ABSTRACT: The probiotic potential of two environmental isolates of Lactic acid bacteria (LAB), Lactobacillus plantarum LR/14 and Enterococcus faecium LR/6 was investigated. The said assessment comprised cell surface hydrophobicity, acid and bile tolerance, bile salt hydrolyzing ability, removal of cholesterol and the production of antimicrobial compounds. Moreover, the survival of these strains in simulated stomach condition with and without the presence of antacids was also looked into. The strain L. plantarum LR/14 showed wide range of probiotic properties and appears to be a good candidate for in vivo testing. E. faecium LR/6, in comparison, was less promising but showed probiotic qualities in terms of acid and bile tolerance. Both the strains showed a good survival in simulated stomach conditions especially in the presence of antacids.

KEYWORDS: Acid Tolerance, Bile Salt Hydrolysis, Bile Tolerance, Cholesterol Reduction, Lactic Acid Bacteria, Probiotics

INTRODUCTION

Probiotics are the live microbial food supplements, which beneficially affect the host by improving the intestinal microbial balance (Schrezenmeir and de Vrese, 2001). The concept of probiotics and their role in human health restoration is not entirely new. In recent years, there has been a renewed interest in functional and health foods mainly because of growing awareness among consumers regarding the linkage between food and good health. As the probiotics could replenish the gut microflora of body under disease-associated (Bhatnagar et al., 1998; Isolauri, 2001) or other medical malfunctioning conditions, they have acquired both prophylactic as well as biotherapeutic status. Probiotics are potentially beneficial in condition like lactose intolerance, lowering of cholesterol and blood pressure, improving immune function and preventing infection, controlling antibiotic-associated diarrhea, improving mineral absorption, preventing harmful bacterial growth under stress (Fooks et al., 1999; Reid, 2001), and irritable bowel syndrome and colitis (Reyed, 2007).

For this reason, many probiotic strains are commercially available as medicinal supplements and many are used in the probiotic food products (Hekmat and McMahon, 1992; Jayamanne and Adams, 2004). These probiotic strains are mainly isolated from natural microflora of the body. Little work has been done on the environmental strains, which could not only be used to extend the list of beneficial microflora, but many may also possess greater variability in these properties. As Lactic acid bacteria (LAB) are generally regarded as safe (GRAS) organisms (Jay, 2000; Holzapfel et al., 2001), other useful strains can also be studied for their potential as probiotics.

A group of requirements has been identified as important properties for any strain to be effective probiotic organism (Reid et al., 2003; Draksler et al., 2004). These include the ability to (i) adhere to intestinal cells based on cell surface hydrophobicity, (ii) persist and multiply, (iii) produce acids, hydrogen peroxide and bacteriocins antagonistic to pathogen growth, (iv) exclude or reduce pathogenic adherence, (v) be safe, non-carcinogenic and non-pathogenic, and (vi) manifest beneficial effects like bile salt hydrolysis and lowering of cholesterol etc. We have studied two environmental isolates of LAB and elucidated some of the properties that make them strong contenders as probiotics.

MATERIALS & METHODS

Two isolates of LAB, Lactobacillus plantarum LR/14 (Tiwari and Srivastava, 2008a,b) and Enterococcus faecium LR/6 (manuscript submitted) were obtained from rhizospheric soil region. The strains were identified by detailed morphological and biochemical characterization (including API-CHL50 analysis). The identification was further confirmed by 16S rDNA
amplification and sequencing (NCBI, Gene Bank accession no. EF 166073 and EU 366176). The cultures were raised in MRS broth (peptone 1%, beef extract 1%, yeast extract 1%, dextrose 2%, tween-80 0.1%, tri-ammonium citrate 0.2%, sodium acetate 0.5%, magnesium sulphate 0.05%, manganese sulphate 0.02%, di-potassium phophate 0.2%, pH 6.0-6.2) using inoculum of 0.5%, magnesium sulphate 0.05%, manganese sulphate 0.02%, 2%, tween-80 0.1%, tri-ammonium citrate 0.2%, sodium acetate broth (peptone 1%, beef extract 1%, yeast extract 1%, dextrose 2%, tween-80 0.1%, tri-ammonium citrate 0.2%, sodium acetate 0.5%, magnesium sulphate 0.05%, manganese sulphate 0.02%, di-potassium phophate 0.2%, pH 6.0-6.2) using inoculum of 0.5% and incubation for 20h at 37ºC. Such a culture was used as inoculum for all the experiments.

**Cell surface hydrophobicity assays**

Various cell surface hydrophobicity assays were done on both the strains as described by Krepsky et al. (2003). Cells were collected by centrifugation at 2500g, washed with 0.01M phosphate buffered-0.15M NaCl (PBS), and resuspended in 5ml of same buffer.

**Spontaneous aggregation assay**

The spontaneous aggregation (SA) of bacterial cells was carried out in MRS culture medium after 22h incubation. A positive test result (SA positive) was recorded when cells remained clumped, and a negative test result (SA-negative) showed a smooth turbid suspension after vortexing the culture gently.

**Auto-aggregation assay**

The auto-aggregation assay was done by dispensing 50 μl of a bacterial suspension (~10^9 cfu/ml), prepared in PBS (pH 6.8) on a glass slide. Auto-aggregation (AA) of cells was observed by manually rotating the droplet for 1 min at room temperature. Bacterial suspension that remained homogeneously turbid was considered AA-negative, whereas when clumped cells were observed, it was considered to be AA-positive.

**Salt aggregation test**

Two-fold serial dilutions of ammonium sulphate (NH₄)₂SO₄ in PBS (pH 6.8) ranging from 0.007M to 4.0 M were prepared. Fifty microliter aliquots of each of these concentrations were placed on clean glass slides and mixed thoroughly with 50 μl of bacterial suspension (~10^9 cfu/ml) in PBS. The lowest concentration of ammonium sulphate giving visible bacterial cell clumping served as the numerical value of the level of the cell surface hydrophobicity (SAT value). A positive test was considered for a strain with a SAT values of < 2.0 M (Krepsky et al., 2003).

**Surface accumulation assay (SAA)**

The strains were evaluated by quantitative polystyrene microtitre plate method. Aliquots of 200 μl of the bacterial suspension (~10^9 cfu/ml) in MRS were dispensed in wells (8 wells per strain) of a 96 well polystyrene, flat-bottomed microtitre plate. The plates were incubated for 24 h at 37°C. The initial OD was taken at 600nm. Plates were then thoroughly washed with PBS twice, after which final OD was taken to evaluate the cells that remained adhered to the well.

**Cell adherence to the glass surface**

The cells were grown in 100ml Erlenmeyer flask in MRS medium under static condition for 24h at 37°C. The culture supernatant was decanted and the cell layer was washed with PBS (pH 6.8) twice. The affinity of cells to adhere to the glass surface gave the indication of cell surface hydrophobicity.

**Microbial adhesion to hydrocarbon**

Cell surface hydrophobicity was also assayed by MATH (microbial adhesion to hydrocarbon) as described by Dufour et al. (2007). The cells were washed three times in ice-cold MilliQ water and resuspended in phosphate buffer (pH 6.8) to an OD₆₀₀ of 0.5. A 4.8 ml volume of cell suspension was mixed with 0.8 ml of n-hexadecane in a glass tube and vigorously shaken for 1 min. After the preparation rested for 30 min, the OD₆₀₀ values of the aqueous phase was determined. The affinity of bacteria for the solvent was evaluated by the formula - % adherence = (1-A/A₀) X 100, Where A₀ is the OD₆₀₀ of the bacterial suspension before mixing and A is the OD₆₀₀ after mixing.

**Acid tolerance**

To simulate the gastric environment of stomach, an electrolyte solution containing 6.2 g/LNaCl, 2.2 g/LCaCl₂, and 1.2 g/LNaHCO₃, adjusted to pH 2.0 to which pepsin was added at a final concentration of 0.3%, was used. Control solution devoid of pepsin was adjusted to pH 7.0. Acid tolerance of the cultures was studied by inoculating these two sets with bacterial suspension (~10^9 cfu/ml) of an overnight culture and incubated at 37°C for 2h. Serially diluted sample of each was plated onto MRS agar, and the plates were incubated at 37°C for 24 h to get the colony count.

**Effect of simulated gastric passage on the viability of strains**

The parameters identified as important for bacterial survival in a model stomach/intestine passage by Vizoso-Pinto et al. (2006), through the Plackett-Burman design (lysozyme, pepsin, acidic condition, and presence of skim milk) were applied to the test bacteria. Reconstituted skim milk (15% w/v) was inoculated with -2 x 10^7 cfu/ml of an overnight culture of both the strains. One millilitre aliquot was removed, serially diluted in normal saline (0.8% NaCl), and spread plated onto MRS agar to determine the cfu/ml at time 0. To simulate the dilution and possible hydrolysis of bacteria in the human oral cavity, the suspension was diluted 1:1 with a sterile electrolyte solution (described above) to which lysozyme was added at a final concentration of 100 ppm, and incubated for 5 min at 37°C. One milliliter aliquot was removed, serially diluted in normal saline and spread plated on MRS agar. The rest of the sample was subsequently diluted 3:5 with an artificial gastric fluid, consisting of the electrolyte solution as described above adjusted to pH 2.5 and with added pepsin. After 1h of incubation at 37°C, another 1 ml aliquot was removed, serially diluted in normal saline and spread plated on MRS agar. To simulate the digestion in the small intestine, the remaining sample was diluted 1:4 using an artificial duodenal secretion
(pH 7.2) consisting of 6.4g/l NaHCO3, 0.239g/l KCl, 1.28g/l NaCl, 0.5% bile salts and 0.1% pancreatin. One milliliter aliquots were again removed after 3 h, serially diluted in saline and spread plated on MRS agar to determine the cfu/ml.

**Acidifying Activity**

Acidifying activity was measured by change in pH during growth as described by Mourad and Nour-Eddine (2006). Ten milliliters of MRS was inoculated with 0.2% (~ 10⁸ cfu/ml) of inoculum and incubated at 37°C. The pH was measured at 0, 2, 4 and 6 h using a pH meter (LABINDIA, µp Controlled pH analyzer, India). The acidification values were expressed as pH decrease, calculated as difference between the value immediately after inoculation and values at 2, 4, and 6 h (ΔpH=pH at time - pHzero time). The culture was considered as fast, medium or slow acidifying when a pH of 0.4 U (ΔpH units) was achieved after 3, 3-5 and > 5 h, respectively.

**Survival in the simulated condition with antacid**

The extent of survival of strains is critical in acidic environment of stomach. Experiments were designed to see the cell survival under similar conditions along with the combination of an antacid. Two antacids (commercially available), Antacid fruit salt (ENO, GlaxoSmithKline, India) and Ranitidine hydrochloride (GlaxoSmithKline, India) were tested. Electrolyte solution was adjusted to pH 2.5 and 0.3% pepsin was added to it. One tablet of Ranitidine hydrochloride (150 mg-Ranitidine) was dissolved in 50 ml of this solution. Similarly one sachet of ENO (~5g having sodium bicarbonate-46%, citric acid-43%, and sodium carbonate-10% w/v) was dissolved in 50 ml of electrolyte solution. These three sets (one control and two with antacid) were inoculated with approximately 2x10⁹ cfu/ml of an overnight culture and incubated at 37°C for 2 h. One milliliter aliquot was taken from each that was serially diluted in saline and spread plated on MRS agar.

**Bile tolerance**

Two types of bile salts, namely oxgall and taurocholic acid (Sigma-Aldrich, USA), were used to study bile tolerance of the organisms. Bile tolerance was assessed according to the method of Gilliland and Walker (1990). MRS broth containing 0.30% of oxgall or taurocholic acid was inoculated with each strain at an OD600 of 0.02, and incubated at 37°C. The control comprised MRS broth without bile salt. Bacterial growth was monitored by measuring OD600 at hourly interval for 8h. The absorbance values obtained were plotted against the incubation time, and the bile tolerance of each strain was monitored based on the time required for the absorbance to increase by 0.3 units.

**Bile salt hydrolase activity**

Bile salt hydrolase (BSH) activity of the cultures was detected using the plate screening procedure described by Franz et al. (2001). Briefly, overnight cultures were streaked onto MRS agar plates containing 0.3% taurodeoxycholic acid and 0.37 g/l CaCl₂. The plates were incubated in anaerobic chamber (filled with CO₂) at 37°C for 24h. Bacterial growth with a precipitation zone was considered BSH-positive.

**Cholesterol removal**

Freshly prepared MRS broth was supplemented with 0.30% oxgall as a bile salt. Water-soluble cholesterol (polyoxyethenyl cholesteryl sebacate, Sigma-Aldrich, USA) was filter-sterilized and added to the broth at a final concentration of 100 µg/mL. Such a medium was inoculated with each strain (at 1% inoculum level), and incubated in anaerobic chamber (filled with CO₂) at 37°C for 20h. After incubation, cells were centrifuged and the cholesterol concentration in the broth was determined using a modified colorimetric method as described by Rudel and Morris (1973). Briefly, 1 ml of culture filtrate mixed with 1 ml of KOH (33% w/v) and 2 ml of absolute ethanol, was vortexed for 1 min., and incubated at 37°C for 15 min. After cooling, 2 ml of distilled water and 3 ml of hexane were added, vortexed for 1 min, and allowed to stand. One milliliter of the hexane layer was transferred into a glass tube and evaporated. The residue was immediately dissolved in 2 ml of o-phthalaldehyde (Sigma-Aldrich, USA) reagent. After complete mixing, 0.5 ml of concentrated sulfuric acid was added and the mixture was vortexed for 1 min. Absorbance was read at 550 nm after 10 min incubation.

**Antimicrobial potential**

For detecting the antimicrobial activity, strains were inoculated in MRS medium to initial OD600 of 0.02. After overnight growth (reflecting the stationary phase), cell-free supernatant was collected by centrifugation (10,000g, 15 min at 4°C), filter-sterilized (0.2µm pore size membrane) and tested for bacteriocinogenic activity by AWDA (agar well diffusion assay) as described previously (Tiwari and Srivastava, 2008 a, b). The strains included were other lactic acid bacteria as well as some food-borne pathogens.

**Statistical analysis**

Each experiment was carried out in two independent replicates with three measurements per replicate. All data are presented as a mean ± standard error of mean.

**RESULTS**

Different tests were carried out to assess the cell surface hydrophobicity of the strains, *L. plantarum* LR/14 and *E. faecium* LR/6. These comprised auto-agglutination (AA) test, salt aggregation test (SAT), glass and polystyrene adherence test, surface accumulation assay (SAA) and adhesion to hydrocarbon. For example, ~90% of the cells adhered to the polystyrene surface in case of *L. plantarum* LR/14 which in case of *E. faecium* LR/6 amounted to only 50%. Interestingly, the adherence of strain LR/14 to the glass surface was so strong that it became difficult to resuspend the culture in the medium again even after vigorous shaking or vortexing (Fig. 1). Moreover, in AA-, SAT-, and SAA- test, strain LR/14 showed a more positive response than strain LR/6. The SAT value for LR/14 is <1
The effect of the pH on viability of the strains under consideration is shown in Table 1. *E. faecium* LR/6 showed greater acid tolerance over the entire incubation period in comparison to *L. plantarum* LR/14. The drop in cell viability ranged from 10^4-fold (LR/14) to 10^5-fold (LR/6), that reflected a decrease in terms of 3.07 log_{10} and 3.69 log_{10} cfu/ml values, respectively in comparison to their control. The survival at low pH was further confirmed by the pH changes during growth and associated acidification of the medium. The ΔpH value in both the strains, as described in Materials and Methods, was achieved with in 3h of growth.

**TABLE 1. Effect of pH 2.0 on viability of *Lactobacillus plantarum* LR/14 and *Enterococcus faecium* LR/6**

<table>
<thead>
<tr>
<th>Period of Incubation</th>
<th><em>Lactobacillus plantarum</em> LR/14</th>
<th><em>Enterococcus faecium</em> LR/6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>8.59 ± 0.06</td>
<td>7.23 ± 0.20</td>
</tr>
<tr>
<td>30 min</td>
<td>7.45 ± 0.35</td>
<td>6.86 ± 0.04</td>
</tr>
<tr>
<td>60 min</td>
<td>6.32 ± 0.08</td>
<td>7.11 ± 0.11</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM; each data point is the average of 3 repeated measurements from 2 independent replicates.

The effect of successive passage through artificial saliva, gastric, and duodenum juices on the cell viability of both the strains was evaluated on the basis of parameters identified by Vizoso-Pinto et al. (2006). Strain *E. faecium* LR/6 showed the higher level of survival, with 99% of cells surviving in simulated gastric and intestinal transit whereas 92.5% of *L. plantarum* LR/14 cells survived these conditions. Almost 100% cells of both the strains survived the first phase that is in the presence of lysozyme. In the second phase, having acidic pH and pepsin, while *E. faecium* LR/6 cells showed a drop of only 1%, ~ 6% loss in viable count in case of *L. plantarum* cells was observed. In the third and final phase, in the presence of pancreatin, there was no loss in viable count in LR/6 cells but 1.5% further loss in viable count was recorded in case of strain LR/14.

The administration of antibiotics generally results in depletion of gut microflora, causing several stomach problems. In order to overcome some of these problems, antacids are prescribed. Thus, it is important to see the effect of antacid on the survival of probiotic strains. The results of this experiment are shown in Table 2. As is clear from the results, almost complete reversal of the effect of acid that led to some drop in cell viability now resulted into 100% survival of both the strains. Addition of the antacids to the electrolyte solution (pH 2.0) led to an increase in pH to 4.5.

**TABLE 2. Effect of pH and Antacid supplements**

<table>
<thead>
<tr>
<th></th>
<th><em>Lactobacillus plantarum</em> LR/14</th>
<th><em>Enterococcus faecium</em> LR/6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH2</td>
<td>10.46±0.12</td>
<td>10.46±0.12</td>
</tr>
<tr>
<td>pH2 + Ranitidine</td>
<td>10.23±0.14</td>
<td>10.46±0.12</td>
</tr>
<tr>
<td>pH2 + ENO</td>
<td>10.08±0.04</td>
<td>10.44±0.12</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM; each data point is the average of 3 repeated measurements from 2 independent replicates.

The growth of a strain in the presence of conjugated bile acid (taurocholic acid) and mixture of conjugated and deconjugated bile acid (oxgall) is an important criteria for assessing its survival in intestine. The effect of oxgall and taurocholic acid on the growth of both the strains along with respective control (without bile salts) is presented in Table 3. Both the strains showed similar growth profile in MRS and MRS with bile salts. The bile tolerance was assessed by the time required for the absorbance (A_{600}) value to increase by 0.3 units, which in case of strain LR/14 and strain LR/6 was around 6 h and 5 h, respectively. Of the two bile salts tested, there was not much of a difference in the tolerance profile. Bile salt tolerance is an important criterion for potentially probiotic strains and could be demonstrated in case of both the strains under study.

**TABLE 3 Bile tolerance of *Lactobacillus plantarum* LR/14 and *Enterococcus faecium* LR/6**

<table>
<thead>
<tr>
<th>TIME</th>
<th><em>Lactobacillus plantarum</em> LR/14</th>
<th><em>Enterococcus faecium</em> LR/6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>0.15±0.02</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td>2h</td>
<td>0.23±0.03</td>
<td>0.22±0.01</td>
</tr>
<tr>
<td>4h</td>
<td>0.48±0.01</td>
<td>0.32±0.01</td>
</tr>
<tr>
<td>6h</td>
<td>0.84±0.02</td>
<td>0.54±0.04</td>
</tr>
<tr>
<td>8h</td>
<td>1.11±0.04</td>
<td>0.80±0.01</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM; each data point is the average of 3 repeated measurements from 2 independent replicates.
Bile salt hydrolase activity is known to confer a selective advantage on probiotic strains in the highly competitive environment of the human intestinal tract (Begley et al., 2006). The two strains were, therefore, tested for this property as well. The bile salt hydrolase activity was evident by the formation of a white precipitation zone around the bacterial growth under anaerobic conditions on MRS agar supplemented with bile salts. Such a precipitation was not observed in MRS agar (control) without bile salts. While strain LR/14 showed the precipitation zone around the growth of bacteria on the plate containing 0.3% oxgall, strain LR/6 failed to grow on such a plate (Fig. 2). This suggested that the former strain possesses bile salt hydrolase activity.

FIGURE 2. Bile salt hydrolase activity as seen by a precipitation zone around the growth of *L. plantarum* LR/14 on the plate containing 0.3% oxgall. Note: Strain *Enterococcus faecium* LR/6 failed to grow on this medium.

High level of serum cholesterol has been associated with the risks of coronary heart disease. The property of probiotic bacteria in reducing serum cholesterol level has, therefore, attracted lots of attention (Jones et al., 2004). When the two strains were tested for this function, cholesterol assimilation was observed during 20h of growth in both the strains. *L. plantarum* LR/14 removed 32.33 µg/ml, whereas *E. faecium* LR/6 could remove only 7.23 µg/ml of cholesterol from the medium.

Crude culture supernatant and purified bacteriocin from *L. plantarum* LR/14 strain showed antimicrobial activity (Tiwari and Srivastava, 2008 a,b,c). When tested, it inhibited 15 related LAB isolates (data not shown). Most importantly, some pathogens such as *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Listeria monocytogenes*, *E. coli* (urogenic), *Yersinia enterocolitica*, *Bacillus* sp., *B. licheniformis* and species of *Staphylococcus*, *Aeromonas* and *Shigella* were also inhibited (Tiwari and Srivastava, 2008b). In comparison, *E. faecium* LR/6 did not inhibit *S. typhimurium*, *E. coli* (urogenic) and *Bacillus* sp. Our previous work has shown that both the strains produce a bacteriocin that explains their antimicrobial potential.

DISCUSSION

The hydrophobicity of the bacterial cell surface is an important determinant in the adherence of the bacteria to both living and non-living surfaces. Different methods are known to test the hydrophobicity of a cell, but these tests are complementary, not restricting. Moreover, the mechanism of adhesion requires the participation of different surface constituents that interact in a sequential manner to overcome repulsive forces (Draksler et al., 2004). For a probiotic strain, this has important implication, as the bacteria need to adhere to the cells of the human gastro-intestinal tract. From all the tests, it appeared that *L. plantarum* LR/14 possessed high cell surface hydrophobicity, whereas in comparison *E. faecium* LR/6 exhibited a moderate level of hydrophobicity. Taken together these properties, LR/14 with a higher hydrophobic potential should have the capacity to adhere to the gut wall (Krepsky et al., 2003; Draksler et al., 2004).

Probiotic bacteria are mostly delivered through the food system and must be acid and bile tolerant to survive in human gastrointestinal tract. It has been reported that many bacteria are subjected to stresses in the stomach, where pH between 2.0 and 3.0 is encountered (Liong and Shah, 2005). Both the strains showed tolerance to pH 2.0 though there was a consequent decrease in viability count. Most probiotic strains are reported to show this kind of drop in viable count due to acidic pH that accounts for a decrease by 3 to 6 log (Liong and Shah, 2005). Thus, a probiotic strain that needs to establish itself in the gastro-intestinal tract should be able to tolerate a pH stress (1.5 to 3.0). Survival at pH 2, therefore, is considered optimal for such strains. Both the strains used in this study survived the harsh acid conditions and thus further exhibited the probiotic potential.

Both the strains show fast acidifying activity, which is perhaps the reason why these cells could sustain themselves at low pH (2.0). These results are in accordance with those shown by Mourad and Nour-Eddine (2006), that most strains of lactic acid bacteria show fast or medium acidifying activity.

Our results, therefore, show that the environment simulating the food vehicle, which the probiotic strains will take, would be supportive of their growth as also described by Vizoso-Pinto et al. (2006).

The effect of antacid with antibiotic is reported in certain clinical trials during the infection with pathogen like *Helicobacter pylori* (Spadacini et al., 1996). Even, there are reports on the comparison of the efficacy of the antacid and probiotics, but there is no report on the effect of antacids on probiotics to the best of our knowledge. This study suggests that the two strains are able to sustain the pH 2.0 environment of stomach more effectively in the presence of antacids perhaps due to increase in pH brought about by the latter.

Of the two strains tested in this study, strain LR/14 clearly possessed bile salt hydrolase (BSH) activity. Bile salt hydrolase activity is known to enable the bacteria to survive and colonize in the intestine. The probiotic strains having BSH activity also confers the property of lowering/removal of bile acids and thus lowering blood serum cholesterol (Jones et al., 2004., Parvez et al., 2006). As is evident from our results, the two strains studied by us, could...
remove cholesterol. By this comparison also, LR/14 appeared to be efficient in cholesterol assimilation. Cholesterol assimilation by lactic acid bacteria is reported to be in the range from 15-40 µg/ml (Liong and Shah., 2005).

There are many reports on probiotic strains producing bacteriocin (Avonts et al., 2004). Like probiotic L. acidophilus 30SC produces bacteriocin, acidocin (Oh et al., 2000) and L. salivarius produces salivaricin P (Barrett et al., 2007). Having a probiotic strain with such an antimicrobial potential could provide a new paradigm by restricting the growth of the unwanted pathogenic microbes and thus proving additionally beneficial.

CONCLUSIONS
In the present scenario of the usage of probiotics as food supplements or biotherapeutics, diverse strains of probiotic bacteria have been studied. The resident gastro-intestinal microbial flora has been seriously considered, as many of them are beneficial in sustaining and benefiting the gut microflora. The present in-vitro study of environmental LAB isolates was undertaken to broaden the range of these bacteria. Study on environmental isolates is attractive because of their diversity and their potential to thrive in adverse conditions. This study shows that L. plantarum LR/14 harbors several properties of a probiotic strain and moreover produces a bacteriocin with a wide antimicrobial host range. The potential of this strain can be further investigated for in vivo testing. The strain E. faecium LR/6 also exhibited some probiotic properties and can be a potential strain for further applications.

ACKNOWLEDGEMENTS
This work was supported by grants from Department of Biotechnology, Government of India. The facilities provided to the Department of Genetics, by University Grants Commission under SAP and by Department of Science and Technology, Government of India under FIST programs are thankfully acknowledged.

REFERENCES


Molecular techniques for the detection of probiotics