to be B-hemolytic.

Effect of feeding with probiotic *Lactobacillus* spp. On growth and liver enzyme *in vivo*

Effect of supplementation with *Lactobacillus* cultures on body weight

Although it has been shown that most *Lactobacillus* species *(L. acidophilus , L. lactis and L. casei* and reference strain *Lactobacillus plantarum*. have no pathogenicity and no acute oral toxicity for animals table 2,3,4. [50] stated that ,it is important to check the

Antibiotic	*Lactobaclluslactis*	*Lactobacillus casei*	*Lactobacillus acidophilus*	No. of isolates
Ampicillin (10mg)	1	3	4	7
Augmentin (30mg)	1	2	3	6
Cefoxitin (30mg)	1	3	3	7
Cephalotoxin (30mg)	1	3	4	8
Oxacillin (1mg)	1	2	2	5
Vancomycin (30mg)	1	3	4	8
Teicoplanin (30mg)	1	3	4	8
Cloranphenicol (30mg)	1	2	3	6
Clindamycin (2mg)	1	3	3	7
Rifampicin (5mg)	1	3	2	6
Tetracycline (30mg)	0	0	0	0
Kanamycin (30mg)	1	3	4	7
Ciprofloxacin (5mg)	0	2	3	5
Nitrofurantoin (300mg)	1	1	2	4
Trimethoprim (5mg)	1	3	4	7

Table 2: Resistance to antibiotics of the representative strains of lactic acid bacteria isolated from dairy products.

Body weight / Group	Gl (positive control) *L. plantarum*	(Negative control)G2	G3*(L. Acidophillus)*	G4 *L. casei*	G5 *L. lactis*
(@zero time)	**14,6**	**13,25**	**15,45**	**15,75**	**16,85**
1	15,25	14,5	16,05	16,2	17,5
2	15.77	16,6	16,6	16,8	18,3
3	15,9	15,15	17	16,06	18,8
4	16.4	17,75	17,25	18,6	19.56
5	15,85	16,4	18,722	19,41	19,81
6	16,75	16,5	18,5	20	19,21
7	18,93	19,5	21,5	23,6	17,125
8	20,25	20,25	21	21,58	18,66
9	20,75	20,35	20,8	21,6	17,8
10	19,57	22,5	22,43	23,1	22,5

Table 3: Effect of *Lactobacillus* cultures on body weight in experimental mice for 10 days.

G3 *(L. Acidophillus,* G4 *L. casei*, G5 *L. lactis*, Gl (positive control, L. *plantarum*), G2 (CONTROL, no bacteria)

Haemoglobin content before Treating with bacteria g/dl	Haemoglobin content after feeding with bacteria	Group
15.25955	14.56092	Gl (positive control)
13.82552	14.19322	G2 (CONTROL)
15.03893	15.55371	G3*(L. Acidophillus)*
13.71521	14.74477	G4 *L. casei*
15.48017	16.1788	G5 *L. lactis*

Table 4: Hemoglobin content in mice feed with *Lactobacillus sp.*

safety of each probiotic strain, since the toxicity profile may be strain dependent. For example, [9] have demonstrated an increase in the liver or spleen weight ratios of mice fed with a strain *of L. plantarum* (dead or live cells). We used young mice in this study, in order to reinforce any potential toxic impact.

We did not detect any chronic toxicity on the basis of gross pathological examination of the viscera, or the analysis of spleen or liver weights ratios under these experimental conditions. These findings indicated that these strains, alone or with their metabolites, had no obvious adverse effect on the general health status of the mice as showed in table 2, 3,4. A successful growth promoter must not only enhance growth performance, it must also be devoid of adverse side-effects. LAB have been expended in fermented foods or nourishes for a few centuries without any self-evident antagonistic impacts 87). They are therefore classified "Generally Recognized As Safe": GRAS [24,27,28,51,58].

The toxicity profile may be strain dependent and among *lactobacilli*, one of the most important genus of LAB, Further *in vivo* studies are necessary, and this study focused on the safety, health and growth performance of mice receiving these two strains daily for 2 or 4 weeks. [7] With a conventional diet, there were no noteworthy contrast in the body weight. between group Using a barley diet, no significant difference WG appeared regardless of the *lactobacilli* strain used in these experiments as showed in table 18, we used the optimal dose (10^8 CFU of *lactobacilli* grown on MRS broth/ml) as determined previously, to study growth performance parameters. The difference between the WG in the mice given water supplemented with *lactobacilli* strains and in those receiving water in previous table group 1 and 3,4,5 supplemented with different species of *Lactobacillus* and group 2 as control no significant difference in body weight and hemoglobin content. from previous table we found that no significant difference between before and after feeding with *Lactobacillus spp*. While in another study, we found a noteworthy useful impact on weight speed and a 45% lower chance of being press insufficient of the utilization of probiotic- and prebiotic-fortified milk for 12 months. No impact was watched on person press insufficiency markers , B12 and folate inc5luded initially a significant proportion of children who were anemic and B12 and folate deficient 158) In 1 study of no anemic healthy young women with low iron status, viable lyophilized *Lactobacillus plantarum* added to 1 test meal did not enhance iron absorption [6].

Effect of *Lactobacillus* spp. On liver enzyme

A successful growth promoter must not only enhance growth performance, it must also be devoid of adverse side-effects. LAB have been expended in matured foods or feeds for a few centuries without any self-evident antagonistic impact.

[35] They are therefore classified "Generally Recognised As Safe": GRAS [24,27,28,51,58]. Nevertheless, From previous. Table 5, 6, 7 and 8 and using statistical analysis by e-test we found no significant difference between before and after feeding with liver enzyme parameter.

Group no.	Gp l +ve control *L. plantarum*	GP2(-ve control)	G3 *(L. Acidophillus)*	G4 *L. casei*	G5 *L. lactis*
1	0,615	0,685	0.577	0.629	0.631
2	0,651	0,637	0.679	0.788	0.798
3	0,615	0,642	0.707	0.648	0.651
Mean	0.627	0,655	0.654	0.688	0.679

Table 5: Liver enzymes GPT (ALT) u/l parameter before feeding with *Lactobacillus spp*.

G3*(L. Acidophillus,* G4 *L. casei*, G5 *L. lactis*, Gl (positive control, L. *plantarum*), G2 (CONTROL, no bacteria)

Group no.	Gpl	Gp2	Gp3	Gp4	Gp5
1	0.591	0.444	0.482	0.480	0.576
2	0.605	0.585	0.665	0.684	0.453
3	0.600	0.541	0.652	0.587	0.581
4	0.447	0.602	0.461	0.579	0.631
5	0.426	0.543	0.565	0.583	0.560
Mean	0.534	0.543	0.565	0.583	0.560

Table 6: Liver enzyme GPT (ALT) u/l parameter after feeding.

G3 *(L. Acidophillus,* G4 *L. casei*, G5 *L. lactis*, Gl (positive control, L. *plantarum*), G2 (CONTROL, no bacteria).

No.	Gpl	Gp2	Gp3	Gp4	Gp5
1	0.416	0.354	0.278	0.312	0.322
2	0.441	0.350	0.302	0.383	0.372
3	0.319	0.379	0.305	0.326	0.325
mean	0.392	0.361	0.295	0.340	0.339

Table 7: Liver enzyme GOT (AST) u/l parameter before feeding.

G3 *(L. Acidophillus,* G4 *L. casei*, G5 *L. lactis*, Gl (positive control, L. *plantarum*), G2 (CONTROL, no bacteria).

NO.	GP1	GP2	GP3	GP4	GP5
1	0.244	0.243	0.250	0.209	0.258
2	0.217	0.265	0.240	0.209	0.218
3	0.314	0.299	0.239	0.223	0.255
4	0.277	0.234	0.265	0.299	0.229
5	0.228	0.255	0.259	0.229	0.353
MEAN	0.256	0.259	0.251	0.234	0.259

Table 8: Liver enzyme GOT (AST) u/l parameter after feeding.

When people involvement persistent insuperable, such as viral contamination, harmful harm, and alcoholic/non-alcoholic greasy liver, the values of AST, ALT, and g-GTP in serum, as hepatic pointers, are significantly expanded. Non-alcoholic fatty liver illness could be a common liver pathology and incorporates a wide histologic range that ranges from straightforward steatosis to non-alcoholic steatohepatitis [47,52]. LAB have been appeared to be compelling in moving forward liver work solely in creature demonstrate tests [2,54]. Within the show think about, we watched that sort B yogurt contributes to a diminish in these hepatic pointers, particularly when the subjects inside the direct ranges (AST 20-80 IU/L, ALT 20-80 IU/L, were analyzed (12-25% diminish). Sort A yogurt decreased the ALT value. This can be the primary report of a human clinical trial in which a certain strain of LAB was found to move forward liver function.

Conclusion

the present study showed that dairy product is a source of potential probiotic strains of LAB and the isolates meet several function features to be considered as suitable probiotic for application in food fermentation where isolated bacteria able to tolerate acidic medium and bile salt a favorable enzymatic activity and no hemolytic activity so we consider it great potential probiotic character and safe for human use. The present study showed the isolates meet several function features to be considered as suitable probiotic for application in food fermentation and the isolated bacteria able to tolerate acidic medium and bile salt a favorable enzymatic movement and no hemolytic movement adjacent to Has been included within the determination criteria for probiotic strains with cholesterol-lowering impacts, so we consider it awesome potential probiotic character and secure for human utilize.

Ethical Approval and Consent to Participate

Manuscript does not report studies involving human participants.

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