Clinical microbiology

Characterization of Lactobacillus isolated from dairy samples for probiotic properties

Ashwani Kumar, Dinesh Kumar*

School of Bioengineering and Food Technology, Shoolini University of Biotechnology and Management Sciences, Solan 173212, H.P., India

ARTICLE INFO

Article history:
Received 21 November 2014
Received in revised form 3 March 2015
Accepted 10 March 2015
Available online 12 March 2015

Keywords:
Lactobacillus characterization
Isolate LBS 2
16S rDNA amplification
Epithelial cell adherence
SEM

ABSTRACT

In the present study twelve Lactobacillus isolates (LBS 1-LBS 12) were characterized for probiotic properties. Out of the twelve, eight isolates (LBS 1–6, 8 and 11) were bile resistant (survival > 50% at 0.3% bile salt w/v) and five isolates (LBS 1, 2, 5, 6 and 11) were found acid pH value resistant (survival > 50% at pH 3). All twelve isolates inhibited the growth of Staphylococcus aureus whereas isolate LBS 2 also inhibited the growth of Escherichia coli and Salmonella typhimurium. Antibiotic susceptibility testing of isolates was also performed and isolate LBS 2 was selected for further study based on its broad spectrum effect in clinical pathogen inhibition. LBS 2 was characterized phenotypically at Institute of Microbial Technology (IMTECH), Chandigarh, India and was confirmed as Lactobacillus rhamnosus by 16S rDNA sequencing and subsequent analysis using BLAST. The gene sequence was deposited in GenBank with accession number KJ562858. Scanning electron microscopy (SEM) study was used to study in vitro epithelial cell adherence and bile salt effect on isolate LBS 2. Epithelial cells adherence assay showed positive results and surface roughness of LBS 2 increased with increase in bile salt (0.15–0.45% w/v).

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host [1]. Most probiotic microorganism belongs to the lactic acid bacteria (LAB) viz. Lactobacillus spp., Enterococcus spp. and Bifidobacterium [2]. Most of the species of the genus Lactobacillus are part of human and animal commensal intestinal flora [3] and consists of a physiologically and genetically diverse group of rod-shaped, Gram-positive, non-pigmented, non-spore forming [4], catalase negative, micro-aerophilic to anaerobic LAB [5] with widespread use in fermented food production [6]. These microorganisms are also called friendly bacteria and are generally recognized as safe (GRAS) microorganisms [7]. The lactobacilli isolated from dairy products have long history of safe use [1] and are widely used as starter cultures in the food industry viz. fermented milk, alcoholic beverages, sourdough and silage preparation [8]. Several health benefits associated with the consumption of probiotic bacteria include; controlling intestinal infections, improving lactose utilization, lowering blood ammonia level [9,10], influencing immune system and low serum cholesterol level [11,12]. There is an increase in use of probiotic bacteria in variety of food products viz. yoghurt, cheese, drinks and dietary supplements based on their therapeutic benefits [13]. Keeping in view the importance of probiotics, the present study is endeavored to screen twelve previously reported Lactobacillus isolates (8 from household curd and 4 from household milk) [14] for the probiotic properties viz. resistance against bile salt, acid pH values, antibiotics, pathogenic microbe inhibition and select a potential isolate for further investigation.

2. Materials and methods

2.1. Bacterial strains

Thirty dairy samples (household milk and curd) were collected from local area of Solan, H.P., India. Serial dilutions of 1 ml dairy samples were prepared in peptone water and then 100 μl sample from different dilutions were spread over the solidified MRS (de Man, Rogosa and Sharpe) medium (HiMedia, India) and incubated at 37 °C for 24–48 h under anaerobic conditions for the isolation of Lactobacillus. Pure cultures of isolated colonies were obtained after re-streaking on MRS agar plate. Each culture was studied for morphological investigation (type of colony, color, margin, elevation, opacity and presence of pigment) and Lactobacillus
specific biochemical tests (motility test, endospore test, catalase and sugar (glucose) fermentation test) as reported in our earlier publication [14] and twelve selected Lactobacillus isolates were given code as LBS 1-LBS 12 [8 from household curd (LBS 1,2,4,5,7,9,10,12) and 4 from household milk (LBS 3,6,8,11)] and used in the present study.

For the antimicrobial activity clinical isolates of Staphylococcus aureus, Escherichia coli and Salmonella typhimurium were obtained from the Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India and maintained on Nutrient agar medium (HiMedia, India) at 4 °C after 24 h growth at 37 °C.

2.2. Characterization of isolates for probiotic properties

All twelve Lactobacillus isolates were screened for their resistance against bile salt, acid pH values, antibiotics and ability to inhibit selected microbial pathogens. Inoculums of each isolates was prepared using standard protocol M7-A7- CLSI (Chemical Laboratory Standard Institute) [15] in test tubes containing 5 ml of broth medium and incubated at 37 °C for 24 h. The turbidity of inoculum was maintained with 0.5 McFarland standards, containing 10^7-10^8 CFU/ml.

2.2.1. Bile and acid tolerance

All isolates were investigated for bile salt tolerance following the method of Vinderola and Reinheimer [16]. Briefly, 0.2 ml of each isolate inoculum suspension (10^7-10^8 CFU/ml) was added to 10 ml of MRS broth containing different concentrations of bile salt (0.15-0.45% w/v) and MRS broth without bile salt as control and incubated at 37 °C for 24 h. Optical density (OD), was recorded at 560 nm after incubation and compared with the control. Lactobacillus isolates showing resistance more than 50% at 0.3% (w/v) bile salt were considered as bile resistant as this is the minimum range for being a probiotic culture.

The ability of isolates to tolerate the acid pH values was determined as described by Sieladie et al. [17]. Briefly, 1 ml of inoculum (10^7-10^8 CFU/ml) of each isolate was transferred into 10 ml of MRS broth at pH 2, 3 and 5 respectively and OD was taken after 24 h incubation at 37 °C against the control (pH 7.0). Isolates showing resistance more than 50% at pH 3 were considered acid tolerant strains.

The percentage resistance in both cases (bile/acid pH values) was calculated as:

\[
\text{% Resistance} = \frac{\text{Increment of OD in MRS broth with bile salt at pH 2, 3, 5}}{\text{Increment of OD in MRS broth without bile salt at pH 7}} \times 100
\]

2.2.2. Antimicrobial activity

Antimicrobial activity of the isolates against pathogenic bacteria viz. E. coli, S. aureus and S. typhimurium was determined by standard well diffusion method using Mueller Hinton Agar (MHA) plates [18]. The 100 μl inoculum (10^7-10^8 CFU/ml) of each bacterium was spread on MHA plates. Wells were made in each inoculated plate and filled with 30 μl of cell free supernatant of Lactobacillus isolates obtained after 24 h growth in MRS broth and centrifugation of the inoculums at 4000 rpm for 10 min. Ciprofloxacin (30 μg/ml) was used as positive control and MRS broth was used as negative control. All supernatant broths were neutralized to pH 6.5 to maintain that the inhibition is not due to lactic acid but by antimicrobial substances. All MHA plates were incubated at 37 °C for 24 h and the zone of inhibition was measured using Hi antibiotic zone scale. The inhibition zone diameter of 10 mm and above was considered as positive antimicrobial effect.

2.2.3. Antibiotic susceptibility test

Antibiotic susceptibility of all isolates was determined by the standard disc diffusion method on MHA plates [19]. After adjusting the turbidity standard of bacterial inoculum (10^7-10^8 CFU/ml) a sterile cotton swab was dipped into each inoculum and swab streaked over the entire surface of respective MHA plates. Now different antibiotic discs (vancomycin, amoxycillin, amikacin, gentamycin, tetracycline, chloramphenicol, co-trimoxazole and ciprofloxacin) were placed on these test plates with the help of sterile forceps and incubated at 37 °C for 24 h and the inhibition zone diameter was measured with Hi antibiotic zone scale.

The isolate LBS 2 was used for the further investigation viz. phenotypic and genotypic characterization and scanning electron microscopy (SEM) study to see in vitro cell adherence assay and effect of bile salt on its morphology by surface roughness study.

2.3. Phenotypic and genotypic characterization of LBS 2

The phenotypic characterization of isolate LBS 2 was performed on the basis of its morphological ( colony morphology, Gram’s reaction, spore formation and motility), biochemical (methyl red, citrate utilization, Voges Proskauer, casein, starch and urea hydrolysis, nitrate reduction, H2S production, cytochrome oxidase, arginine dihydrolase, lysine decarboxylase and indole test) and physiological characteristics (growth at varying temperature 25-42 °C, pH 5-9 and 2.5-5% (w/v) NaCl) at Institute of Microbial Technology (IMTECH), Chandigarh, India [20,21]. The isolate LBS 2 was further identified by 16S rDNA gene sequencing. Briefly, genomic DNA was extracted by the standard chloroform-isooamyl alcohol method as described by Sambrook et al. [22]. PCR amplification of the 16S rDNA was performed using the following forward and reverse primers: 5’ AGA GTT TGA TCC TGG CTC AG 3’ and 1492R: 5’ ACG GCT ACC TTG TTA CCA CTT 3’.

The polymerase chain reaction (PCR) mixture consisted of 7.50 μl of DNase-RNase free water, 12.50 μl (1 × ) of 2 × PCR master mix, 1.0 μl (10 pmole) of each primer and 30 ng of DNA template. The PCR reaction was performed in 25 μl volumes for 30 cycles at 95 °C, 30 s at 52 °C, and 1 min at 72 °C with additional extension for 10 min at 72 °C. Amplified DNA was examined by electrophoresis in 1% agarose with 5 μl aliquots of PCR product using non-amplified DNA as a negative control. The 16S rDNA PCR product was purified by QAquick Gel Extraction Kit (Qiagen, India). The purified PCR product was sequenced (forward and reverse sequence) by Xcelris lab, Ahmadabad, India. In the present study only the forward sequence was used. The sequence was compared with the nucleotide database from the NCBI GenBank using the BLAST program. A similarity of >98% to the 16S rDNA sequence of the reference Lactobacillus rhamnosus was used as a criterion for the identification. The obtained 16S rDNA sequence was deposited in the NCBI GenBank database. A phylogenetic tree was generated from the alignment of the deposited sequence by the Neighbor-joining method using Sea-View version 4 [23].
2.4. In vitro epithelial cells adherence assay

The in vitro adherence of LBS 2 cells to epithelial cells was studied using rat ileum as a model. Epithelial cells were prepared according to the method described by Annika et al. [24]. Small segment of rat ileum was opened and washed thrice with sterilized phosphate buffer saline (PBS) (0.1 mol/l, pH 7.2). It was further held in PBS and incubated at 4 °C for 30 min to remove the surface mucus and then washed thrice with PBS. Epithelial cells were scraped into sterilized PBS. One ml of bacterial inoculum (10^7–10^8 CFU/ml) was mixed with 1 ml of the cell suspension of epithelial cells. The mixture was incubated at 37 °C for 30 min. The in vitro adhesion of LBS 2 to epithelial cells was observed using FEI Nova NanoSEM 450, Scanning Electron Microscope.

2.5. Effect of bile salt on morphology of LBS 2

Effect of different concentrations of bile salt on morphology (roughness) of bacteria was analyzed using SEM as described by Soo-Hwan et al. [25] with some modifications. Bacterial cells after treatment with bile salt (0.15–0.45% w/v) were fixed in 3% (v/v) glutaraldehyde buffered with 0.1 M sodium phosphate buffer (pH 7.2) for 3 h and then washed three times in the same buffer. Further cells were dehydrated in a graded alcohol series (30, 50, 70, 90, 100%). The specimens were dried, coated with thin layer of gold and mounted onto stubs using double-sided carbon tape and examined for change in surface roughness using SEM.

2.6. Statistical analysis

All characterization experiments were done in triplicates and standard deviation was calculated. Statistical evaluation was also carried out using GraphPad Prism 6.0. A p-value below 0.05 was considered to be statistically significant.

3. Results

Thirty bacterial isolates were initially obtained from thirty dairy samples collected from Solan district in Himachal Pradesh, India. Out of thirty, 14 isolates were from milk sample and 16 isolates were from curd samples. Characterization of isolates was performed on the basis of their morphological characteristics viz., type of colony, color, margin, elevation, opacity and presence of pigment and Lactobacillus specific biochemical tests. Based on morphological, cultural and biochemical characteristics, 12 bacterial isolates (8 from curd and 4 from milk) were considered to belong to the genus Lactobacillus as reported in our previous publication [14] and these were further characterized for the probiotic potential and the results are discussed in the following sections.

3.1. Characterization of isolates for probiotic properties

3.1.1. Bile and acid tolerance

Bile salt resistance is one of the criteria for any microbial strain to be used as a probiotic culture. The presence of bile in the intestine affects the viability of LAB. All twelve isolates were grown in different concentrations of bile salt and results showed that only eight isolates were resistant at 0.3% (w/v) of bile salt i.e. percentage of resistance ≥50%. Maximum resistance was observed with LBS 2 (76.88%, 72.36%, 68.62%) and minimum with LBS 3 (67.42%, 57.61%, 50.67%) respectively at 0.15%, 0.3% and 0.45% (w/v) of bile salt (Fig. 1).

The survival of LAB in low pH of stomach is important for bearing the initial acid stress. The effect of acidity on the viability of all isolates was assessed by their growth in different incubation pH in MRS broth. Out of twelve isolates, five were resistant to acid pH value 3 (with resistance ≥50%). Highest resistance was reported with LBS 2 (60.52%) and lowest with LBS 11 (52.62%) at pH 3 (Fig. 2). However, no isolate was found resistant at pH 2 (with percentage resistance <50%).

3.1.2. Antimicrobial activity

One of the major properties for an isolate to be used as probiotic is its ability to inhibit microbial pathogens. In the present study, antimicrobial effects of all isolates against selected pathogenic bacteria were studied. All isolates inhibited the growth of S. aureus whereas only one isolate (LBS 2) inhibited the growth of E. coli, S. typhimurium with inhibition zone diameter of 10.66 ± 0.57 mm and 10.33 ± 0.57 mm respectively in addition to S. aureus (11.33 ± 0.57 mm). However, highest zone of inhibition (14 ± 1 mm) against S. aureus was observed with the isolate LBS 8 (Table 1).

3.1.3. Antibiotic susceptibility test

The susceptibility to antibiotics is a common feature of probiotic bacteria. The antibiotic susceptibility of all isolates was assessed by disc diffusion method using MHA medium and results are shown in the Table 2. The isolate LBS 5 was found resistant to six antibiotics and the isolate LBS 2 was resistant to five antibiotics. The antibiotic

![Fig. 1. Effect of different concentration of bile salt 0.15–0.45% (w/v) on survival of Lactobacillus isolates (LBS1–12) after 24 h incubation at 37 °C in MRS broth.](image-url)
resistance traits among probiotic microorganisms are advantageous to survive the gastrointestinal tract during antibiotic treatment. Some resistance genes might be present in resistant microorganisms and mechanism associated with this resistance is still unknown.

3.2. Identification and characterization of selected isolate LBS 2

The isolate LBS 2 showed maximum resistance with bile salt (72.36%) at 0.30% (w/v), acid pH value 3 (60.52%), inhibited growth of all tested microbial pathogens and was resistant to five antibiotics and was selected for further identification and characterization.

3.3. Phenotypic characterization and genotypic characterization of LBS 2

The selected Lactobacillus (LBS 2) with maximum probiotic potential properties was further characterized at IMTECH, Chandigarh, India for its phenotypic characteristics viz. morphological, biochemical and physiological properties. Morphological investigation of LBS 2 showed that colonies were round, smooth, raised, entire margins, Gram positive rods arranged in clusters, non-spore forming and non-motile. Various biochemical tests viz. methyl red, citrate utilization, Voges Proskauer, casein, starch and urea hydrolysis, nitrate reduction, H2S production, cytochrome oxidase, arginine dihydrolysis, lysine decarboxylase and indole test were found negative for this isolate. This isolate showed positive growth at 25–42 °C, pH 5–9 and 2.5–5% NaCl (w/v), however, no growth was observed with increase in temperature beyond 55 °C, pH 9 and 6% NaCl respectively. The isolate LBS 2 showed positive results for fermentation with dextrose, fructose, galactose, lactose, maltose, mannitol, rhamnose, sorbitol, sucrose, trehalose and mannose and no fermentation was observed with xylose, adonitol, inulin, cellobiose, dulcitol, inositol, melibiose, raffinose and salicin. Based on morphological, biochemical and physiological characteristics the isolate LBS 2 showed similarity in phenotypic properties with L. rhamnosus.

The PCR amplification of 16S rDNA of LBS 2 resulted in a single band of about 1500 bp product which corresponds to the expected size of the 16S rDNA gene. Both forward and reverse sequence was obtained after sequencing and assembly sequence was generated but in the present study only the forward sequence was used. BLAST analysis of the obtained sequence with the available nucleotide database in the GenBank, showed 99% similarity of the LBS 2 isolate with the L. rhamnosus (Fig. 3). The sequence was deposited in GenBank with accession no. KJ562858. The comparative analysis of LBS 2 sequences with the published sequences of different Lactobacillus species, using the SeaView Version 4 program confirmed its similarity with the L. rhamnosus KF806539.1 and demonstrated the phylogenetic distances in a generated Neighbor-joining rooted tree (Fig. 3).

3.4. In vitro epithelial cell adherence assay and bile effect analysis using SEM

The ability of microbe to adhere to intestinal mucosa is an important selection criterion for its probiotic use. The Lactobacillus isolates with more than 15 adhered cells per epithelial cell were considered positive and the results of adherence of LBS 2 to in vitro epithelial cells is shown in Fig. 4.

The effect of different concentration of bile salt after incubation of Lactobacillus rhamnosus LBS 2 at 37 °C for 24 h on its cell morphology (roughness) was studied with SEM. The results showed that with an increase in amount of bile salt from 0.15% to 0.45% the roughness of bacterial cell surface also increased (Fig. 5).

4. Discussion

In the present study twelve Lactobacillus spp. isolated from dairy
Table 2
Antibiotic susceptibility of *Lactobacillus* isolates using disc diffusion method with MHA after 24 h incubation at 37 °C.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th><em>Lactobacillus</em> isolate (LBS 1–12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12</td>
</tr>
<tr>
<td>Co-trimoxazole (25 mcg)</td>
<td>R R R R R R R ++ ++ ++ ++ ++ ++ ++</td>
</tr>
<tr>
<td>Amikacin (30 mcg)</td>
<td>++ R ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
</tr>
<tr>
<td>Amoxycillin (10 mcg)</td>
<td>R R R R R R R ++ ++ ++ ++ ++ ++ ++</td>
</tr>
<tr>
<td>Penicillin-G (10 mcg)</td>
<td>+ + ++ ++ ++ ++ ++ ++ ++ ++ ++ ++</td>
</tr>
<tr>
<td>Ciprofloxacin (10 mcg)</td>
<td>++ + +++ + R ++ ++ +++ ++ +++ ++ +</td>
</tr>
<tr>
<td>Vancomycin (10 mcg)</td>
<td>R R ++ R R R R ++ ++ R ++ ++ R ++</td>
</tr>
<tr>
<td>Tetracycline (10 mcg)</td>
<td>++ + +++ + R R R ++ +++ ++ ++ ++</td>
</tr>
<tr>
<td>Gentamycin (10 mcg)</td>
<td>R R ++ R R R ++ +++ ++ ++ ++ ++</td>
</tr>
</tbody>
</table>

R = resistant, + = less sensitive, ++ = moderately sensitive, +++ = highly sensitive.

---

**Fig. 3.** Phylogenetic tree (neighbor-joining) of isolate LBS 2 based on the 16S rDNA sequence. The scale bar represents relative sequence similarities.

**Fig. 4.** In vitro epithelial cell adherence of *Lactobacillus rhamnosus* LBS 2. A) Control (only crop epithelium), B) crop epithelium with positive adhered cells.
samples were characterized for probiotic properties and results were compared with the existing literature. In screening study eight, out of twelve isolates were bile resistant and Lactobacillus isolates showing resistance ≥50% at 0.3% (w/v) bile salt were considered as bile resistance strains [16]. Highest resistance (72.36% and 68.62%) was observed with isolate LBS 2 at 0.3% and 0.45% of bile salt respectively. Gilliland [26] reported that lactobacilli isolated from animal intestines showed high tolerance to bile salt than isolates from milk products. Similar results were reported by Patel et al. [27]. Gilliland et al. [28] observed high variability among Lactobacillus acidophilus strains isolated from calf intestinal contents to grow in vitro in the presence of bile salts. Garriga et al. [29] reported the selected LAB strains with resistant to 4% bile salts. The resistance variability among lactobacilli is due to the presence of bile salt hydrolase (BSH), an enzyme that reduces toxic effects by conjugating bile [30].

The acid pH stability results of tested isolates in the present study are in accordance with the reported literature. Isolates showing ≥50% resistance at acid pH value 3 were considered acid tolerant [17]. In present study five isolates were acid resistant and highest resistance was observed with LBS 2 (60.52%) at acid pH value 3. Idoui et al. [31] also showed resistance of Lactobacillus plantarum BJ0021 at pH 3. Idoui [32], showed that in comparison to the Lactobacillus isolated from the gastrointestinal tracts of human, strains of Lactobacillus Fermentum, Lactobacillus gasseri and Lactobacillus delbrueckii subsp. bulgaricus were having better acid tolerance.

In present study, antimicrobial activity of twelve selected isolates was tested against selected clinical isolates. Isolate LBS 2 inhibited the growth of E. coli, S. typhimurium and S. aureus and showed broad spectrum antimicrobial activity. Garriga et al. [29] reported inhibition of one or more enteric indicator strains (E. coli, Salmonella enteritidis) by Lactobacillus paracasei subsp. Paracasei. L. acidophilus strains isolated from infant faeces had weak antibacterial activity on E. coli and Yersinia enterocolitica [33]. Daeschel [34] reported that the antimicrobial effect of LAB is due to the production of lactic acid, reduction of pH, acetic acid, diacetyl, fatty acids, aldehydes and other compounds. Whereas, as per another report the antimicrobial action is due to the potential of LAB bacteriocin and peptides having inhibitory properties [35].

In antibiotic test all isolates showed varying antibiotic susceptibility pattern. Isolate LBS 5 was resistant to six antibiotics and LBS 2 was resistant to five antibiotics. As reported in literature, specific antibiotic resistance traits among probiotic strains may be desirable [36]. In case of co-administration of probiotics with antibiotics they should be resistant to certain antibiotics to survive in the gastrointestinal tract [37]. The presence of antibiotic-resistance genes in many LAB and transfer of plasmids and conjugative transposons to and from LAB, have been reported in Lactobacillus species [39]. In general, glycopeptide, aminoglycoside (aminicin, kanamycin, streptomycin and gentamycin) and sulfamethoxazole resistance has been described in LAB, and in most cases it is associated with their natural and intrinsic resistance due to membrane impermeability, probably complemented by potential efflux mechanism resistance [40–42]. Intrinsic resistance is not horizontally transferable and poses no risk in non-pathogenic bacteria [43]. Hoque et al. [44] found that the Lactobacillus spp. were resistant to β-lactams due to presence of β-lactamase in the isolates. Zhou et al. [45] reported that new probiotic strains; L. rhamnosus HN001 and HN067 were resistant to fusidic acid, kanamycin, nalidixic acid, neomycin, polymyxin B and vancomycin due to their intrinsic resistance and without transmissible antibiotic resistance genes. In another report L. rhamnosus BFE 7442 was resistant against ciprofloxacin, gentamycin and streptomycin and demonstrated that the resistant genes might be present in probiotic strains but are silent. Genetic basis and associated resistance mechanisms towards some antibiotics are still unknown and need to be explored [46,47].

The isolate LBS 2 was selected for further study as it showed highest resistance against bile salt and acid pH value in vitro experiments. It also showed broad spectrum microbial pathogen inhibition, resistance to five antibiotics and was further investigated for phenotypic and genotypic characterization and was identified as L. rhamnosus LBS 2 by 16S rDNA sequencing.

In vitro epithelial cell adherence and bile salt effect was studied with SEM analysis. L. rhamnosus LBS 2 showed positive epithelial cell adherence test. The results of the epithelial cell adherence were compared with the existing literature and isolates with an adhesion efficacy of 15 bacteria per epithelial cells are considered positive [48]. Idoui [32], reported adherence specificity of L. fermentum HG3 and L. gasseri HG8, to chicken intestinal epithelium. Jamaly et al. [48] reported that all the tested strains were able to adhere to rat ileum epithelial cells. Effect of bile salt on surface morphology of L. rhamnosus showed that with an increase in concentration of bile salt the smoothness of bacterial surface changed.

5. Conclusion

In conclusion one potential probiotic isolate LBS 2 was obtained after screening of twelve Lactobacillus isolates for the probiotic properties. This isolate LBS 2 from the household curd sample of
Solan, district of Himachal Pradesh was identified as *L. rhamnosus* LBS 2 after phenotypic and genotypic characterization. High resistance to bile salt, tolerance to acid pH values, broad spectrum microbial pathogen inhibition and adherence to epithelial cells seems to be a potential advantage of this culture for use as probiotic culture. Isolate LBS 2 was resistant to five antibiotics, out of tested eight. Antibiotic resistant traits among probiotic strains may be desirable if administered alongwith antibiotics. However, detailed molecular investigation has to be conducted to establish the mechanism of the antibiotic resistance genes in this isolate.

Acknowledgments

The authors are thankful to Dr. Rohit Goyal, Faculty of Pharmaceutical Sciences, Shoolini University, Solan, India for his help in epithelial cells adherence assay and Dr. Sandeep, Indian Institute of Technology, Mandi, India for SEM analysis in the present study.

References


