Probiotic Characterization of Lactobacilli and Yeast Strains Isolated from Whey Beverage and Therapeutic Potential of *Lactobacillus Yoghurt* in Murine Giardiasis

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Abstract

In the present study, an attempt has been made to assess the probiotic characteristics of lactobacilli and yeast strains isolated from whey beverage under conditions simulating the in vivo stresses encountered in the gastrointestinal tract i.e. acid, alkaline, proteolytic enzymes and bile stress. The therapeutic potential of probiotics *Lactobacillus casei* or *Lactobacillus yoghurt was* assessed in *Giardia* infected mice. All the isolated strains well tolerated the gastrointestinal stresses. It was found that probiotic supplementation of either *L. casei or L. yoghurt,* one day after *Giardia* infection, reduced the severity of infection compared to *Giardia* infected mice. Pathophysiologically, the morphological and cellular changes in the small intestine were least in probiotic treated mice compared to severely inflamed, oedematous, vacuolated epithelial cells in *Giardia* infected mice. It can be concluded that *L. yoghurt* possessed better probiotic properties and has the potential to reduce the severity of *Giardia* infection in mice.

Keywords*: Giardiasis; Probiotic; Therapeutic; Microbial Interference Therapy (MIT).*

1. Introduction

Giardia lamblia (synonyms: *Giardia intestinalis* or *Giardia duodenalis*) is the flagellated intestinal protozoan which causes waterborne diarrhea worldwide. Prevalence rate of giardiasis ranges from 20-30% in most developing countries and 2-7% in developed countries [1,2,3]. School children, malnourished individual, common variable immunodeficiency (CVID) patients, hypogammaglobulineamic patients and people with blood group-A are highly susceptible

to giardiasis [3,4]. The clinical manifestations of *Giardia* infection are varied, with diarrhea being the predominant symptom, occurring in almost 90% of symptomatic patients.

Giardia is generally treated with antibiotics like nitroimidazoles and nitrofurans. However, clinical failures, occurrence of resistant strains, and side effects of anti-*Giardia* drugs [5, 6] have encouraged research on alternative biotherapeutic strategies such as plant extracts, products derived from bees and probiotics that are safe, inexpensive and effective in improving the cause of intestinal parasitosis [7]. Recently, Shukla et al. 2008 (8) have shown that probiotic *L. casei* MTCC 1423, modulates murine *Giardia* infection by minimizing or preventing the adherence of *Giardia* trophozoites to the mucosal surface, suggesting that probiotics offer a safe and effective mode to prevent / or treat the *Giardia* infection.

The term 'probiotic' is defined as 'microbial cell preparations or components of microbial cells that have a beneficial effect on the health and wellbeing [9]. Moreover, probiotics are emerging as an important new therapy for prevention and treatment of infectious diseases mainly gastrointestinal infections and are also known as microbial interference therapy (MIT). The main characteristics of a probiotic are resistance to digestion by enteric or pancreatic enzymes, gastric acid, bile, ability to prevent the adherence, establishment and replication of pathogens in the gastrointestinal tract [10]. However, the protective mechanism of a probiotic appears to be species and strain specific [11]. Thus, the present study was designed to study the probiotic characteristics of lactobacilli and yeast strains isolated from whey beverage vi*s a vis* therapeutic potential of *L. yoghurt* on the establishment of murine giardiasis.

2. Materials and Methods

2.1 Bacterial and Yeast strains

Lactobacillus casei MTCC 1423, a standard lactic acid bacterial strain (LAB) was procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. *Lactobacillus choozit*, *Lactobacillus yoghurt,* yeast strains P82, P84 and V84 isolated

from whey beverage were kindly provided by Dr. Parampal Sahota, Punjab Agricultural University, Ludhiana, Punjab, India.

2.2 Growth and maintenance of bacterial/yeast strains

L. casei was grown in De Mann Rogosa Sharpe (MRS) broth at 37^0C for 48 h and maintained on MRS agar plate by regular subculturing at an interval of 15 days. *L. choozit* and *L. yoghurt* were grown in Tomato Juice Broth (TJB) at 37^0C for 24h and maintained on Tomato Juice Agar (TJA) plates by regular subculturing. Yeast strains P82, P84 and V84 were grown in Glucose Yeast Extract (GYE) broth at 30^0C in Biological Oxygen Demand (BOD) incubator for 30 h and maintained on GYE agar slants by regular subculturing.

2.3 Phenotypic characterization of bacterial and yeast strains

Morphological (Gram staining, colony morphology, motility) and biochemical characterization of the isolated strains was performed.

2.4 Probiotic characteristics

Isolated bacterial and yeast strains were screened for their probiotic characteristics under gastrointestinal stresses like bile, acid and enzyme tolerance. In addition, cholesterol assimilation of these strains in vitro was also assessed.

2.5 Bile tolerance

Bile tolerance of all the isolated strains was studied as per Gilliland et al. 1984 (12). Briefly, TJB supplemented with different concentrations of bile salts (sodium deoxycholate and sodium taurocholate; 0.3%, 1% and 2%) was inoculated with 1% inoculum and incubated at 37°C for various time intervals (1h, 2h and 3h)**.** After each hour of incubation, samples were drawn, serially diluted, plated on TJA plates and incubated at 37°C for 24h. Growth on the plates indicated their tolerance to bile salts. The control comprised of TJB without bile salt. Yeast strains were also processed as above using GYE broth and GYE agar**.**

2.6 Viability at pH extremes

The isolated strains were screened for their survival and stability at pH extremes i.e**.** acidic pH 2.0 (homologous to stomach) and alkaline pH 8.0 (homologous to intestinal tract).

2.7 Acid tolerance

TJB with different pH (2.0, 6.3, and 8.0) were prepared. TJB (pH 2) was inoculated with lactobacilli culture (1%) grown overnight in TJB (pH 6.7). Samples at 0h and after 4h of incubation were drawn, serially diluted, plated on TJA plates and incubated at 37 ℃ for 24h.

2.8 Alkaline tolerance

After 4h, 1% inoculum from TJB (pH2.0) was transferred to fresh TJB (pH 8.0) and the above steps were repeated (as for acid tolerance) to monitor the response of lactobacilli to alkaline pH. Acid and alkaline tolerance of yeast strains were processed in the same way as above using GYE broth and GYE agar.

Growth of lactobacilli and yeast strains on the plates indicated their tolerance to extreme pH.

2.9 Enzyme tolerance

Sensitivity of lactobacilli and yeast strains to oral and intestinal enzymes (lysozyme, trypsin and α-amylase) was studied as per Nowroozi et al. 2004 (13). Lactobacilli and yeast strains were grown in the presence of various enzymes: lysozyme (22 IU mg^{-1}ml^{-1}), trypsin (0.5mg ml^{-1}) and α-amylase (220 IU mg^{-1} ml^{-1}) for 1h at 30 ℃, thereafter transferred to 37 ℃ and was incubated for another 1h. Both optical density (OD $_{600 nm}$) and colony forming units (CFU) were recorded at 0 h and after 2 h of incubation. A control (without enzyme) was also processed.

2.10 Cholesterol assimilation from culture media

Assay was performed as per Liong & Shah, 2005 (14). Briefly, freshly prepared TJB was supplemented with 0.3% oxgall (bile salt) and cholesterol (100 μg ml^{-1}) and was inoculated with 1 % of respective cultures, incubated at 37 ℃ for 20 h. After incubation, cultures were centrifuged and unutilized cholesterol was estimated in the supernatant by the modified method of Zlatkis et al.1953 (15). Optical density (OD$_{540 nm}$) was measured and results were expressed as follows:

$$\% \text{ cholesterol assimilated} = \frac{(a-b)}{a} \times 100$$

a = initial concentration of cholesterol in the medium (μg).

b = final concentration of cholesterol left in the medium after 20 hours of incubation (μg)

2.11 In vivo studies

After assessing the probiotic characteristics of the isolated strains, therapeutic potential of *L. casei* and *L. yoghurt* in *Giardia* infected mice was assessed.

2.12 Parasite

Giardia lamblia trophozoites (Portland strain I) were grown auxenically in TYI-S-33 medium supplemented with antibiotic solution and pH was adjusted to 6.9, before sterilization with 0.22 μm seitz filter. Actively growing trophozoites (48-72 h old culture) were sedimented by chilling the tubes in ice for 15 min followed by centrifugation at 2000 rpm for 15 min. Trophozoites were suspended in phosphate buffer saline (PBS- 7.2) to a final concentration of 1×10^6 trophozoites / 0.1 ml.

2.13 Bacterial strains, preparation and inoculation

For experimental inoculation, 18 h old cultures of *L. casei* and *L. yoghurt* were centrifuged at 8000 rpm for 10 min and pellets were washed with PBS- 7.2. Finally, suspended in PBS-7.2 to contain 1x 10^9 lactobacilli / 0.1ml and were fed intra oesophageally via catheter [8].

2.14 Animals

BALB/c mice aged 5 – 6 weeks old (18-20 gm) obtained from Central Animal House, Panjab University, Chandigarh, India, were housed under standard conditions of light and dark cycle with free access to feed (Ashirwad Industries Pvt. Ltd., Ropar, India) and water *ad libitum*. Both water and feed were monitored for any bacterial or parasitic contamination by Gram's staining and Lugol's iodine staining techniques before

providing to animals [16]. Animals were also screened for any protozoal infection via stool examination for three consecutive days [8]. Mice free from parasitic infections were used. Care and use of animals were in accordance with the guidelines of the institutional ethical committee.

2.15 Experimental design

To carry out the study animals were divided into 4 groups, each containing 15 animals. **Group I (Control):** These animals were fed with a single dose of PBS -7.2 via orogastric gavages daily for 30 days. **Group II (*Giardia*–infected):** These mice were challenged orally with a single dose of 1×10^6 *Giardia* trophozoites. **Group III (*Giardia*–*L.casei*):** These animals were challenged orally with a single dose of 1×10^6 *Giardia* trophozoites. A day after *Giardia* challenge, mice were fed orally with a single dose of *L. casei* (1×10^9). The probiotic treatment was continued for 30 days. **Group IV (*Giardia*–*L. yoghurt*):** Animals belonging to this group were challenged orally with a single dose of 1×10^6 *Giardia* trophozoites. A day after *Giardia* challenge, a single dose of *L. yoghurt* (1×10^9 cfu/ 0.1ml) was administered daily for 30 days.

2.16 Follow up of animals

After respective treatments in different groups, *Giardia* cysts counts, lactobacilli counts, trophozoites counts and pathological changes in the small intestine were studied.

2.17 Enumeration of *Giardia* cysts in faeces

Cysts in the faecal samples of mice were enumerated as per Shukla et al. 2008 (8). Briefly, one gram of freshly passed fecal sample was dissolved in 10 ml of normal saline, homogenized using pestle and mortar. Cysts stained with iodine were counted on every third day using hemocytometer and were expressed as cysts ml^{-1}.

2.18 Enumeration of lactobacilli in faeces

To confirm whether the lactobacilli species were able to survive the stress within the gastrointestinal tract, freshly voided faecal samples from each group of mice were homogenized in normal saline and serially diluted, plated on MRS agar, incubated at 37 ℃ for 24 –

48hrs and colony forming units (cfu) were counted [8].

2.19 Enumeration of *Giardia* trophozoites in the small intestine (jejunum)

The trophozoites were counted in the intestinal fluid of animals belonging to groups II, III, IV respectively [17]. Mice were sacrificed and the proximal 10cm section, mainly the jejunum was removed and placed in 5ml of ice chilled saline. The small intestine sections were minced, kept for 15–20 minutes in ice chilled saline and trophozoites were counted using haemocytometer.

2.20 Histopathological studies

Mice were sacrificed by cervical dislocation and upper part (jejunum) of the small intestine was removed aseptically, fixed in 10% buffered formalin, processed, stained with haematoxylin and eosin and were examined under the light microscope.

2.21 Statistical analysis

The inter group variation was assessed by one way analysis of variance (ANOVA). Statistical significance of the result was calculated.

3. Results and Discussion

The emergence of antibiotic resistant bacteria and natural ways of suppressing the growth of pathogens has contributed to the concept of 'Probiotics'. Probiotic bacteria not only compete and suppress 'unhealthy fermentation' in human intestine but also have a number of health benefits [18]. Moreover, the health benefits of probiotic vary with the species and strains of probiotics. Hence, it becomes pertinent to isolate and identify probiotics that have disease specific health benefits [19]. Therefore, the present study was designed to delineate the probiotic characteristics of the bacterial and yeast strains isolated from whey beverage vis a vis therapeutic potential in murine giardiasis.

3.1 Identification of different bacterial and yeast isolates

The different phenotypic characteristics such as colony morphology, Gram reaction, catalase, motility, and sugar fermentation of different bacterial and yeast isolates (from whey beverage) were studied. All lactic acid bacteria were Gram positive rods, catalase negative, non motile but had different sugar fermentation ability (Table1). Similarly, all yeast strains also had different sugar fermentation ability (Table 2).

Table 1: Morphological characteristics and sugar fermentation by lactobacilli strains. (+) = utilization of sugars; (-) = Non utilization of sugars.

Characteristics	*Lactobacillus casei*	*Lactobacillus Choozit*	*Lactobacillus Yoghurt*
Gram reaction	Gram +ve, thin bacilli in chains	Gram +ve, thin, long and short rods	Gram +ve, thin short rods
Colony characteristics	White, pin head, round, entire margins, smooth texture, slightly elevated, non lustrous.	White, pin head, round, irregular margins, smooth texture, flat, non lustrous.	Creamy white, translucent, pin head, round, entire margins, smooth texture, flat, non lustrous, glistening.
Catalase	-	-	-
Motility	-	-	-
Sugar utilization			
Glucose	+	+	+
Arabinose	+	+	+
Cellobiose	+	+	+
Lactose	+	+	+
Maltose	+	+	+
Mannose	+	+	-
Mannitol	+	-	+
Mellibiose	+	+	+
Raffinose	+	-	+
Rhamnose	+	+	+
Sorbitol	+	-	-
Sucrose	+	+	+
Xylose	+	+	+
Galactose	+	+	-
Fructose	+	+	+

3.2 Bile tolerance

Gastrointestinal systems have varying concentrations of bile ranging 0.5% to 2.5% in the first hour of digestion and the levels may decrease further in subsequent hours. Moreover, tolerance to bile salts is considered to be a main prerequisite for growth, colonization and metabolic activity of bacteria in the host's gut [14]. Therefore, it is generally included in the selection criteria of potential probiotic. In the present study it was observed that all the isolated strains survived and tolerated bile salts (0.3%) quite effectively. But a marginal decrease in the viability of all the isolated strains was found at 0.3% and 1% bile salt concentrations for different time intervals (Figure 1 and 2). However, at higher concentration (2%) bile tolerance of all the strains decreased significantly ($p < 0.05$, Figure 3). The difference in the level of bile tolerance of strains in the present study may probably be due to differences in their ability to grow and colonize the intestinal tract and is in accordance with the earlier studies [20-22].

Table 2: Morphological characteristics and sugar fermentation by yeast strains. (+) = utilization of sugars (-) = Non utilization of sugars

Characteristics	Yeast P82	Yeast P84	Yeast V84
Colony characteristics	Pure white, pin head, round, entire margins, smooth texture, elevated, shiny.	Pure white, pin point, round, entire margins, smooth texture, elevated, shiny.	Creamy white, pin head, round, entire margins, smooth texture, elevated, shiny.
Sugar utilization			
Glucose	-	-	+
Arabinose	-	-	-
Cellobiose	-	-	-
Lactose	-	+	-
Maltose	-	+	+
Mannose	+	-	+
Mannitol	+	+	-
Mellibiose	-	+	-
Raffinose	+	-	-
Rhamnose	+	-	-
Sorbitol	-	+	+
Sucrose	+	-	+
Xylose	-	+	-
Galactose	-	-	-
Fructose	+	+	+

Table 3: Viability of lactobacilli and yeast strains at extreme pH values.

Organism	2.0		8.0	
	0 hour	4hours	0 hour	4hours
L.casei	8.20 ±0.18	7.76±0.20	6.96 ± 1.15	5.97±0.56
L.yoghurt	7.72 ±0.90	7.14±0.15	6.61 ± 0.91	5.65±2.15
L.choozit	7.530 ±2.15	6.23±0.40	5.74 ±1.25	4.87±1.55
P82	7.8 43 ±1.26	7.1±1.05	6.877 ±0.66	5.56±0.88
P84	7.732 ±0.99	6.84±0.25	6.23 ± 0.43	5.74±0.01
V84	7.515 ±1.16	6.66±0.31	5.93 ±0.31	5.48±0.75

Values are expressed as $\log_{10}$ cfu/ml.

3.3 Viability at extreme pH

Beneficial aspects of probiotic strains can be expected only when they are able to survive passage through the human stomach, digestive system and colonize the human gut. Of all the strains, *L. choozit* was most susceptible to acidic and alkaline pH while *L.casei* appeared to be resistant to both pH extremes (Table 3).   It is reported that some probiotic strains are more tolerant to acidic conditions than others either due to high cytoplasmic buffering capacity (pH 3.72–7.74) or membrane ATPases , that in turn may resist changes in the cytoplasmic pH and gain stability under acidic conditions [23,24].
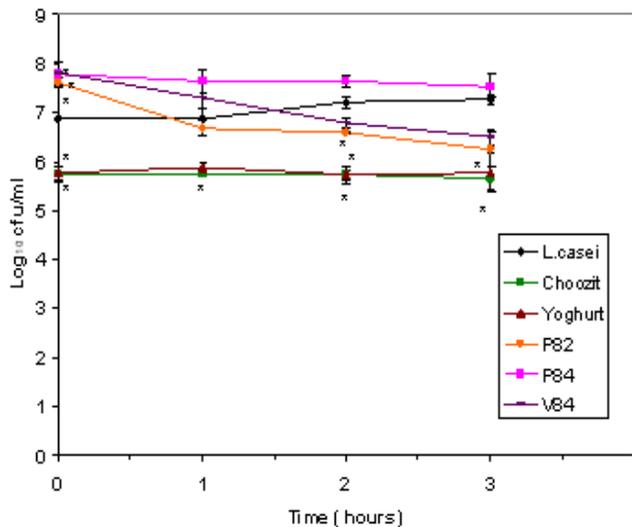
Figure 1: Survivability of lactobacilli and yeast strains in culture medium containing 0.3% bile salts. Values are Mean $\pm$SD. *$p<0.05$ v/s *L. casei*.
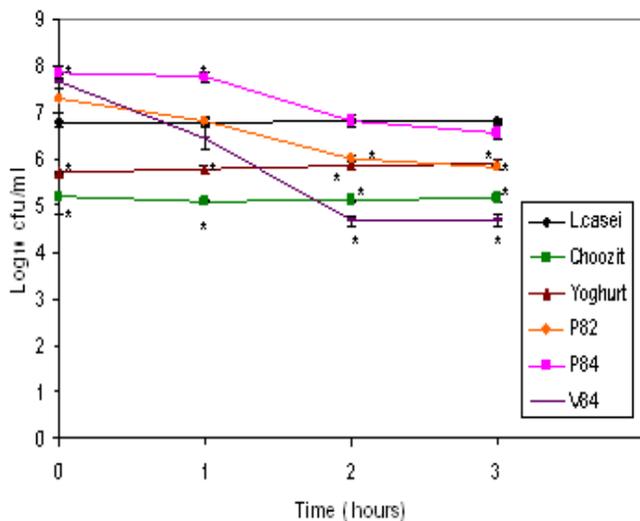

Figure 2: Survivability of lactobacilli and yeast strains in culture medium containing 1% bile salts. Values are Mean $\pm$SD. *$p<0.05$ v/s *L. casei*.
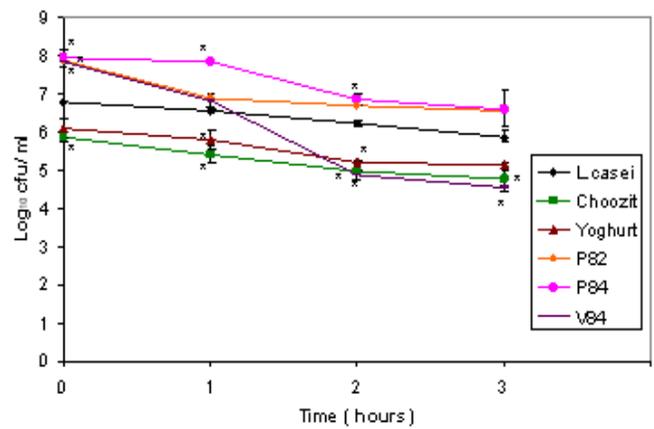

Figure 3: Survivability of lactobacilli and yeast strains in culture medium containing 2% bile salts. Values are Mean $\pm$SD. *$p<0.05$ v/s *L.casei*.

3.4 Enzyme tolerance

Bacteria and yeast used as probiotic adjuncts are commonly delivered in a food system and begin their journey to the lower intestinal tract via mouth. Therefore, these useful organisms should be resistant to the enzymes in the oral cavity (e.g. lysozyme) and in the intestine (α–amylase, lysozyme and trypsin). Interestingly, we found that all the strains grew equally well in the presence of either of these enzymes (Figure 4, 5, 6) and clearly suggests that these enzymes do not interfere with the survival and colonization of these organisms in the gut. This is the first study to report the enzyme tolerance of probiotic strains.
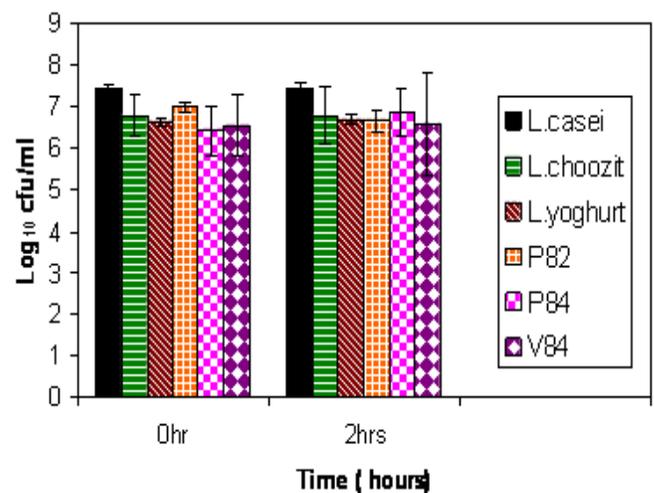
Figure 4: Survivability of lactobacilli and yeast strains in culture medium containing α- Amylase. Values are Mean $\pm$SD. *$p<0.05$ v/s *L. casei*.
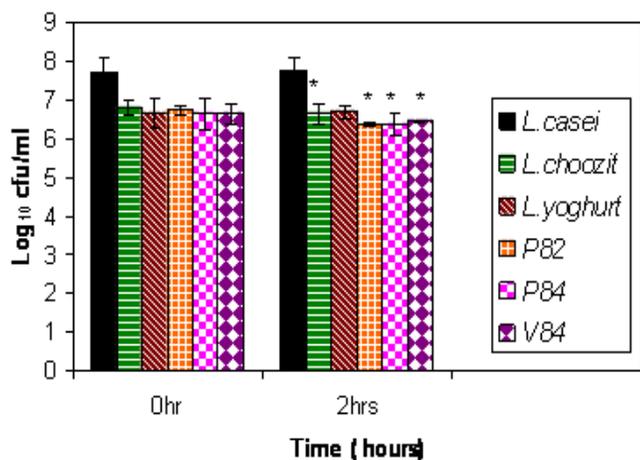
Figure 5: Survivability of lactobacilli and yeast strains in culture medium containing lysozyme. Values are Mean ±SD. *p<0.05 v/s *L. casei*.
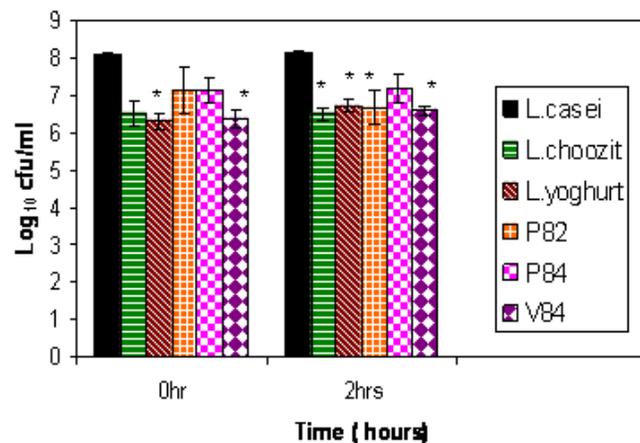

Figure 6: Survivability of lactobacilli and yeast strains in culture medium containing trypsin. Values are Mean ±SD. *p<0.05 v/s *L. casei*.

3.5 Cholesterol Assimilation from culture media

Various studies have strongly suggested that fermented milk could lower total cholesterol and low density lipoprotein (LDL) cholesterol, thus having a hypocholesterolemic effect [25, 26]. These strains were also examined for their ability to assimilate cholesterol in vitro. The amount of cholesterol assimilation by these organisms revealed a wide variation (29 to 57%) among the strains. Interestingly, *L. yoghurt* and P84 had significantly highest (p<0.05) cholesterol assimilating ability while *L. casei* exhibited moderate, and *L. choozit* and V84 the least (Figure 7). The present observation corroborates with earlier studies where it has been reported that different strains of lactobacilli had different cholesterol assimilating ability and warrants further clinical studies [14, 20]. Therefore, it can be suggested that probiotic can also be used to treat hyper-cholesterolemic patients, thus reducing the heart attacks. The observed hypocholesterolemic effect of these strains may either be due to their ability to deconjugate bile acids into free acids which are excreted more rapidly (from the intestinal tract) than conjugated bile acids as the synthesis of new bile acids from cholesterol either reduces the total cholesterol concentration in the body or can be due to co-precipitation of cholesterol with bile acids at low pH [14, 27].
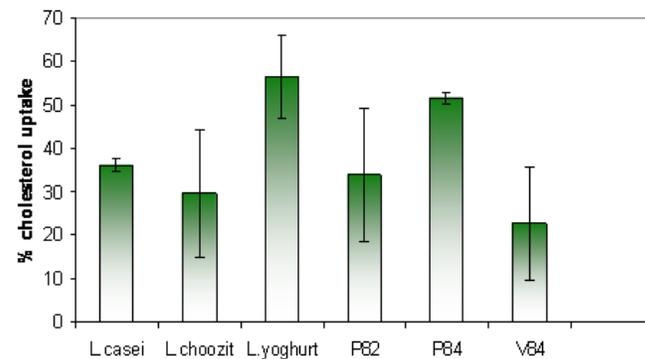

Figure 7: Percentage uptake of cholesterol by lactobacilli and yeast strains. Values are Mean ± SD. *p<0.05 v/s *L. casei*.

3.6 Assessment of therapeutic potential of *L. casei* and *L. yoghurt* in murine giardiasis

Among all the five isolated strains *L. yoghurt* exhibited better probiotic characteristics hence was employed to assess its therapeutic potential in murine giardiasis.

3.7 Enumeration of *Giardia* cysts

It was observed that the excretion of cysts increased gradually from day 0 onwards and was significantly (p<0.05) more on day 7 post inoculation (PI). Thereafter, the cyst count started decreasing and mice became *Giardia* free by day 30 PI. However, oral feeding of *L. casei* or *L. yoghurt* one day after infection significantly (p<0.05) reduced the cyst excretion from day 3

onwards in *Giardia- L. casei* and *Giardia – L. yoghurt* mice (Group III and IV, Figure 8). Moreover, none of the *Giardia* infected mice had any other clinical symptoms like diarrhea, weight loss. Interestingly, daily feeding of either *L. casei or L. yoghurt* after *Giardia* challenge reduced the severity of infection but was unable to reduce its duration. This may be due to the fact that *Giardia* trophozoites adhered to the microvilli and their establishment. However, after probiotic administration, G*iardia* infection did not increased significantly. This may either be due to competition for resources [28], bacterial exocellular factors [29-31], or binding ability of the lactobacilli to the intestinal epithelial cells [32-33].
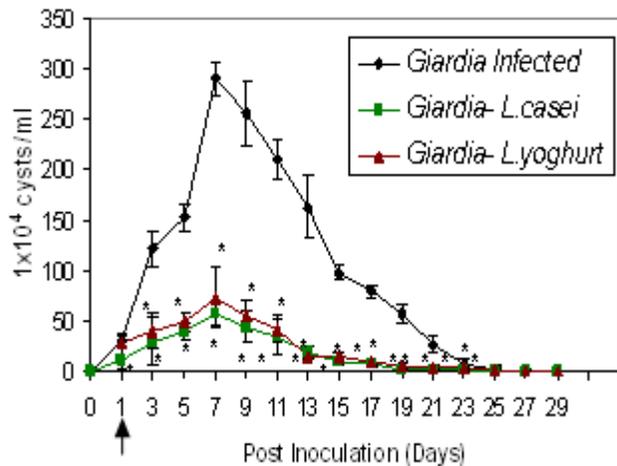
Figure 8: Enumeration of *Giardia* cysts in faeces of different groups of mice treated with *L. casei* and *L. yoghurt* as the probiotic. Values are Mean ± SD, *p<0.05 v/s *Giardia* infected, Probiotic treatment ↑.

3.8 Enumeration of lactobacilli in faeces

Intact intestinal mucosa acts as a barrier to exclude pathogen translocating into intestinal organs and tissues. However, intestinal inflammation due to intestinal disorders could alter the permeability of this barrier, which could lead to invasion of pathogens, foreign antigens and other harmful substances [34]. Therefore, probiotic bacteria that are able to survive gastric conditions and colonize the intestine can be used to treat patients with intestinal disorders. In the present study ability of lactobacilli to persist in gastrointestinal tract was monitored by counting

their number in feces of mice. Interestingly, the faecal lactobacilli counts increased significantly (p<0.01) in *Giardia – L. casei* and *Giardia – L. yoghurt* (Group III and IV) compared to *Giardia* infected mice (Group II, Figure 9). This observation corroborates with the earlier studies carried out by Yuki et al. 2000 (35), Oyetayo et al. (36) and Shukla et al. 2008 (8), suggesting that lactobacilli not only survived the gastrointestinal passage but also adhered effectively to the epithelial lining of animal gut, thus providing a continuous inoculum of specific lactobacilli that may have additive effects.
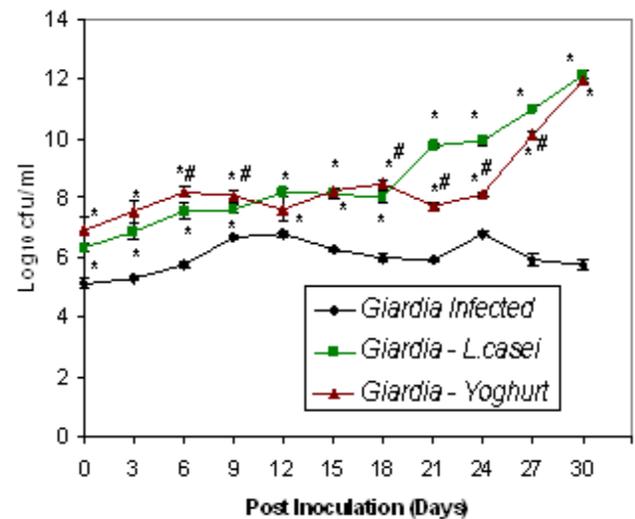

Figure 9: Lactobacilli count ($\log_{10}$ cfu/ml) in faeces of different groups of mice treated with *L. casei* and *L. yoghurt* as the probiotic. Values are Mean ± SD, *p<0.001 v/s *Giardia* infected group, #p<0.05 v/s *L. casei*.

3.9 Enumeration of *Giardia* trophozoites

It was observed that the administration of either *L. casei* or *L. yoghurt* significantly (p<0.05) reduced the trophozoites counts in the gut of *Giardia*-Probiotic treated mice (Group III and IV) compared to *Giardia* infected mice (Group II, Figure 10) and is in accordance with Humen et al. 2005 (37) and Shukla et al. 2008 (8).
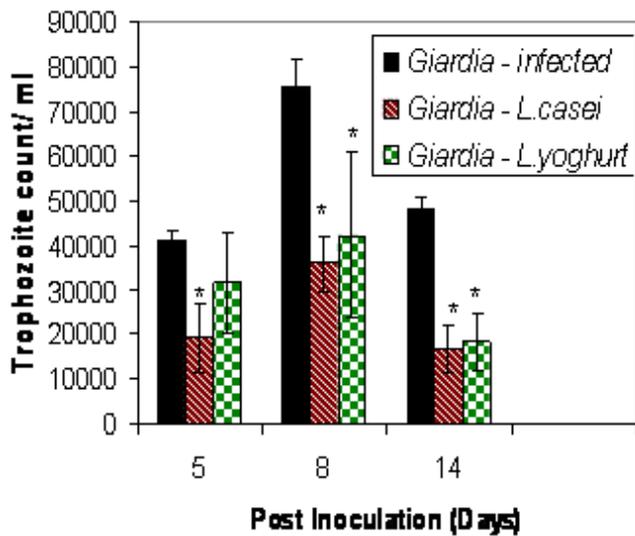
Figure 10: Enumeration of *Giardia* trophozoites in small intestine of different groups of mice using *L. casei* and *L. yoghurt* as the probiotic supplement. Values are Mean ±SD, *p<0.05 v/s *Giardia* infected.
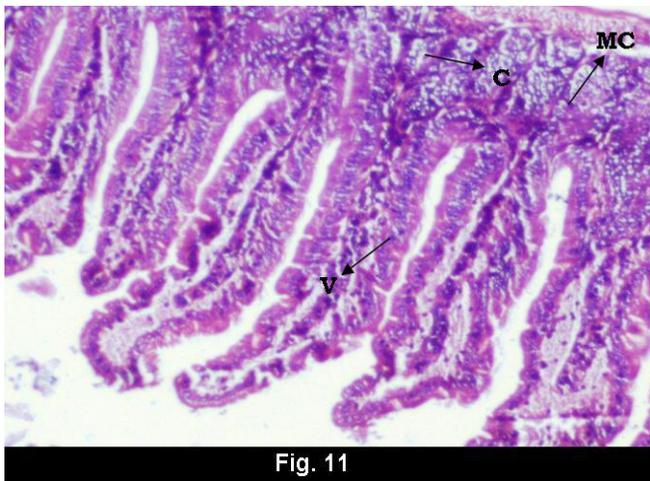

Figure 11: Photomicrograph of the small intestine of Control (Group I) mouse showing a healthy muscle coat (MC), intact mucosal epithelial lining, basal crypts (C) and normal villi (V) (H&E stain, 100 x).

3.10 Histopathological studies

Histologically, the small intestine of the control (Group I) mice had healthy muscle coat, intact mucosal epithelial lining, basal crypts and normal villi compared to varying degrees of villous atrophy, inflammation, lymphocytic infiltration and lymphocytic hyperplasia in *Giardia* infected mice (Figure 11 and 12 a, b, c). Interestingly, probiotic supplementation after

Giardia infection reduced the inflammation of the small intestine but the damage was less in *Giardia* - *L. casei* mice compared to *Giardia* - *L. yoghurt*
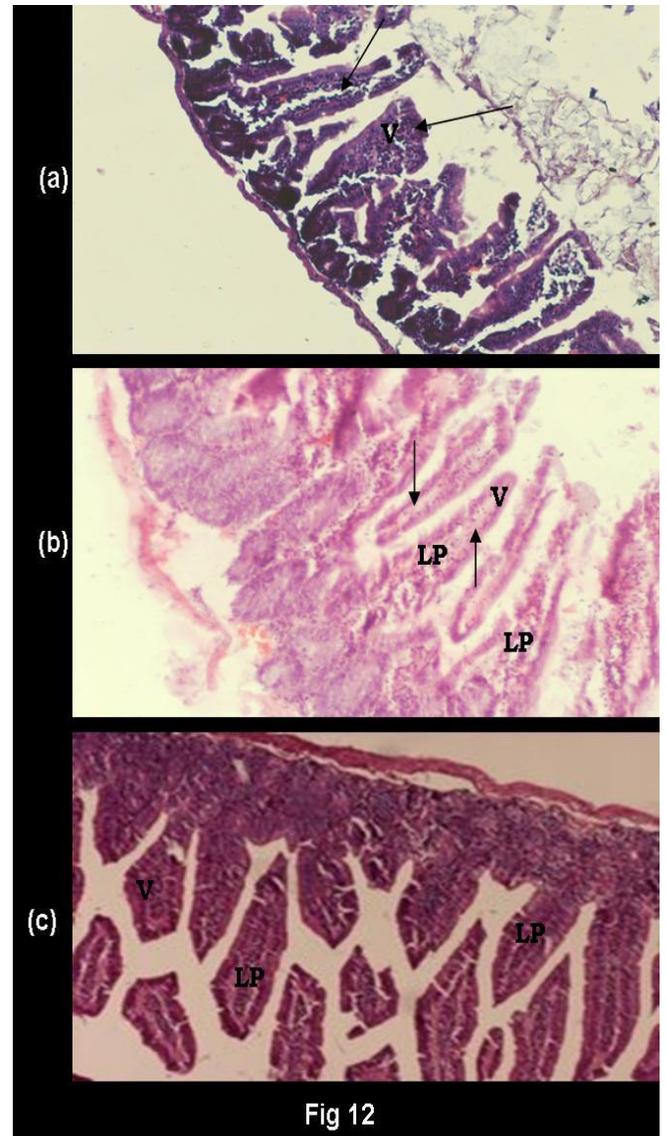

Figure 12: Photomicrograph of the small intestine of *Giardia* infected (Group II) mouse; **a)** day 5 showing inflammation of villi (V) along with lymphocytic infilteration in the lamina propria (single arrow)and normal epithelium; **b)** day 8 showing inflamed and disrupted villi (V) with high number of inflammatory cells in the lamina propria (LP) and intact epithelium (single arrow); **c)** day 14 showing damaged microvilli (V), reduced villus height, increased lymphonuclear cell infilteration in lamina propria (LP) and mild ileitis (H&E stain, 100x).
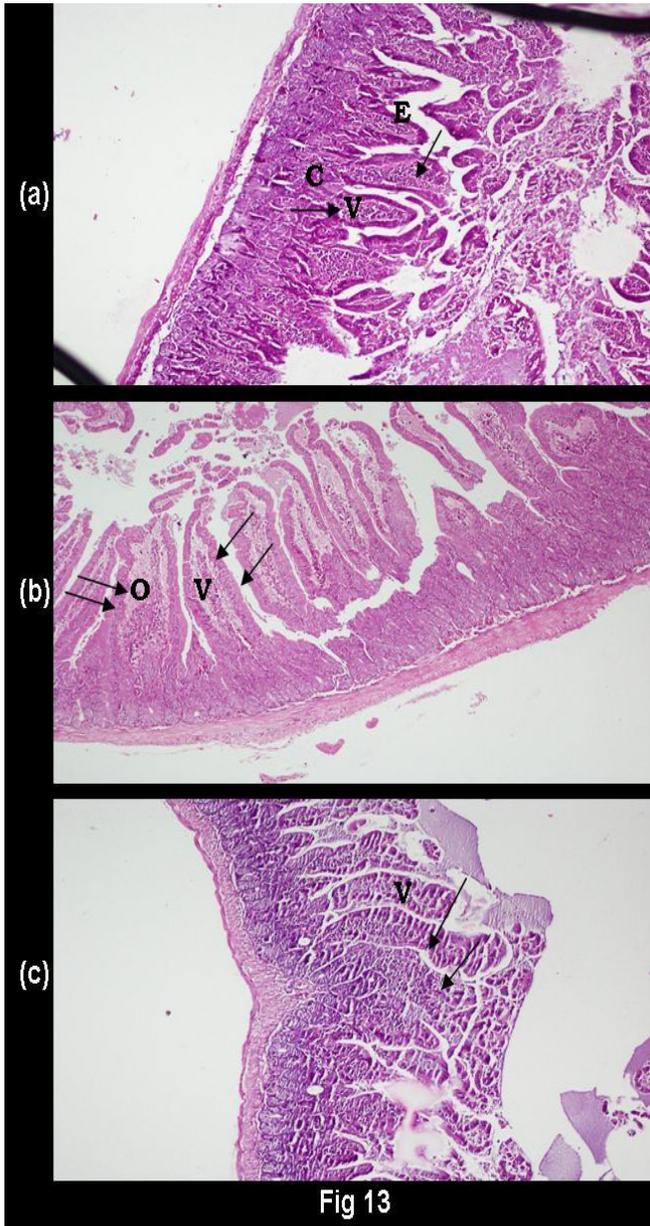
Figure 13: Photomicrograph of the small intestine of *Giardia - L. casei* (Group III) mouse; **a)** day 5 showing mild swelling of villi (V single arrow), cell infilterates devoid of mononuclear histiocytes with abundant plasma cells in lamina propria, healthy epithelium (E) with crypts (C) showing paneth cell hyperplasia, a case of mild plasma cell rich ileitis; **b)** day 8 showing swollen villi (V) with oedema (O), normal epithelium and brush border of the villi (double arrow) and inflammed villi; **c)** day 14 showing slightly disrupted villi (V) but normal in height with mild increase of mononuclear cells in lamina propria (single arrow, H&E stain, 100x).
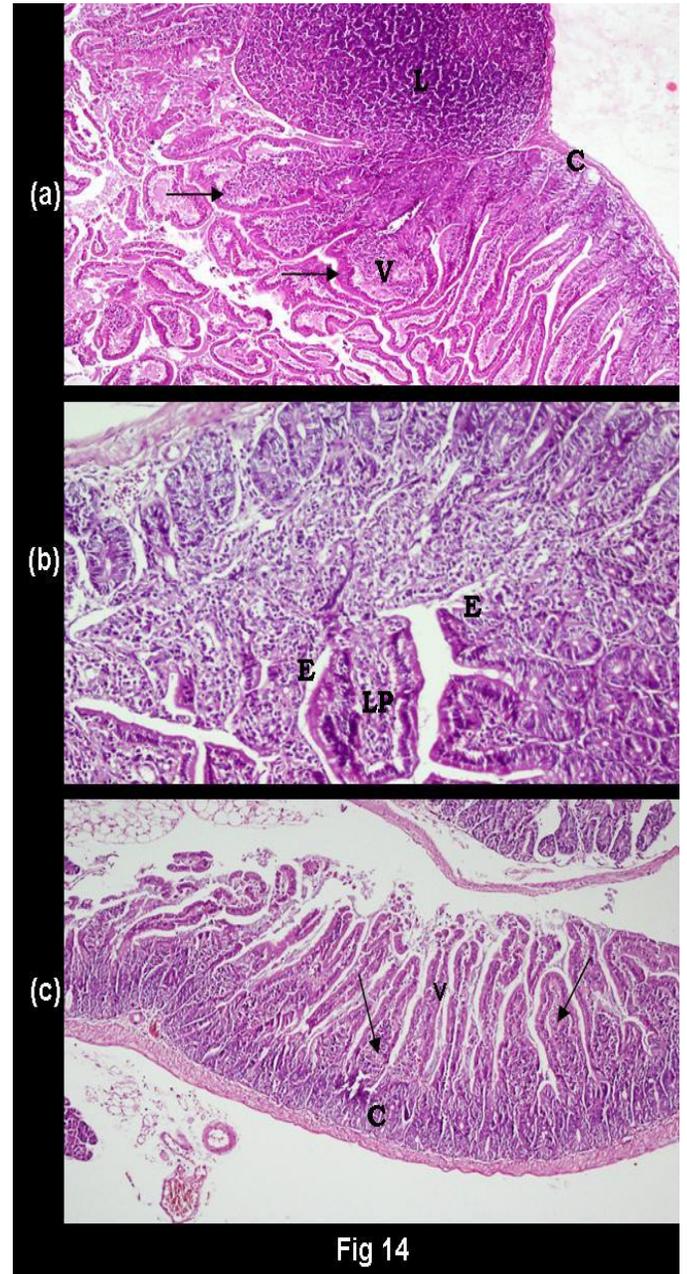

Figure 14: Photomicrograph of the small intestine of *Giardia - L. yoghurt* (Group IV) mouse; **a)** day 5 showing normal crypts (C), hyperplasia of paneth cells, a possibly large lymphoid aggregate (Peyer's Patch L), bulbous swelling of villi (V) and normal epithelium/ brush border (single arrow); **b)** day 8 showing disrupted villi due to inflammatory cells in the lamina propria (LP) and eroded epithelium (E); **c)** day 14 showing normal crypts (C), long villi (V) with less number of inflammatory cells in the lamina propria (single arrow, H&E stain, 100x).

(Figure 13 a, b, c and 14 a, b, c). Initially, the small intestine of *Giardia - L. yoghurt* (Group IV) mice showed inflamed villi with excess of lymphomononuclear cells in the lamina propria along with fibrinous exudates beneath the epithelium (Figure 14b) while *Giardia - L. casei* mice (Group III) had more number of plasma cells in the lamina propria, swollen villi and oedema on day 8PI (Figure 13b). Interestingly, villous height increased with mild inflammation in *Giardia- L. yoghurt* mice (Group IV, Figure 14c) but the villous height were normal in *Giardia- L. casei* mice (Group III) on day 14 PI.

4. Conclusion

It has been found from the study that the isolated lactobacilli and yeast strains fulfill the probiotic characteristics and can be used as functional foods for human health. The cholesterol assimilating property of these strains in vitro signifies their role in reducing the risk of coronary heart diseases and warrants further in vivo studies. The *L. yoghurt* supplementation to *Giardia* infected mice reduced the severity of *Giardia* infection.

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References

1. Tripathi DM; Gupta N.; Lakshmi V.; Saxena KC.; Agarwal AK. Antigiardial and immunostimulatory effect of Piper longum on giardiasis due to *Giardia lamblia, Phytother Res*, 1999, 13, 561-565.
2. Upcroft P.; Upcroft JA. Drug targets and mechanisms of resistance in the anaerobic protozoa, *Clin Microbiol Rev,* 2001, 14, 150-164.
3. Taherkhani H.; Shariati S.; Abdolahi N.; Roshandel GH. Clinical manifestations of Giardiasis in Iran, *Journal of Clinical and Diagnostic Research*, 2009, 3, 1416-1418.
4. Ganayni GA.; Attia RA.; Motawea SM. The relation between ABO blood group, HLA typing and giardiasis in children, *J Egypt Soc Parasitol*, 1994, 24, 407-412.
5. Lemee V.; Zaharia I.; Nevez G.; Rabodonirina M.; Brasseur P.; Ballet JJ .; Favennec L. Metronidazole and albendazole susceptibility of 11 clinical isolates of *Giardia duodenalis* from France, *J Antimicrob Chemother,* 2000 46, 819–821.
6. Gardner TB.; Hill DR. Treatment of giardiasis, *Clin Microbiol Rev,* 2001, 14(1), 114-2.
7. Calzada F.; Meckes M.; Cedillo-Rivera R. Antiamoebic and antigiardial activity of plant flavonoids, *Planta Med,* 1999, 65: 78-80.
8. Shukla G.; Devi P.; Sehgal R. Effect of *Lactobacillus casei* as a probiotic on modulation of giardiasis, *Dig Dis Sci*, 2008, 53, 2671-2679.
9. Marteau P.; Cuillerier E.; Meance S.; Gerhardt MF.; Myara A.; Bouvier M.; Bouley C.; Tondu F.; Bommelaer G .; Grimaud JC. Bifidobacterium animalis strain DN-173 010 shortens the colonic transit time in healthy women: a double-blind, randomized, controlled study, *Aliment Pharmacol Ther*, 2002, 16, 587-593.
10. Kim HJ.;Shin HS.; Ha WK.; Yang IH.; Lee SW. Characterization of lactic bacterial strains isolated from raw milk, *Asian-Aust J Anim Sci,* 2006 19,131-136.
11. Lewis SJ.; Freedman AR. Review article: the use of biotherapeutic agents in the prevention and treatment of gastrointestinal disease, *Aliment Pharmacol Ther*, 1998, 12, 807–822.
12. Gilliland SE.; Staley TE; Bush LJ. The importance of bile tolerance of *Lactobacillus acidophilus* used as a dietary adjunct, *J Dairy Sci,* 1984, 67, 3045-3051.
13. Nowroozi J.; Mirzaii M.; Norouzi M. Study of *Lactobacillus* as probiotic bacteria, *Iranian J Publ Health*, 2004, 33(2), 1-7.
14. Liong MT.; Shah NP. Acid and bile tolerance and cholesterol removability of lactobacilli strains,*J Dairy Sci,* 2005, 88:55-66.

15. Zlatkis A.; Zak B.; Boyle AJ. A new method for the direct determination of serum cholesterol, *J Lab Clin Med*, 1953, 41, 486-492.

16. Tiwari R.P.; G.S. Hoondal and R.Tewari. Laboratory Techniques in Microbiology and Biotechnology, Abhishek Publication, 2004.

17. Singer SM.; Nash TE. The role of normal flora in *Giardia lamblia* infections in mice, *J Infect Dis,* 2000, 181, 1520–1522.

18. Glodin BR. Health benefits of probiotics, *Br J Nutr*, 1998, 80(suppl.2), 5203-5207.

19. Kaur P.; Bhatia A. The recent developments in molecular techniques for the detection of probiotics, *Int J Probiotics Prebiotics*, 2008, 3, 65 -70.

20. Usman.; Hosono A. Bile tolerance, taurocholate deconjugation, and binding of cholesterol by *Lactobacillus gasseri* strains, *J Dairy Sci*,1999, 82, 243–248.

21. Dunne C.; O'Mahony L.; Murphy L.; Thornton G.; Morrissey D.; O'Halloran S.; Feeney M.; Flynn S.; Fitzgerald G.; Daly C.; Kiely B.; O'Sullivan G.; Shanahan F .; Collins K. In vitro selection criteria for probiotic bacteria of human origin: correlation with *in vivo* findings, *Am J Clin Nutr,* 2001, 73, 386S-392S.

22. Kheadr EE. Impact of acid and oxgall on antibiotic susceptibility of probiotic lactobacilli, *Afr J Agri Res,* 2006, 1(5), 172-181.

23. Ritus N.; Sole M.; Francis A.; Loren JG. Buffering capacity and membrane H^+ conductance of lactic acid bacteria, *FEMS Microbiol Lett,* 1994, 120, 291-296.

24. Berada N.; Lemeland JF.; Laroch G.; Thouvenot P.; Piala M. Bifidobacterium from fermented milks: Survival during gastric transit, *J Dairy Sci*,1991, 74, 409- 413.

25. Anderson JW.; Gilliland SE. Effect of fermented milk (yoghurt) containing *Lactobacillus acidophilus* L1 on serum cholesterol in hypercholesterolemic humans, *J Am Coll Nutr*, 1999, 18, 43- 50.

26. Sanders ME. Considerations for use of probiotic bacteria to modulate human health, *J Nutr,* 2000, 30, 384S- 390S.

27. Pereira DIA .; Gibson GR. Cholesterol assimilation by lactic acid bacteria and biofidobacteria isolated from the human gut, *Appl Environ Microbiol,* 2002, 68(9), 4689-4693.

28. Cebra JJ. Influences of microbiota on intestinal immune system development., *Am J Clin Nutr,*1999, 69: 1046S–1051S.

29. Bernet-Camard MF.; Lievin V.; Brassart D.; Neeser JR.; Servin AL.; Hudault S. The human *Lactobacillus acidophilus* strain LA1 secretes a nonbacteriocin antibacterial substance(s) active in vitro and in vivo, *Appl Environ Microbiol*, 1997, 63, 2747-2753.

30. Coconnier MH.; Lievin V.; Hemery E .; Servin AL. Antagonistic activity against *Helicobacter* infection in vitro and in vivo by the human *Lactobacillus acidophilus* strain LB, *Appl Environ Microbiol,* 1998, 64, 4573–4580.

31. Le Moal V.; Amsellem R.; Servin AL.; Coconnier MH. *Lactobacillus acidophilus* (strain LB) from the resident adult human gastrointestinal microflora exerts activity against brush border damage promoted by a diarrhoeagenic *Escherichia coli* in human enterocyte-like cells, *Gut,* 2002, 50, 803-811.

32. Bernet MF.; Brassart D.; Neeser JR .; Servin AL. Lactobacillus acidophilus LA 1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria, *Gut,*1994, 35, 483-489.

33. Bibiloni R.; Pérez PF.; De Antoni GL. Will a high adhering capacity in a probiotic strain guarantee exclusion of pathogens from intestinal epithelia? *Anaerobe*, 1999, 5,519-524.

34. Salminen S. ; Bouley C. ; Boutron-Ruault MC *et al.* Functional food science and gastrointestinal physiology and function, *Br J Nutr*, 1998, 80, S147–171.

35. Yuki N.; Shimazaki T.; Kushiro A.; Watanabe K.; Uchida K.; Yuyama T .; Morotomi M. Colonization of the stratified squamous epithelium of the nonsecreting area of horse stomach by lactobacilli, *Appl Env Microbio,*2000, 66, 5030-5034.

36. Oyetayo VO.; Adetuyi FC.; Akinyosoye FA. Safety and protective effect of *Lactobacillus*

acidophilus and *Lactobacillus casei* used as probiotic agent *in vivo, Afr J Biotechnol*, 2003, 2(11), 448-452.

37. Humen MA.; De. Antoni GL.; Benyacoub J.; Costas MB.; Cardozo MI.; Kozubsky L.; Saudan K.; Doenzli-Bruand A.; Blum S.; Schiffrin EJ.; Perez PF. *Lactobacillus johnsonii* La1 antagonizes *Giardia intestinalis in vivo, Infect Immun*, 2005,7,1265–1269.