Probiotic Attributes of Lactobacillus rhamnosus of Dairy Origin and Effectiveness of Almond in Stimulation of its Growth in vitro

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Three strains of Lactobacillus rhamnosus of dairy origin and Lactobacillus rhamnosus GG (LGG) as a reference organism, were compared for their probiotic attributes viz., acid tolerance, bile salt tolerance, cell surface hydrophobicity and antimicrobial activity. Among the different organisms, Lactobacillus rhamnosus NCDC 17 was found to be the best strain in acid tolerance and antimicrobial activity and exhibited good bile salt tolerance and cell surface hydrophobicity. Prebiotic effectiveness of almond for stimulation of growth of L. rhamnosus NCDC 17 and LGG was also investigated in vitro. Almond supplementation to basal medium at the level of 2% (w/v) exhibited stimulatory effect on proliferation of both the strains. However, LGG fared comparatively better than L. rhamnosus NCDC 17 as indicated by an increase in viable counts to the extent of 1.3-1.5 log cycles as compared to an increment of around 0.9-1.1 log cycles in case of L. rhamnosus NCDC 17 after 12 h of incubation. Inulin was not found to support the growth of any of the L. rhamnosus strains. Almond may serve as a natural source of prebiotic which may be used in combination with health promoting probiotic lactobacilli in symbiotic formulations for a wide range of health attributes.

Keywords: Almond, inulin, prebiotic, probiotic, Lactobacillus rhamnosus NCDC 17, LGG

INTRODUCTION

Functional foods, which provide health benefits beyond traditional nutritional value, are becoming popular worldwide and there is increasing interest in exploring the health benefits associated with ingestion of such foods and food components. A number of food products containing probiotics, prebiotics, synbiotics & other functional ingredients of plant origin are widely being promoted by food industry. Prebiotics can play an important role in the stimulation of proliferation of probiotic bacteria in different types of fermented milk products and also the determining factor for their survivability during storage. Prebiotics pass largely undigested through the upper portion of gastrointestinal tract and serve as substrate for the selective stimulation of beneficial bacteria in colon. Therefore, there is increasing interest in the identification of new prebiotics for development of functional foods with added functionality (Mandalari et al. 2008b).

Almond is utilized in various types of food preparations all over the world, and reported to have beneficial effect on lipid profile. There is inverse relationship between incidence of cardiovascular disease (CVD) and consumption of almond (Chen et al. 2006). Almond contains more than 50% fat, 21 to 25% protein and 11 to 12% dietary fiber of total weight of the seed. Monounsaturated fatty acids (MUFA) constitute more than 63% of total almond fat. These have been shown to improve the lipid profile & insulin sensitivity (Tierney and Roche, 2007). Recent studies showed that encapsulation of intracellular lipids by the cell walls of almond restrict their digestion in the stomach and small intestine (Ellis et al. 2004; Mandalari et al. 2008a). Different microscopical examinations of digested almond in fecal sample showed that undigested lipid
from almond tissue reaches the large intestine and could be used by resident microbiota (Ellis et al. 2004). Mandalari et al. (2008b) investigated the prebiotic potential of almond seeds in vitro by using finely ground almonds (FG) already subjected to a combined model of the gastrointestinal tract (in vitro gastric and duodenal digestion) and fermentation with mixed fecal bacterial cultures. FG almond significantly increased the population of bifidobacteria and Eubacterium rectale in comparison to that with a commercial fructooligosaccharide as substrate. In a later study, Mandalari et al. (2010a) showed prebiotic potential of almond skin.

Lactobacilli constitute the important category of health promoting bacteria being added to different types of fermented foods. There is increased interest to exploit the different lactobacillus strains for various health benefits to the consumer. According to Boyle et al. (2006) indigenous cultures may have better colonization, longer transient time and more healthful effects for local population as probiotics are highly strain and host specific. The aim of present study was to evaluate the probiotic attributes of three indigenous cultures of Lactobacillus casei of dairy origin (found to be Lactobacillus rhamnosus by species specific PCR in our experiments), and also to study the effectiveness of finely ground almond for in vitro stimulation of growth of the strain exhibiting best probiotic attributes.

**MATERIALS AND METHODS**

**Bacterial Strains**

Three strains of *Lactobacillus* casei viz., NCDC 17, 297 and 298 were obtained from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute, Karnal, India. *Lactobacillus rhamnosus* GG was used as a reference probiotic. *Bacillus cereus* NCDC 66, *Enterococcus faecium* NCDC 124, *Staphylococcus aureus*, NCDC 109, *Pediococcus acidilactici* LB 42 and *Listeria monocytogenes* ATCC 15303 were used as indicator strains to determine the antimicrobial property of probiotics.

**DNA Isolation and Species Specific PCR**

Genomic DNA of lactobacilli was isolated according to the method of Pospiech and Neumann (1995). Amplification of 16S rRNA gene by PCR was carried out using three different species specific primers for *casei*, *paracasei* and *rhamnosus* (Ward and Timmins, 1999).

**Probiotic Attributes**

The cultures of lactobacilli were activated by subculturing three times and used for study of different probiotic attributes.

**Acid Tolerance**

Acid tolerance of lactobacilli was studied at different pH according to Bhardwaj et al. (2010). MRS (HiMedia, India) medium was adjusted with HCl to pH 2 and 3. Sterilized 10 ml medium was inoculated with 200 μl of 24 h activated culture and incubated at 37°C. One ml sample was taken immediately (0 h) and after 0.5, 1 and 2 h and serially diluted with peptone water. Suitable dilutions were poured in MRS agar and incubated aerobically at 37°C for 48 h.

**Bile Salt Tolerance**

Bile salt tolerance of dairy cultures was studied according to Bhardwaj et al. (2010) in MRS medium containing different concentrations viz., 0.5, 1 and 2% (w/v) of oxgall (HiMedia, India). Ten ml medium was inoculated with 200 μl of 24 h activated culture and incubated at 37°C. One ml samples were taken at 0, 3, 6 and 12 h and serial dilutions prepared with peptone water. Suitable dilutions were poured in MRS agar and bile tolerance of each strain determined by comparing the bacterial counts with 0 h control.

**Cell Surface Hydrophobicity**

Cell surface hydrophobicity was determined as described by (Rosenberg et al. 1980) using three hydrocarbons viz. n- hexadecane, xylene and octane.

**Antimicrobial Activity**

Antimicrobial activity of all the lactobacilli strains was determined by well diffusion assay according to Mishra and Prasad (2005). Surface of the solidified and dried (overnight at 37°C) MRS agar plates was overlaid with 7 ml of soft MRS agar inoculated with 20 μl of overnight activated culture of pathogenic test strains viz., *Bacillus cereus* NCDC 66, *Enterococcus faecium* NCDC 124, *Staphylococcus aureus* NCDC 109, *Pediococcus acidilactici* LB 42 and *Listeria monocytogenes* ATCC 15303. Wells were made in agar plates and filled with 50 μl of cell free broth of 24 h activated dairy cultures which was obtained by
centrifugation at 10000 g for 10 min at 4°C, heat treated (90°C for 5 min) and neutralized to pH 6.5. A clear zone of 1 mm or more extending laterally around the well was considered as positive inhibition.

**In Vitro Stimulation of Probiotics by Almond**

Stimulation of *L. rhamnosus* NCDC 17 and LGG was studied in basal medium (containing all the components of MRS broth except dextrose) supplemented with different substrates. Basal medium (BM) served as negative control while BM with 2% dextrose was used as positive control. Inulin (a reference prebiotic, HiMedia, India) or almond powder prepared by dry grinding of almonds (Alm1, local name-American/California almond, average length 2.7 cm or Alm2, local name-Gurbandi almond, average length 1.4 cm) was added to BM @ 2% (w/v) to evaluate its prebiotic potential. Activated cultures were inoculated at 1% (v/v) and fermentation was carried out at 37°C. Three independent experiments were conducted in each case with pour plating in triplicate. The pH of medium and viable counts were determined in aliquots drawn aseptically at 0, 6, 12 and 24 h of incubation. To determine viable counts, aliquots were serially diluted and pour plated in MRS agar and incubated at 37°C for 48 h. The colonies were counted and expressed as log cfu/ml.

The data obtained on viable counts were analyzed statistically by two-way ANOVA with Bonferroni post tests using software graph pad prism.

**RESULTS AND DISCUSSION**

In the present investigation, the probiotic potential of three strains of *L. rhamnosus* of dairy origin was evaluated. In addition, potential of finely ground almond for stimulation of proliferation of *L. rhamnosus* NCDC 17 and a widely recognized probiotic *L. rhamnosus* GG was also examined.

**Species Specific PCR**

All three indigenous strains of lactobacilli along with the reference LGG were subjected to species specific PCR and gave single specific band of approximately 295 bp size with primer pairs for *rhamnosus* spp. (Fig. 1).

**Acid Tolerance**

Human stomach secretes about three liters of gastric juice (around pH 2) each day, and the ingestion of food or dairy products raises the pH in stomach to 3 or higher (Martini *et al*. 1987; Vernazza *et al*. 2006). Therefore, the survival of probiotic bacteria at low pH during transit through stomach is an important consideration. In the present study, at pH 3, all four strains of *L. rhamnosus* viz., NCDC 17, 297, 298 and LGG showed sufficiently high (>8 log cfu/ml) residual counts after 2 h incubation. Further, *L. rhamnosus* NCDC 17 exhibited maximum survivability among all strains tested with viable counts up to 9 log cfu/ml (Table 1). At pH 2 also, *L. rhamnosus* NCDC 17 exhibited maximum survivability with residual viable counts around 8 and 6 log cfu/ml, while NCDC 297 retained 5.46 and 5 log cfu/ml after 0.5 and 1 h incubation, respectively. No culture could survive at pH 2 when incubated for 2 h. Mishra and Prasad (2005) also reported *L. rhamnosus* NCDC 17 to be good tolerant at pH 2. LGG was found to exhibit comparatively poor acid tolerance and could not survive at pH 2 when incubated for 30 min. Schillinger *et al*. (2005) exposed different strains of *L. casei* group to simulated gastric buffer containing pepsin at pH 2.0 and reported a significant decrease in viable counts of all *L. paracasei* strains and LGG from about 10⁸ cfu/ml to a level of 10 cfu/ml within 30 min. A higher survival percentage of probiotic lactobacilli at pH 3 as compared to that at pH 2 observed in the present investigation is in conformity with the reports by various other researchers (Maragkoudakis *et al*. 2006; Pan *et al*. 2009).

**Bile Salt Tolerance**

Before performing probiotic function in colon, probiotic organisms need to tolerate bile salts for longer time. Bile acid concentration in intestine ranges from ~0.2 to 2% in different persons depending upon dietary intake and health status (Gunn, 2000). Maximum
survivability was found at 0.5% (w/v) and least at 2% (w/v) of oxgall added to MRS medium for all the lactobacilli strains tested. A decrease in viable counts of LGG and NCDC 298 was observed after 3 h, whereas no significant effect could be observed in case of L. rhamnosus NCDC 17 and 297 (Table 2). All four strains exhibited increase in viable counts of bacteria at 6 and 12 h. L. rhamnosus NCDC 17 and 297 had > 9 log cfu/ml counts at 12 h at all bile salt concentrations. However, LGG and NCDC 298 were found to be comparatively less tolerant to bile salt and had < 8 log cfu/ml at 1% and < 7 log cfu/ml at 2% bile salt concentrations. Many studies have also suggested that survival of probiotic bacteria in acid and bile is highly strain specific (Charteris et al. 1998; Maragkoudakis et al. 2006; Vernazza et al. 2006). In the present study, different L. rhamnosus strains seemed to adapt to bile salt environment after 3 h which was reflected in term of increase in viable counts after 6 and 12 h of incubation. Adaptation to bile with time has been reported by other researchers also (Bhardwaj et al. 2010). Mishra and Prasad (2005) reported low bile salt tolerance of lactobacilli in their study. However, they used more stringent conditions in their experiments as bile salt was dissolved in water alone which could be the reason for low bile salt tolerance. The higher bile tolerance of probiotics observed in the present investigation may be due to the MRS ingredients serving as protectants to the organisms.

**Cell Surface Hydrophobicity**

Cell surface hydrophobicity is considered as measure of adhesiveness and colonizing property of bacteria to the intestinal lumen (Rosenberg et al. 1980; Prakash et al. 1997). It has been reported to vary from 2 to 95% for different probiotic bacteria (Rijnaarts et al. 1993; Schillinger et al. 2005). With n-hexadecane as a test solvent, L. rhamnosus NCDC 298, 17 and LGG exhibited better cell surface hydrophobicity in comparison to L. rhamnosus NCDC 297 (Table 3). With xylene, L. rhamnosus NCDC17 showed maximum cell surface hydrophobicity while the minimum was observed in case of LGG and NCDC 298. When octane was used as test solvent, LGG showed maximum cell surface hydrophobicity followed by L. rhamnosus NCDC 298 and NCDC 17. It was minimum in case of L. rhamnosus NCDC 297. In our study, we found a wide range (13-53%) of cell surface hydrophobicity with different hydrocarbons for different L. rhamnosus strains. L. rhamnosus NCDC 17 exhibited consistently good cell surface hydrophobicity (24-31%) with all hydrocarbons. Hexadecane has been maximally used for determination of cell surface hydrophobicity of

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**Table 1: Acid tolerance of different strains of L. rhamnosus (expressed in log cfu/ml)**

<table>
<thead>
<tr>
<th>Strain</th>
<th>pH 3</th>
<th>pH 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>0.5 h</td>
</tr>
<tr>
<td>17</td>
<td>9.45±0.06</td>
<td>9.38±0.06</td>
</tr>
<tr>
<td>297</td>
<td>9.08±0.21</td>
<td>8.62±0.27</td>
</tr>
<tr>
<td>298</td>
<td>9.45±0.09</td>
<td>8.75±0.15</td>
</tr>
<tr>
<td>LGG</td>
<td>7.79±0.15</td>
<td>7.75±0.04</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of three independent experiments, pour plating in triplicate

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**Table 2: Bile tolerance of different strains of L. rhamnosus (expressed in log cfu/ml)**

<table>
<thead>
<tr>
<th>Bile salt concentration</th>
<th>0.5%</th>
<th>1%</th>
<th>2%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>6h</td>
<td>12h</td>
</tr>
<tr>
<td>17</td>
<td>8.03±0.03</td>
<td>7.79±0.02</td>
<td>8.49±0.04</td>
</tr>
<tr>
<td>297</td>
<td>9.19±0.01</td>
<td>9.33±0.01</td>
<td>9.72±0.03</td>
</tr>
<tr>
<td>298</td>
<td>8.40±0.20</td>
<td>8.00±0.22</td>
<td>8.59±0.28</td>
</tr>
<tr>
<td>LGG</td>
<td>7.42±0.03</td>
<td>6.55±0.14</td>
<td>6.74±0.05</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of three independent experiments, pour plating in triplicate
probiotic organisms. The results in present investigation indicated the percentage hydrophobicity ranging from 13 to 27% when hexadecane was used as the test solvent. Contrary to our findings, Harty and Knox (1991) reported a maximum 9% hydrophobicity with hexadecane for \textit{L. rhamnosus} ATCC 7469 out of the four \textit{L. rhamnosus} strains tested by them. However, Pelletier \textit{et al.} (1997) showed cell surface hydrophobicity ranging from 5.8% to a level of 26.5% for different \textit{L. rhamnosus} strains.

**Antimicrobial Activity**

Another desirable property for probiotic bacteria is antimicrobial activity against pathogenic bacteria. All the cultures exhibited good antimicrobial activity against sensitive strain \textit{Pediococcus acidilactici} LB 42 and \textit{Bacillus cereus} NCDC 66 (Table 4). With \textit{Listeria monocytogenes} ATCC 15303 as test pathogen, \textit{L. rhamnosus} NCDC 17 and LGG showed maximum zone of inhibition while NCDC 297 and 298 showed poor activity. With \textit{Enterococcus faecium} NCDC 124 and \textit{Staphylococcus aureus} NCDC 109, three organisms; \textit{L. rhamnosus} GG, NCDC 17 and 298 showed good zone of inhibition while \textit{L. rhamnosus} NCDC 297 had poor inhibitory activity against the pathogens. The results suggest \textit{L. rhamnosus} NCDC 17 to possess maximum inhibitory activity against all potential pathogens tested. Although, three pathogens among a total of five test organisms used in the present study were different than those used by Mishra and Prasad (2005), they also reported \textit{L. rhamnosus} NCDC 17 to exhibit higher antimicrobial activity in comparison to that of \textit{L. rhamnosus} NCDC 297(C1) and 298(Y).

On the basis of probiotic attributes studied, \textit{L. rhamnosus} NCDC 17 was found to be the best among the three cultures evaluated, and was taken with LGG for further \textit{in vitro} experiments on stimulation of proliferation of the probiotic with finely ground almond.

**Stimulation of Growth of Probiotics by Almond**

Prebiotics are gaining increased importance not only for their physiological effects but also in terms of their role in enhancing the activity and survivability of probiotic organisms added to different functional food products. Different prebiotics have been tested in this regard including inulin and other fructo-oligosaccharides (Kaplan and Hutkins, 2000; Rossi \textit{et al.} 2005) for fermentation by lactic acid bacteria. To the best of our knowledge, there are no reports available on efficacy of almond as a substrate for proliferation of probiotic lactobacilli in vitro. Almond is a repository of various functional ingredients; an excellent source of vitamin E and manganese, a good source of magnesium, copper, phosphorous, fiber, riboflavin, protein and the MUFA rich fat. It is being used in different types of confectionary items, milk based beverages, and other food preperations. Effect of almond and its skin as a prebiotic has been shown by one research group based on \textit{in vitro} evaluation of colonic fermentation using mixed fecal bacterial cultures (Mandalari \textit{et al.} 2008b; Mandalari \textit{et al.} 2010a). In the present investigation, \textit{L. rhamnosus} NCDC 17 along with LGG were used to examine their proliferation in presence of finely ground almond and also with inulin.

### Table 3: Cell surface hydrophobicity (%) of different strains of \textit{L. rhamnosus}

<table>
<thead>
<tr>
<th>Strain</th>
<th>Hexadecane</th>
<th>Xylene</th>
<th>Octane</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>23.98±1.45</td>
<td>31.29±3.92</td>
<td>27.99±4.22</td>
</tr>
<tr>
<td>297</td>
<td>13.46±2.20</td>
<td>27.80±2.50</td>
<td>18.70±3.68</td>
</tr>
<tr>
<td>298</td>
<td>26.52±4.39</td>
<td>22.35±2.39</td>
<td>37.45±6.88</td>
</tr>
<tr>
<td>LGG</td>
<td>21.70±1.12</td>
<td>22.95±1.43</td>
<td>53.06±1.91</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of three independent experiments

### Table 4: Antimicrobial activity of \textit{L. rhamnosus} against common pathogens

<table>
<thead>
<tr>
<th>Strains</th>
<th>\textit{Pediococcus acidilactici}</th>
<th>\textit{Listeria monocytogenes}</th>
<th>\textit{Staphylococcus aureus}</th>
<th>\textit{Bacillus cereus}</th>
<th>\textit{Enterococcus faecium}</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>297</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>LGG</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

Interpretation of zone of inhibition (diameter):-

+ = 1.0-2.5 mm; ++ = 2.5-4.0 mm and +++ = more than 4.0
No decrease in pH of medium was observed with increase in time of incubation in case of negative control (BM, basal medium), basal medium supplemented with inulin (BM-Inu) and two types of finely ground almond (BM-Alm1 and BM-Alm2) in case of both _L. rhamnosus_ NCDC 17 and LGG (Fig. 2A and 2B). A decrease in pH could be observed only in dextrose supplemented medium (BM-Dext) which reached a level of around pH 3.8 with _L. rhamnosus_ NCDC 17 as well as LGG after 24 h incubation at 37°C.

Effect of supplementation of BM with dextrose, inulin or finely ground almond on stimulation of growth of _L. rhamnosus_ NCDC 17 and LGG was studied at different time intervals. Both the organisms were found to exhibit maximum proliferation with 2% (w/v) dextrose supplementation to the basal medium and a continuous increase in viable counts was observed up to 12 h. A significant difference in viable counts of positive control (BM-Dext) in comparison to other treatments (BM, BM-Inu, BM-Alm1 and BM-Alm2) was observed after an incubation period of 12-24 h, both in case of _L. rhamnosus_ NCDC 17 and LGG (Table 5). However, there was no significant difference in viable counts between BM and BM-Inu with either _L. rhamnosus_ NCDC 17 or LGG throughout the period of incubation suggesting the inability of inulin to serve as an effective substrate for the stimulation of growth of any of the two strains of _L. rhamnosus_ which was further corroborated by no decrease in pH of medium. Non fermentation of inulin by LGG and other lactobacilli has been reported by other researchers also (Makras _et al._ 2005). Moreover, nonfermentation of FOS mixture by LGG has also been reported (Kaplan and Hutkins, 2000). However, they reported FOS fermentation by 12 out of 16 Lactobacillus strains. This further suggests strain dependent variations in utilization of fermentable substrates by probiotic lactobacilli.

Finely ground preparation from two types of almond was found to support the growth of _L. rhamnosus_ NCDC 17 as well as LGG. A significant difference (P< 0.001) in viable counts of _L. rhamnosus_ NCDC 17 with almond supplemented media (BM-Alm1 and BM-Alm2) was observed at 12 and 24 h of incubation in comparison to BM as well as BM-Inu. In case of LGG, the significant difference in viable counts could be observed within 6 h of incubation. It fared comparatively better than _L. rhamnosus_ NCDC 17 as indicated by an increase in viable counts to the extent of 1.3-1.5 log cycles as compared to an increment of around 0.9-1.1 log cycles in case of _L. rhamnosus_ NCDC 17 after 12 h of incubation. However, the two varieties of almond were equally effective in stimulation of growth of two strains of _L. rhamnosus_ and no statistically significant difference in viable counts could be observed at different time intervals during fermentation. The reason for no decrease in pH with increase in viable counts is not known. Possibly the contributing factors may be the different minerals

**Figure 2:** Effect of different substrates on the change in pH of medium during growth of _L. rhamnosus_ NCDC 17 (A) and _L. rhamnosus_ GG (B).
functioning as alkaline forming agents or it may be the cumulative effect of other ingredients present in almond. Another probable reason may be the complexity of fermentation process of almond in comparison to the simple carbohydrates like dextrose.

Almond contains 3.60% sucrose out of 3.89% total sugars (USDA National Nutrient Database for Standard Reference, Release 23, 2010). LGG has been shown as a non fermentor of sucrose (Meurman et al. 1995). Therefore, fermentation study on almond with LGG suggests that the active component for prebiotic effect of almond may not be a sugar as main sugar component is sucrose. Mandalari et al. (2008b) observed prebiotic effect of finely ground almond seeds but not of defatted seeds indicating relevance of fat for prebiotic effect. Oleic acid, the main fatty acid of almond fat has been shown to be metabolized by the ruminal bacterium Selenomonas ruminantium and strains of Streptococcus, Enterococcus, and Lactobacillus (Hudson et al. 1995; Hudson et al. 2000). It has also been reported to increase the survival of probiotic lactobacilli in gastric juice (Corcoran et al. 2007). Linoleic acid, another unsaturated fatty acid in almond has also been shown to be metabolized in the human colon by Roseburia species (Devillard et al. 2007). Mandalari et al. (2010b) have reported that pectic substances encasing the cellulose microfibrils are the major component of almond skin walls, with small amounts of hemicelluloses such as xyloglucan and α-glucans. In another study, Mandalari et al. (2010a) investigated the prebiotic effect of natural and blanched almond skins using a model of gastrointestinal tract including in vitro gastric and dudodenal digestion, followed by colonic fermentation using mixed fecal bacterial cultures and reported a significant increase in population of bifidobacteria and Clostridium coccoides/ Eubacterium rectal group. They suggested the beneficial effects on colonic microbiota due to fermentation of the nonglycaemic carbohydrates, mainly pectin present in almond skin.

It appears that the prebiotic effect of almond may be due to the cumulative effect of well protected fat and the nonglycemic carbohydrates reaching the colon and becoming available for fermentation. This could be of great significance in explaining the health properties of almond. The addition of almond in combination with probiotics is also of great relevance for preparation of different types of value added dairy foods and harnessing the benefits of these functional ingredients.

**CONCLUSIONS**

Through the present investigation, we have been able to establish that *L. rhamnosus* NCDC 17 exhibits good probiotic attributes and has better acid and bile tolerance in comparison to well known probiotic LGG. Finely ground almond could be well utilized by the lactobacilli, however, LGG fared better than *L. rhamnosus* NCDC 17 in terms of the viable counts. Our study is adding support to the prebiotic potential of almond. It could be used as a natural prebiotic source. A combination of almond and health promoting probiotic lactobacilli as a synbiotic may be used in product development or explored for a wide range of health attributes. In this regard, both animal and human studies targeting the gut microflora, immune function and prevention of disorders associated

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>BM</th>
<th>BM-Dext</th>
<th>BM-Inu</th>
<th>BM-Alm1</th>
<th>BM-Alm2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.03 ± 0.04</td>
<td>8.21 ± 0.04</td>
<td>8.08 ± 0.03</td>
<td>8.08 ± 0.02</td>
<td>8.15 ± 0.90</td>
</tr>
<tr>
<td>6</td>
<td>8.30 ± 0.06</td>
<td>9.54 ± 0.05***</td>
<td>8.44 ± 0.11</td>
<td>8.44 ± 0.06</td>
<td>8.55 ± 0.04</td>
</tr>
<tr>
<td>12</td>
<td>8.68 ± 0.05</td>
<td>9.92 ± 0.08 ***</td>
<td>8.65 ± 0.03</td>
<td>9.19 ± 0.03***</td>
<td>9.02 ± 0.02 ***</td>
</tr>
<tr>
<td>24</td>
<td>8.71 ± 0.03</td>
<td>9.81 ± 0.08 ***</td>
<td>8.86 ± 0.10</td>
<td>9.41 ± 0.05 ***</td>
<td>9.35 ± 0.04 ***</td>
</tr>
</tbody>
</table>

All comparisons are made with BM (Negative control) and BM-Inu. Values are mean ± SEM of three independent fermentations, pour plating in triplicate. *** p<0.001
with lifestyle diseases like obesity and type 2 diabetes can be the subject of further research.

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Stimulation of \textit{L.\textit{rhamnosus}} by Almonds


