Functional Properties of Heavy Metal Tolerant Probiotic Strains Isolated from Curd

Nivedita Prasad*†, Manikant Tripathi‡†, Sangeeta Shukla¹, P. W. Ramteke³ and Ramesh Chandra¹

¹Department of Dairy Microbiology, Sam Higginbottom Institute of Agriculture, Technology and Sciences (SHUATS), Allahabad, India.
²Department of Microbiology, Dr. Ram Manohar Lohia Avadh University, Faizabad, India.
³Department of Biological Sciences, Sam Higginbottom Institute of Agriculture, Technology and Sciences (SHUATS), Allahabad, India.

Authors’ contributions

This work was carried out in collaboration between all authors. Authors NP and MT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SS managed the analyses of the study. Authors PWR and RC managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

The two heavy metal tolerant bacterial isolates L. fermentum SN_4 and L. rhamnosus SN_6 were identified (isolated from curd samples) which were found to be potentially resistant to Cr⁶⁺, Pb²⁺ and Cd²⁺ under study. Both the isolates were resistant to simulated gastric juices at pH 2.0 and 3.0 at 0 hours and survived well more than 50% in 0.2%, 0.3%, 0.5% and 1.0% bile salt solution but the isolate L. rhamnosus SN_6 showed the best survival at all the concentrations of bile salt. These two isolates showed poor antagonistic agent activity against the four pathogenic bacteria viz. E. coli, B. cereus, S. typhimurium and V. cholera. L. fermentum SN_4 showed resistant to all antibiotic except clindamycin and azithromycin, on the other hand, L. rhamnosus SN_6 was found resistant to clindamycin and tetracycline only. Also, they were found to be haemolytic negative which proved them to be a potential probiotic.

*Corresponding author: E-mail: nivedita.prasad01@gmail.com
† Both authors are equally contributed
Keywords: Probiotic; heavy metal; Lactobacillus species; curd; antibiotic.

1. INTRODUCTION

In recent years, different investigations support the importance of probiotics as a part of healthy diet for humans and animals and as a way to provide a natural, safe and effective barrier against microbial infections [1]. According to the definition by the World Health Organization (WHO), probiotics are “live microbial food supplements which, when administered in adequate amounts confer a health benefit on the host” [2]. Among the usually used microorganisms, lactic acid bacteria (LAB) are regarded as a major group of probiotic bacteria [3]. They are non-pathogenic, technologically suitable for industrial processes, acid tolerance, bile tolerance and produce antimicrobial substances [4]. They are classified as generally recognized as safe (GRAS) microorganisms because of their long and safe use as starter cultures in fermented products.

The majority of probiotic bacteria belong to the lactic acid bacteria (LAB) group. LAB exhibit fermentation activities and have been used in food preservation for thousands of years. The species of Lactobacillus genus are the most famous group of LAB that is recognized as probiotics [5]. Lactobacillus strains exhibit health-promoting and preservation activities [6]. They are used as a starter culture to enhance the texture, flavour, and nutrition value of some products, such as cheese, sourdough, wine, beer, silage, fermented plant, and meat [7]. Specific probiotic strains have positive effects on atopic eczema, irritable bowel syndrome, diarrhoea, antibiotic-related diarrhoea, vaginal infections, inflammatory bowel disease, and cancers by stimulating the immune mechanisms and balancing the human microbiota composition [8-14]. Moreover, certain strains significantly affect the bioavailability of such nutrients like magnesium and calcium in the human body [15].

Probiotic bacteria are resistant to gastrointestinal conditions (low pH and high concentrations of bile salts) [16]. Some LAB carries antibiotic resistance genes and thus exhibit high resistance against antibiotics [17]. For probiotics to be capable of inducing to promote effects on host health, they must tolerate environments with high concentrations of bile salts and low pH and display high antimicrobial activities [18].

To carry out their beneficial effects, probiotic strains must survive passage through the upper gastrointestinal tract (GIT), by tolerating gastric acidity and bile toxicity and colonizing the GIT by adhering to mucin or intestinal-derived epithelial cells [19,20]. Moreover, probiotic strains of antibiotic susceptibility should be investigated to assess their safety before their use as food additives [21].

Currently, researchers are interested in developing efficient techniques for screening and selecting probiotics bacteria, but many challenges remain. In the present study, appropriate strategies were used for the characterization of potential probiotic Lactobacillus strains isolated from indigenous dairy product curd which were tolerant to heavy metals (Pb, Cd and Cr) in order to evaluate their suitability for the addition in popular traditional fermented milk products, and/or to develop new dairy/non-dairy probiotic foods.

2. MATERIALS AND METHODS

2.1 Bacterial Strains and Isolation

Fifty curd samples from local vendors of Allahabad city were collected and transported to the laboratory and were stored in a refrigerator. One gm of sample was serially diluted in Ringer’s solution then sequential decimal dilutions of the homogenate were obtained. A volume of 0.1 ml of the dilutions was plated on MRS agar medium (Hi-Media) supplemented with heavy metals (Cd^{2+}, Pb^{2+} and Cr^{6+}) at 50 mg/l and incubated in anaerobic conditions at 37°C for 48 h [22]. The stock solution of heavy metals was prepared using their salts, viz., cadmium chloride, lead acetate and potassium dichromate, respectively. Isolated colonies were selected randomly and purified. The obtained purified colonies were tested for Lactobacilli by microscopic examination using Gram stain and catalase production techniques. The Gram-positive, catalase-negative rods were selected and stored at -20°C in MRS broth (Hi-Media), supplemented with 15% (v/v) glycerol for further studies.

2.2 Lactobacilli Identification

The selected Lactobacilli isolates were cultivated in MRS broth and incubated under anaerobic conditions for 24 hours at 37°C. The best selected two bacterial isolates on the basis of their tolerance to all the three heavy metals (Pb, Cd and Cr) were identified at the morphological, biochemical and molecular levels. Molecular
characterization was done at genomic level by using 16S rRNA gene sequencing technique. Genomic DNA of bacterial strains was isolated. The isolated DNA of each isolate was used in PCR to amplify small subunit of 16S rRNA genes using universal primers having an expected product size of 1500 bp. The PCR products so obtained after amplification were visualized using ethidium bromide on 1.5% agarose gel. These, after purification had got sequenced by Eurofins Genomics India Private Limited to identify the isolates. The strains were then compared with the sequences deposited in NCBI and GeneBank.

2.3 Probiotic Properties

As probiotics are usually administered orally, they must have the ability to survive passage through the stomach and small intestine. Thus, the following are some tests that provided the necessary conditions of intestinal flora.

2.3.1 Tolerance to simulated gastric juice

The tolerance of the strains to simulated gastric juices was tested as described by with slight modifications [23]. The stationary phase grown cells were harvested by centrifugation at 6,000 g for 20 min at 4°C, were washed twice with 50 mmol/l phosphate buffer (pH 6.5) and were suspended in 3 ml of the same buffer. Then, 1 ml of washed cell suspension was harvested by centrifugation at 12,000 g for 5 min under 4°C and resuspended in 10 ml of simulated gastric solution containing NaCl (125 mmol/l), KCl (7 mmol/l), NaHCO\textsubscript{3} (45 mmol/l), and pepsin (3 g/l). Final pH was adjusted to 2.0 and 3.0 using 1 mol/l HCl solution. A total viable count was determined before and after three h incubation period at pH 2.0 and 3.0, at 37°C under anaerobic conditions.

2.3.2 Ox-bile cells resistance bacteria

The resistance of the strains to bile was performed according to [24]. The results were expressed as the percentage of bile salts resistance compared to the control.

Percentage of resistance = (Increment of OD in MRS broth with Ox-bile / increment of OD in MRS broth without Ox-bile) × 100.

Strains showing resistance percentage at the value of 0.3% of Ox-bile more than 50% were considered as bile resistance strains.

2.3.3 Antimicrobial activity assay

A modified agar diffusion method was used to determine antimicrobial activity [25]. The clear zone formation indicated a positive antimicrobial activity of isolated metabolites on the pathogens as described by [25]. This experiment was performed against some clinically important human pathogens including E. coli, B. cereus, S. typhimurium and V. cholera.

2.3.4 Antibiotic susceptibility

The antibiotic susceptibility pattern of the isolates was studied on MRS Agar medium plates by disc diffusion method [25]. The antibiotics (mcg) (Hi-media) used were as follows: Streptomycin {10}, Erythromycin {15}, Trimethoprim {25}, Gentamicin {30}, Chloramphenicol {30}, Tetracycline {10} and Azithromycin {15}. The zone of inhibition was observed and recorded after 24 hours when the visible lawn culture of bacteria contrasted against the clear zone of inhibition around the discs.

2.3.5 Haemolytic activity

To determine bacteria haemolytic activity, blood haemolysis was evaluated on Columbia agar plates (Oxoid) supplemented with 5% sheep blood. Each bacterial suspension was streaked on the blood agar plates. After 24 hours of incubation at 37°C, the plates were examined for signs of β-haemolysis (clear zones around colonies), α-haemolysis (a green-hued zone around colonies) or γ-haemolysis (no halo around colonies) [26].

2.4 Statistical Analysis

Data were analyzed by one-way ANOVA. Significant differences in means (P < 0.05) were then compared by Duncan's test using the SPSS (SPSS Inc, Chicago, IL, USA) 19.0 software. All graphs were prepared using Microsoft Office Excel.

3. RESULTS AND DISCUSSION

3.1 Isolation and Characterization of Heavy Metal (Cd, Pb and Cr) Resistant Lactobacilli

A total of 110 heavy metal-resistant colonies were picked up from Cd\textsuperscript{2+} (8 colonies), Pb\textsuperscript{2+} (80 colonies) and Cr\textsuperscript{6+} (22 colonies) supplemented plates of three heavy metals were selected as
given in the Table 1. These selected colonies were further subjected to the high heavy metal concentration of all the three heavy metals by gradually increasing their concentration in MRS plates (Table 2). Only two bacterial isolates (CD5 and CD8) were found to be potentially resistant to the high heavy metal concentration of all the three heavy metals. Based on their morphological, biochemical and genome sequencing, the two isolates were identified as *L. fermentum* SN_4 and *L. rhamnosus* SN_6 (Tables 3-5). Phylogenetic tree of *L. fermentum* SN_4 and *L. rhamnosus* SN_6 are presented in Figs. 1 and 2.

### Table 1. Incidence of high heavy metal tolerant isolate in dairy samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total no. of samples</th>
<th>No. of Pb resistant colonies</th>
<th>No. of Cd resistant colonies</th>
<th>No. of Cr resistant colonies</th>
<th>Total no. of heavy metals resistant colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curd (CD)</td>
<td>50</td>
<td>80</td>
<td>8</td>
<td>22</td>
<td>110</td>
</tr>
</tbody>
</table>

### Table 2. Minimum Inhibitory Concentration (MIC) of isolated bacterial strains against heavy metals

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cd** (ppm)</th>
<th>Cr** (ppm)</th>
<th>Pb** (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1</td>
<td>250</td>
<td>350</td>
<td>2000</td>
</tr>
<tr>
<td>CD2</td>
<td>150</td>
<td>350</td>
<td>1500</td>
</tr>
<tr>
<td>CD3</td>
<td>250</td>
<td>350</td>
<td>1000</td>
</tr>
<tr>
<td>CD4</td>
<td>250</td>
<td>150</td>
<td>2000</td>
</tr>
<tr>
<td>CD5</td>
<td>300</td>
<td>400</td>
<td>2400</td>
</tr>
<tr>
<td>CD6</td>
<td>150</td>
<td>150</td>
<td>1000</td>
</tr>
<tr>
<td>CD7</td>
<td>150</td>
<td>250</td>
<td>1500</td>
</tr>
<tr>
<td>CD8</td>
<td>300</td>
<td>400</td>
<td>2400</td>
</tr>
<tr>
<td>CD9</td>
<td>250</td>
<td>350</td>
<td>1500</td>
</tr>
<tr>
<td>CD10</td>
<td>250</td>
<td>250</td>
<td>2000</td>
</tr>
<tr>
<td>CD11</td>
<td>250</td>
<td>350</td>
<td>1000</td>
</tr>
<tr>
<td>CD12</td>
<td>250</td>
<td>350</td>
<td>1000</td>
</tr>
</tbody>
</table>

Fig. 1a. Molecular phylogenetic analysis of *L. fermentum* SN_4 by maximum likelihood method

Fig. 1b. Sanger sequence chromatogram of *L. fermentum* SN_4
### Table 3. Morphological characteristics of bacterial isolates

<table>
<thead>
<tr>
<th>Strains</th>
<th>Colony colour</th>
<th>Cell shape</th>
<th>Configuration</th>
<th>Margin</th>
<th>Elevation</th>
<th>Surface</th>
<th>Opacity</th>
<th>15°C</th>
<th>37°C</th>
<th>45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD5</td>
<td>White</td>
<td>Rod</td>
<td>Round</td>
<td>Entire</td>
<td>Convex</td>
<td>Smooth</td>
<td>Opaque</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CD8</td>
<td>White</td>
<td>Rod</td>
<td>Round</td>
<td>Entire</td>
<td>Convex</td>
<td>Smooth</td>
<td>Opaque</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 4. Biochemical characteristics of bacterial isolates

<table>
<thead>
<tr>
<th>Strains</th>
<th>Catalase</th>
<th>Indole test</th>
<th>Methyl red test</th>
<th>Vogues proskauer</th>
<th>Citrate utilisation</th>
<th>Gram Reaction</th>
<th>Maltose</th>
<th>Sorbitol</th>
<th>Sucrose</th>
<th>Xylose</th>
<th>Fructose</th>
<th>Lactose</th>
<th>Ribose</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD8</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Fig. 2a. Molecular phylogenetic analysis of *L. rhamnosus* SN_6 by maximum likelihood method

![Molecular phylogenetic analysis](image)

Fig. 2b. Sanger sequence chromatogram of *L. rhamnosus* SN_6

Table 5. Identification of finally screened *Lactobacillus* strains

<table>
<thead>
<tr>
<th>Name of the isolate</th>
<th>Source</th>
<th>Closest homology</th>
<th>Identity (%)</th>
<th>16S rRNA identification</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD5</td>
<td>Curd</td>
<td>KF030779.1</td>
<td>100</td>
<td><em>L. fermentum</em> SN_4</td>
<td>MG461543</td>
</tr>
<tr>
<td>CD8</td>
<td>Curd</td>
<td>KJ702504.1</td>
<td>100</td>
<td><em>L. rhamnosus</em> SN_6</td>
<td>MG461545</td>
</tr>
</tbody>
</table>

It was also observed that all the isolates demonstrated the highest degree of resistance for Pb followed by Cr and least for Cd. These findings were in support as it was observed that isolated heavy metal tolerant LAB from mud and sludge samples whose phylogenetic analysis of their 16S rDNA sequences revealed that the strains Cd70-13 and Pb71-1 belong to *Lactobacillus reuteri* which were more resistant to Pb as compared to Cd by [27]. Whereas, isolated heavy metal tolerant *Pseudomonas aeruginosa* from wastewater of El-Malah canal located in Assiut [28].

3.2 Probiotics Properties

Survival of probiotic bacteria upon ingestion through the transit to GI tract is crucial to confer any health benefits to the host; consequently, their survival when subjected to pH and bile conditions similar to the human GI region should be assessed as these are important factors to be investigated while selecting potential probiotic bacteria. Additionally, these should be able to aggregate with each other and should efficiently adhere to gastrointestinal mucus and epithelial cell lines as these are essential characteristics.
for effective colonization and enhanced persistence of probiotic bacteria in the GI system.

### 3.2.1 Tolerance to simulated gastric juice

The results presented in the Table 6 showed that both the isolates were resistant to simulated gastric juices at pH 2.0 and 3.0 at 0 hours. However, the growth of culture was lesser at pH 2.0 as compared to pH 3.0. Moreover, cell survival was also assessed for 0-3 hours. The results showed that increase in time have an adverse effect on the viable count of bacteria due to decreased pH as compared to control. A study was conducted on the resistance of Lactic Acid Bacteria with a high number of Lactobacilli to gastrointestinal transit and found that most of the isolates showed a low % survival rate ranging from 0.00% and 0.05% after the simulated gastric digestion (SGD) while some of the isolates showed high survival rates [29].

A higher survival rate to the pancreas than to the gastric digestion was noticed, and it was found to be 56.7% for isolate GGAS-T1-111, 39.4% for isolate GG2F-T0-13, 36.7% for isolate GM2S-T0-36 and 36.1% for isolate GM2F-T5-327. In a similar finding a study was conducted on the influence of pepsin (3 g/l) at pH 1.5, 2, 2.5 and 3, as well as of pancreatin (1 g/l) on the survival of L. rhamnosus IL4.2 and recorded that viability of L. rhamnosus IL4.2 was decreased after treatment with gastric juice by 26% within 1 h of exposure and the same pattern was found with intestinal fluid where viability was dropped by 26% after 2 h of exposure [30].

### 3.2.2 Ox-bile cells resistance bacteria

To evaluate the potential of using LAB as effective probiotics, it is generally necessary to evaluate their ability to resist the effects of bile acid. Oxgall is a natural dried bovine bile component containing both conjugated and unconjugated bile salts [31]. The results are presented in Table 7. Both the strains survived well more than 50% in 0.2%, 0.3% 0.5% and 1.0% bile salt solution. There was a gradual decrease of viable cells when the concentration of bile salt was increased from 0.2% to 1.0%. Isolate L. rhamnosus SN_6 showed the best survival at all the concentrations. The gradual decrease of viable cells was observed when the concentration of bile salt was increased up to 1.0% [16].

The protective effect of food matrix may prevent the bacteria from bile exposure and hence, give rise to the increased bile resistance [32]. It was also determined the level of bile resistance exhibited by several of the Lactobacillus and Bifidobacterium strains isolated from the human ileum on the solid media supplemented with bovine bile to final concentration between 0.3% and 7.5% and found that the tested Lactobacillus and Bifidobacterium strains exhibited resistance to the bovine bile used while the porcine bile used in these assays proved to be significantly more inhibitory to both of the bacterial groups [20]. Similarly, a study was conducted on Lactobacillus spp. Isolated from yoghurts (Bogra and Khulna regions of Bangladesh) and it was found that all the isolates were able to tolerate bile acid at

---

### Table 6. Tolerance of Lactobacillus isolates to simulated gastric juices

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Gastro intestinal juices</th>
<th>Incubation time (h)</th>
<th>Cell survival (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>L. fermentum SN_4</td>
<td>pH 2</td>
<td>8.33</td>
<td>5.36</td>
</tr>
<tr>
<td></td>
<td>pH 3</td>
<td>9.21</td>
<td>7.43</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>11.46</td>
<td>11.20</td>
</tr>
<tr>
<td></td>
<td>CD(0.05)= 4.939</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. rhamnosus SN_6</td>
<td>pH 2</td>
<td>8.41</td>
<td>6.78</td>
</tr>
<tr>
<td></td>
<td>pH 3</td>
<td>9.56</td>
<td>7.80</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10.59</td>
<td>11.12</td>
</tr>
<tr>
<td></td>
<td>CD(0.05)= 3.209</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 7. Percentage of resistance of isolated Lactobacillus species at different concentrations of ox-bile

<table>
<thead>
<tr>
<th>Isolates</th>
<th>0.2% ox-bile</th>
<th>0.3% ox-bile</th>
<th>0.5% ox-bile</th>
<th>1.0% ox-bile</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. fermentum SN_4</td>
<td>92.64</td>
<td>80.69</td>
<td>64.33</td>
<td>63.41</td>
</tr>
<tr>
<td>L. rhamnosus SN_6</td>
<td>94.23</td>
<td>83.26</td>
<td>77.69</td>
<td>69.46</td>
</tr>
</tbody>
</table>
the rate of 0.3% [33]. Bile salt tolerance (0.3%) of Lactic acid bacteria isolated from traditional fermented ‘Dahi’ was studied, and during the tolerance assay, it was observed that L. fermentum B5 40-2 exhibited the highest bile acid tolerance (95.53±0.79%) followed by E. faecium D3 25-1 (88.66±0.76%), L. lactis subsp. lactis B 25-3 (74.40±1.09%), L. raffinolactis D4 25-3 (72.34±1.20%) and P. pentosaceus B2 25-5 (65.67±1.58%) [23].

3.2.3 Antimicrobial activity

The antimicrobial activity of Lactobacillus spp. was checked against pathogenic bacteria E. coli, B. cereus, S. typhimurium and V. cholerae and it was observed that both the isolates showed poor antagonistic agent activity against the above pathogenic bacteria and the results are presented in Table 8. A good probiotic should present their antimicrobial actions particularly to the pathogens in the GI system [34].

A study was conducted in which three potentially probiotic Lactobacillus isolates were subjected to antibacterial activity assay in which the isolate number B20 showed the most antibacterial potency to S. aureus and E. coli whereas the isolate no. B2 demonstrated the highest potency to L. innocua [35]. The production of organic acid and hydrogen peroxide by Lactobacilli was reported to inhibit both Gram-positive and negative bacteria, whereas bacteriocin affects only the growth of Gram-positive bacteria [34].

3.2.4 Antibiotic susceptibility

The results for antibiotic susceptibility are given in Table 9. Both the isolates were subjected to antibiotic susceptibility tests using 8 different antibiotics Streptomycin (10), Erythromycin (15), Trimethoprim (25), Gentamicin (30), Clindamycin (2), Chloramphenicol (30), Tetracycline (10) and Azithromycin (15).

Mostly, Lactic Acid bacteria (LAB) are generally sensitive to inhibitors of protein synthesis such as Tetracycline, Chloramphenicol, Erythromycin and Clindamycin and resistant to glycopeptides (Gentamicin, Kanamycin, Streptomycin, etc.) [23]. In the present study, L. fermentum SN_4 showed resistant to all antibiotic except clindamycin and azithromycin, on the other hand, L. rhamnosus SN_6 was resistant to clindamycin and tetracycline only.

The antibiotic susceptibility of all these isolates turns them safe and thus suggests their successful use as potential probiotics. According to World Health Organization (WHO), and European Food Safety Authority-EFSA, bacteria used as probiotics for human and animal use should not carry any transferable antimicrobial/antibiotic resistance gene [2].

The occurrence of a large number of transferable resistance genes within the intestinal microbiota is undesirable due to the potential risk of acquisition by pathogens present in GI tract and subsequent antibiotic treatment failure [36]. Therefore, it is very important to verify that probiotic strains consumed on a daily basis lack acquired antibiotic resistance properties prior to considering them safe for human and animal consumption [37]. Thus, the sensitivity of these lactic acid bacteria to the clinically important antimicrobials is favourable as it minimizes the chances of disseminating resistance genes to pathogens both in food matrix and in the GI tract.

### Table 8. Antagonistic spectrum of Lactobacillus isolates against pathogens

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Isolates</th>
<th>E. coli (mm)</th>
<th>B. cereus (mm)</th>
<th>S. typhimurium (mm)</th>
<th>V. cholerae (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>L. fermentum SN_4</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>2.</td>
<td>L. rhamnosus SN_6</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Diameter of inhibition zones (mm) Zone size < 10 mm = Poor activity Zone size > 15 mm = Good activity Zone size > 20 mm = Strong activity

### Table 9. Antibiotic susceptibility pattern in Lactobacillus isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>S (10mcg/disc)</th>
<th>E (15mcg/disc)</th>
<th>TR (25mcg/disc)</th>
<th>GEN (30mcg/disc)</th>
<th>CD (2mcg/disc)</th>
<th>C (30mcg/disc)</th>
<th>TE (10mcg/disc)</th>
<th>AZM (15mcg/disc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. fermentum SN_4</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>L. rhamnosus SN_6</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
</tbody>
</table>

S=Streptomycin; E=Erythromycin; TR=Trimethoprim; GEN=Gentamicin; CD=Clindamycin; C=Chloramphenicol; TE=Tetracycline; AZM=Azithromycin
3.2.5 Haemolytic activity

In the present investigation both the screened isolates viz. _L. fermentum_ SN_4, _L. rhamnosus SN_6 were found negative for haemolysis on blood agar plates. Because, neither clear zones (α-haemolysis) nor green-hued zones (β-haemolysis) (negative test) were observed around colonies, thereby proving their safe and non-virulent nature. Studies on blood haemolysis were conducted to evaluate the blood haemolysis activity of 11 _Lactobacillus plantarum_ strains isolated from fermented foods and it was found that none of the isolate were able to produce zone of lysis on Columbia agar plates supplemented with 5% sheep blood [38]. An investigation of the safety of _Lactobacillus plantarum_ DU10 isolated from Algerian raw camel milk was done and found that _L. plantarum_ showed no positive haemolytic activity [39].

4. CONCLUSION

Two potential heavy metal tolerant (Pb, Cd and Cr) probiotic isolates were characterized from curd collected from different regions of Allahabad city. These isolates were identified as _L. fermentum_ SN_4 and _L. rhamnosus_ SN_6 which were found to be tolerant to all the three heavy metals. Also, these isolates were found to be tolerant to simulated gastric juices and ox-bile. However, they are showed poor resistant against selected pathogens and showed negative hemolytic activity. Their heavy metal tolerant activity and probiotics potentials can be considered for heavy metal sequestration and also can be used to develop a dairy probiotics food.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

13. Geier MS, Butler RN, Howarth GS. Inflammatory bowel disease: Current insights into pathogenesis and new therapeutic options; probiotics, prebiotics


© 2018 Prasad et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/26072