

## ***Lactobacillus plantarum* Isolates from Homemade Dahi as a Potential Probiotic with *In vitro* $\alpha$ -amylase Inhibitory Activity**

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### **Abstract**

Postprandial hyperglycemia (PPG) is among the earliest signs related to type 2 diabetes. Targeting the  $\alpha$ -amylase enzyme responsible for the initial stage of carbohydrate digestion can be an effective strategy to control the PPG. With this objective, about 300 Lactic acid bacteria were obtained from different ethnic fermented foods of Sikkim and screened for  $\alpha$ -amylase inhibitor (AAI) activity. Five isolates of *Lactobacillus (Lb.) plantarum* were found to inhibit  $\alpha$ -amylase whose inhibitory potential was similar to that of copper sulfate and acarbose. Maximum AAI activity was observed within 24 hours of incubation. All the isolates were also assessed for other probiotic properties including bacterial adherence to hydrocarbons, utilization of prebiotic components, etc. Among the safety features of the isolates tested, none of the isolates was hemolytic and neither could hydrolyze gelatin. Most of the isolates were sensitive to ampicillin, chloramphenicol, and erythromycin out of twelve antibiotics tested. These isolates also showed good response to *in vitro* simulation of oro-gastric-intestinal transit which confirms its ability to survive in the gastrointestinal tract. Therefore, these *Lb. plantarum* isolates can be potential probiotic candidates with  $\alpha$ -amylase inhibitory activity which can be further exploited for the management of postprandial hyperglycemia.

**Keywords:** *Lactobacillus plantarum*, dahi, probiotic, alpha-amylase inhibitor

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## INTRODUCTION

The incidence of Diabetes is increasing each year throughout the world affecting about 46.3 crores people worldwide<sup>1</sup>. International Diabetes Federation (IDF) has predicted the rise of diabetes by 57.3 crores in 2030 and 70.0 crores by 2045 which is a 51% increase within 25 years<sup>2</sup>. Diabetes is a multi-factorial physiological condition with many complicated and unknown risk factors. Postprandial hyperglycemia (PPG) is one of the earliest abnormalities associated with type 2 diabetes<sup>3</sup>. The role of PPG on diabetes has always been neglected by medical practitioners because of its complexity. Studies have indicated that control of PPG is more important than controlling fasting hyperglycemia. An individual destined to develop diabetes remains in a PPG state for 10-12 years before the complete onset of diabetes. Thus controlling PPG at the earlier stage can present a permanent cure for the dreaded disease.

During diabetes development, structural and functional changes in the alimentary canal result in the increased activity of major carbohydrate digestive enzymes, i.e. amylase and glucosidase. Inhibitors of  $\alpha$ -amylase ( $\alpha$ -1,4-glucan-4-glucanohydrolases), an enzyme responsible for initial stages of carbohydrate digestion, have emerged as the molecules of choice due to their unambiguous and unique mechanism of action on PPG<sup>4,5</sup>. Moreover, the enzyme inhibitor drugs prescribed to inhibit  $\alpha$ -amylase and glucosidase for the treatment of diabetes are not free from side effects<sup>6</sup>. Therefore, an alternative and complementary method for the management of PPG and diabetes is necessary.

Probiotics are the microorganisms when ingested or used at appropriate cell density imparts a health benefit to the host<sup>7</sup>. Probiotic microorganisms belonging to genus *Bifidobacteria*, *Lactobacillus*<sup>8</sup>, and *Saccharomyces*<sup>9,10</sup> are the most studied ones. Probiotics have been reported to be helpful in irritable bowel syndrome, inflammatory bowel disease, constipation, antibiotic-associated and acute diarrhoea, hypertension, and diabetes<sup>11,12</sup>. Therefore, the blend of microbial enzyme inhibitors and probiotic *Lactobacillus* sp will have twin benefits of the nutritional and pharmacological solution for PPG. As lactobacilli have 'generally regarded as safe' (GRAS) status<sup>13</sup>, enzyme inhibitor producing

probiotic organisms would be added feature. So far *Lactobacillus* isolates from traditional ethnic fermented vegetable and milk products from North-East part of India including Sikkim have not been studied for such property. Hence, the study aims to screen  $\alpha$ -amylase inhibitors and other probiotic characteristics from various *Lactobacillus* isolates from the traditional fermented vegetables and dairy products of Sikkim.

## MATERIALS AND METHODS

### Collection of samples

Altogether thirty samples where five samples each of human stool and milk, two samples each of bamboo shoot, *mesu* (fermented bamboo), *kinema* (fermented soybean), *gundruk* (fermented leafy vegetable of brassica family), cow milk, yak *ghew* (butter) and eight samples of homemade cow *dahi* were obtained from different locations of Sikkim. The samples were collected in sterile bags/bottles and were taken to the laboratory in an ice-box.

### Isolation of lactic acid bacteria (LAB)

Isolation of LAB was carried out by taking 5 g of sample in a 45 ml sterile saline water (0.85%) and was homogenized in a stomacher (Seward, UK)<sup>14</sup>. Serial dilutions in the same diluents were prepared and then plated into the Petri-dishes followed by pouring of melted de Man, Rogosa, and Sharpe (MRS) Agar (HiMedia, India) containing 1.5% CaCO<sub>3</sub>. The inoculated plates were incubated by maintaining the anaerobic condition in Gas Pack System (HiMedia, India) at 37 °C for 72 h. The pure cultures were obtained by streaking on sterile MRS agar plates. The pure cultures of each LAB isolate were preserved –80 °C in MRS broth containing 15 % (v/v) glycerol.

### Screening for $\alpha$ -amylase inhibitory (AAI) activity

About 300 LAB isolates were examined for AAI activity as described by Feng et al. with some modifications<sup>15</sup>. Cell-free supernatant of 18-20 h MRS broth culture of each LAB isolates was obtained by removing the cells by centrifugation. 40  $\mu$ L of cell-free supernatant was added to 50  $\mu$ L 0.02 M sodium phosphate buffer (pH 6.9) containing 0.5 mg/ml porcine pancreatic  $\alpha$ -amylase (Sigma, US) solution and kept at room temperature for 10 min. 40  $\mu$ L of 1% starch solution was added to the inhibitor-enzyme mixture and allowed to stand under the same

condition. The reactions were then quenched by the addition of 10 µL Lugol's iodine solution. The intensity of the developed colour was measured in the micro-plate reader (Bio-rad, Switzerland). 40 µL of MRS broth was incubated with the enzyme instead of cell-free supernatant in the control and the blank tube consisted of MRS broth without the enzyme. Acarbose and copper sulfate (1%) was used as a positive control.

The inhibition of α-amylase was calculated as:

$$\text{Inhibition \%} = \left\{ \frac{(\text{Test} - \text{Control})}{(\text{Blank-Control})} \right\} \times 100$$

#### Production of α-amylase inhibitor

The selected LAB isolates were cultured in 100 ml MRS broth in 250 ml culture flask under shaking condition (60 rpm) at 37 °C. 250 ml of MRS broth in 250 flasks were kept as control under a similar condition. AAI activities of selected LAB isolates were determined by withdrawing the samples at a regular interval of eight hours from 0 to 96 h. The AAI activities were assayed as per the method described in the screening section. Along with AAI activities, pH and optical density of the culture medium and uninoculated MRS broth was also measured at regular intervals.

#### Stability of crude α-amylase inhibitor

18-20 h MRS broth culture of LAB isolates were centrifuged and the supernatant was collected. The culture supernatant showing AAI activities were stored at room temperature (RT), 8°C and -20°C and AAI activities were checked at regular intervals of seven days to check the stability of AAI active constituent.

#### Hydrophobicity test

Hydrophobicity was estimated by taking 4 ml of the 24 hr LAB cultures and were centrifuged at 4 °C, 7500 rpm for 5 min. Then the cell pellet was washed twice with Ringer's solution. Finally, a 9 ml Ringer's solution was added to resuspend the cells. OD<sub>580</sub> was taken by pipetting 1 ml of the cell suspension. Again, an equal volume of the suspension was mixed with an equal volume of the hydrocarbons (chloroform, n-hexadecane, n-octane, p-xylene) and vortexed vigorously. After allowing for 30 min for the separation of the aqueous and the hydrocarbon phases, the aqueous phase was pipetted into a clean cuvette and the OD<sub>580</sub> was measured in the spectrophotometer (Lambda-25, Perkin Elmer)<sup>16</sup>. The hydrophobicity

percentage was calculated as

$$\text{Hydrophobicity \%} = \left\{ \frac{(\text{OD before mixing with hydrocarbons} - \text{OD after mixing with hydrocarbons})}{\text{OD before mixing with hydrocarbons}} \right\} \times 100$$

#### Utilization of prebiotics

The utilization of prebiotic components was examined by inoculating the LAB isolates into the MRS broth containing filter-sterilized raffinose, xylitol, inulin, and fructo-oligosaccharides at the level of 2% (w/v) instead of glucose and incubated at 37 °C for 5 days.

#### Hemolytic activity and gelatin hydrolysis

The overnight grown LAB isolates were streaked on the blood agar plates containing 5% blood and incubated at 37 °C for two weeks. The plates were observed for the formation of hemolytic zones around the LAB colonies<sup>17</sup>. Gelatin hydrolysis was carried out by inoculating LAB isolates in Brain Heart Infusion (BHI) agar stabs containing 3 % gelatin<sup>18</sup>. The cultures were kept at 4 °C for half an hour followed by recording the liquid gelatin hydrolysis daily during the incubation period.

#### Antibiotic susceptibility test

The antibiotic susceptibility was studied by using the method of Bauer et al.<sup>19</sup>. MRS Broth was inoculated with the LAB isolates and incubated at 37 °C for 24 h. Inoculum density of all the bacterial cultures was maintained as per 0.5 McFarland standard. *Staphylococcus aureus* and *E. coli* lawn for reference were made on Mueller Hinton Agar (MHA) while for LAB isolates, LAB susceptibility test medium (LSM) agar plates were used for making bacterial lawn. Using sterile forceps, the antibiotic discs were placed on the agar medium aseptically. Plates were incubated at 37 °C for 24-48 hours under an anaerobic Gas Pack system (HiMedia, India). The zone of inhibitions was measured and was expressed as sensitive, intermediate, and resistant<sup>20</sup>.

#### Survival studies during oro-gastric-intestinal transit

LAB isolates showing the best AAI activities and other probiotic properties were analyzed for their ability to survive the oro-gastric-intestinal transit following the method of Marteau et al<sup>21</sup> with some modifications. Actively growing 5 ml overnight culture of LAB isolates was centrifuged at 10,000 rpm and cells were resuspended and washed with 0.85% physiological

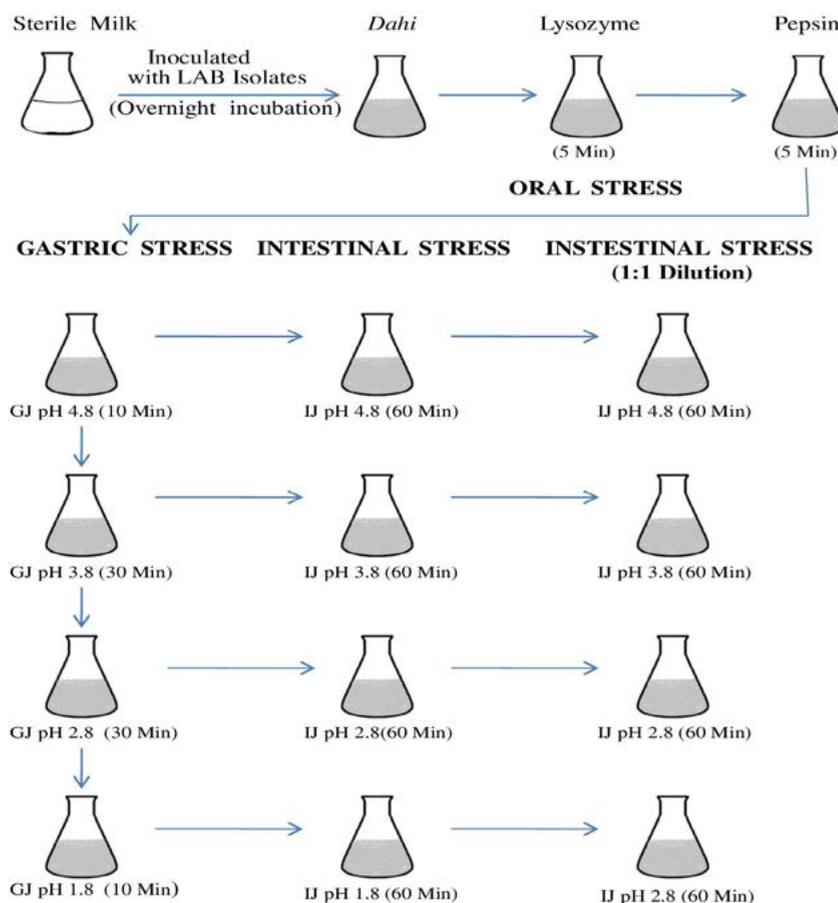
saline. Finally, 5 ml of cells in physiological saline were inoculated into sterile 500 ml milk and incubated at 37 °C until the milk was coagulated and turned into *dahi*. The *in vitro* survival of LAB isolates during oro-gastric-intestinal transit was performed at 37 °C as shown in Fig. 1.

Oral stress was simulated by dilution of the *dahi*-bacterial mixture with 150 mg/L lysozyme (Sigma-Aldrich) in a sterile electrolyte solution. The gastric environment was mimicked by further dilution and acidification by the addition of gastric juice. pH of the mixture was acidified (addition of 1M HCl) to lower the pH from 4.8 to 3.8, 2.8 and 1.8 and kept in the water bath at 37 °C for 10, 30, 30, and 10 min for each pH value respectively. Intestinal stress in the small intestine was mimicked by treating 5ml aliquot with intestinal

juice for 90 min after and maintaining the pH to 6.5 for 90 min<sup>22</sup>. Then, 1mL aliquots were withdrawn and analyzed immediately. Subsequently, intestinal juice stressed mixtures were diluted with an equal volume of intestinal electrolyte solution<sup>23</sup> reducing the bile to 3g/L. After 90 min, the samples were recovered for analysis. Surviving bacterial populations were enumerated after exposure to the stress conditions.

#### Identification of lactic acid bacterial isolates

Phenotypic identification of *Lactobacillus* isolates was done as per Bergy's Manual of Systematic Bacteriology<sup>24</sup>. The genotypic identification was done based on 16S rDNA sequences. Phenol chloroform method<sup>25</sup> was performed to obtain the genomic DNA from the bacterial isolates. 16S rDNA region was amplified



**Fig. 1.** Flow chart showing the plan for oro-gastric-intestinal stress. Time of incubation after each stress is indicated in the parenthesis and all incubations were carried at 37°C. Each pH stress was followed by intestinal stress. GJ, gastric juice; IJ, intestinal juice.

with 27F and 1492R primers. 1 µl of purified PCR products were subjected to sequencing in automated DNA sequencer (3500 Genetic analyzer, Applied Biosystems, Japan). A sequence similarity search was carried out using the BLAST tool from the NCBI website. Phylogenetic analysis of the obtained sequences was performed using UPGMA method<sup>26</sup> and the evolutionary distances were analyzed through the Maximum Composite Likelihood method<sup>27</sup> using the MEGA7 software<sup>28</sup>. All the 16S rRNA gene sequences were submitted in GenBank (<https://www.ncbi.nlm.nih.gov>) and the accession numbers were obtained<sup>25</sup>.

**Analysis of data**

At least three independent experiments were conducted to collect the data and the means and standard deviation/error were calculated. Kruskal-Wallis Test was performed to determine the significant differences between means at  $p < 0.05$  followed by Mann-Whitney-Wilcoxon post hoc analysis. All statistical analyses were performed using RStudio.

**RESULTS AND DISCUSSIONS**

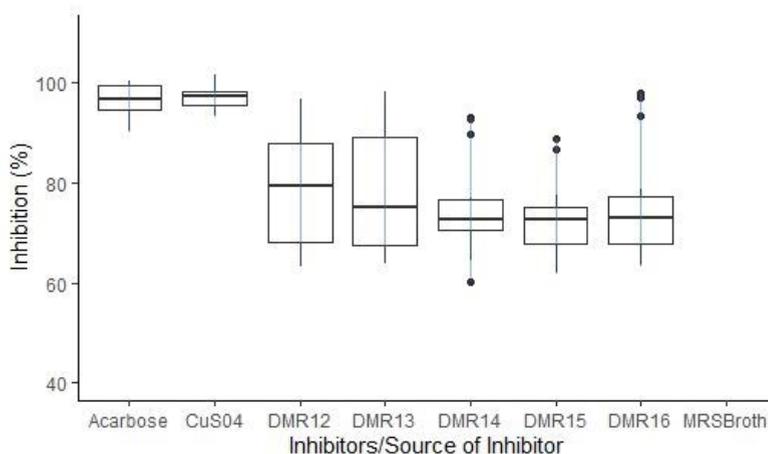
**Screening for AAI activity**

Out of 300 LAB isolates screened for AAI activities, only five isolates from home-made cow *dahi* were found positive. These isolates showed variable amylase inhibition pattern (Fig. 2) among which the isolate DMR12 and DMR13 had consistent AAI activities while DMR14, DMR15 and DMR16 had some outliers. Though

the mean AAI activities of DMR12 (79.2%) were found to be higher than those of DMR13 (78.3%), DMR14 (75.4%), DMR15 (73.1%), and DMR16 (75.5%), statistically, all the AAI activities were similar ( $p < 0.05$ ). Acarbose (96.8%) and copper sulphate (97.2%) were taken as positive control shown in Table 1. All the isolates showed excellent AAI activities which were only slightly less and statistically significant ( $p < 0.05$ ) than those of acarbose and copper sulfate. Similar action on  $\alpha$ -amylase and  $\alpha$ -glucosidase by the cellular extracts of *Lactobacillus* sp. isolated from infant faeces and other sources have been reported which were comparable to that of acarbose<sup>29,30</sup>. The acarbose can show up to 98% inhibition on  $\alpha$ -amylase in a dose-dependent manner<sup>31,32</sup>. This is the reason for its use as one of the prescription drugs for the management of post-prandial hyperglycemia in diabetic patients<sup>33</sup>. Copper sulfate is also the potent inhibitor of  $\alpha$ -amylase enzyme and regularly used as a positive control for the screening of  $\alpha$ -amylase inhibitors<sup>34</sup>.

**Production of  $\alpha$ -amylase inhibition**

When the amylase inhibitory properties of the isolates were checked during the growth of the AAI positive isolates, DMR15 showed the highest inhibitory property (Fig. 3a). The isolate DMR15 showed 59% of inhibition after 16 hours and reached 62.5% in 24 hours. The isolate DMR08 was considered as negative control which had no AAI activities. All the six LAB isolates including DMR08 decreased the pH of the growth medium



**Fig. 2.** Boxplot showing the distribution pattern of AAI activities of the inhibitor from different LAB isolates. AAI activities of MRS broth was below 2% and hence not shown in the plot.

as shown in Fig. 3b. The optical density of the growing cells was also similar for all the isolates. AAI activity of all the isolates decreased after 32 hours. The AAI production corresponds to

**Table 1.** AAI activities of culture-free supernatant of LAB isolates

Inhibitor	AAI Activities (%)
Acarbose	96.8 ± 2.98 <sup>a</sup>
Copper sulfate	97.2 ± 2.31 <sup>a</sup>
DMR12	79.2 ± 11.7 <sup>b</sup>
DMR13	78.3 ± 12.4 <sup>b</sup>
DMR14	75.4 ± 9.58 <sup>b</sup>
DMR15	73.1 ± 7.27 <sup>b</sup>
DMR16	75.5 ± 11.5 <sup>b</sup>
MRS Broth	1.97 ± 0.77 <sup>c</sup>

Data represent the means of five independent replicates. Different superscripts indicate the means are significantly different ( $p < 0.05$ )

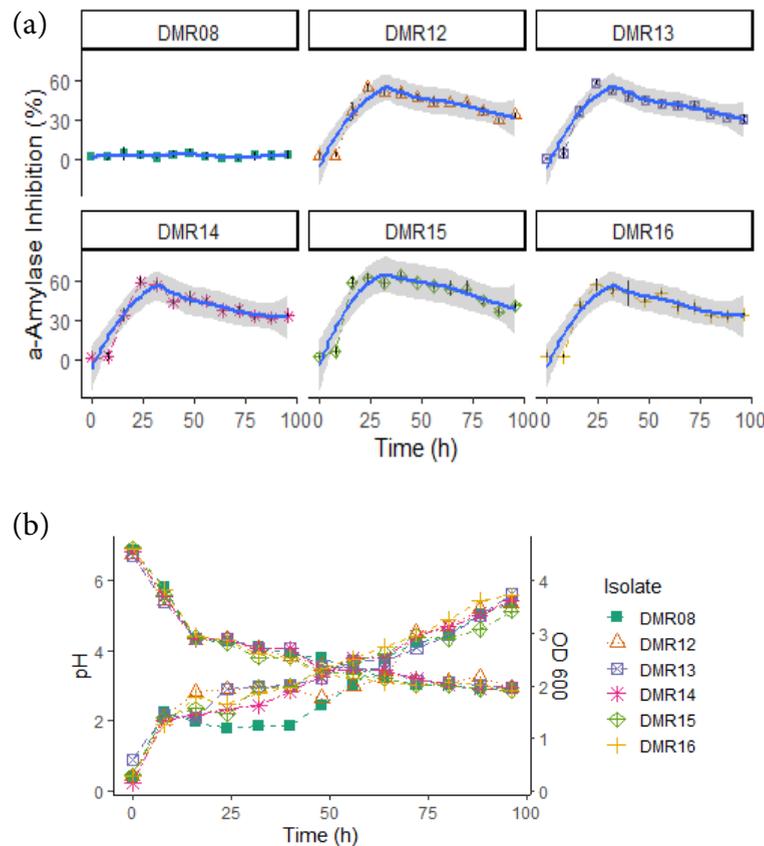
a late exponential phase of the growth for all the isolates. Though DMR08 could not produce  $\alpha$ -amylase inhibitor, its growth characteristics and acidification of the medium were similar to those of AAI positive isolates. Moreover, the MRS medium was also negative for amylase inhibitory activity. This indicates that the AAI activity is not because of the acidification of the growth medium or due to the components of the growth medium.

**Stability of crude  $\alpha$ -amylase inhibitor**

Cell-free culture supernatant showed a decrease in AAI activities at different storage conditions (Fig. 4). AAI activities of crude extract were preserved best at -20°C which remained around 50% after 28 days whereas in 8°C it showed around 45% AAI activities and at room temperature only 35% AAI activities.

**Hydrophobicity test**

The probiotic bacteria should stay in

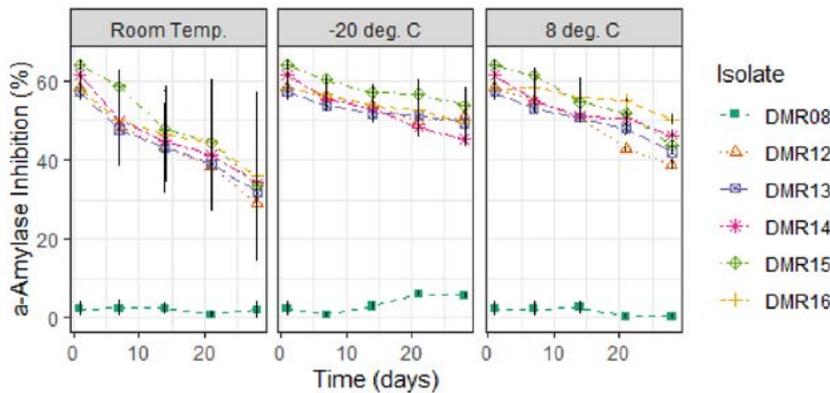


**Fig. 3.** (a) AAI activities during the growth of LAB isolates. Gray ribbon indicates the local regression line calculated using Loess method (b) pH are shown by descending lines and optical density measured during the growth of the isolates are indicated by ascending lines.

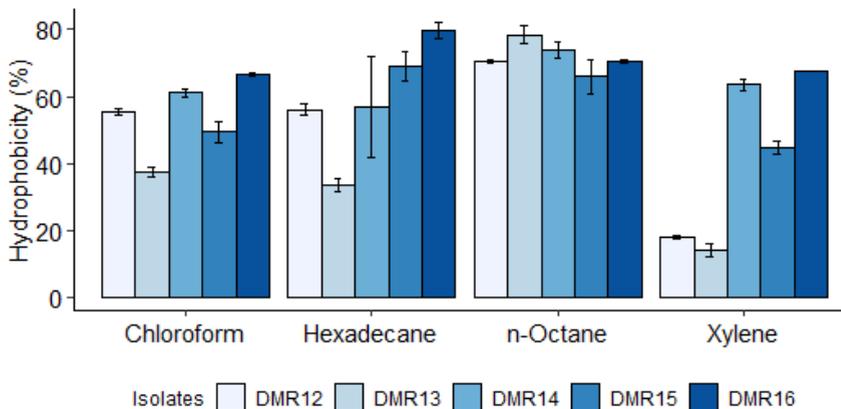
the GI tract, though for a short period of time<sup>35</sup>. Therefore, to attach to the intestinal mucosa, the cell surface of the probiotic microorganism should be hydrophobic in nature<sup>36,37</sup>. All the isolates showed moderate to high ability to adhere to chloroform, n-hexadecane, and n-octane (fig 5). The highest hydrophobicity was shown by DMR16 in n-hexadecane and n-octane which was more than 70%. While in p-xylene, most of the isolates showed high adherence ability except DMR12 and DMR13 (<20%). The adherence to hydrocarbons is dependent on incubation time, the composition of culture media, the presence of organic acids, and on the nature of the solvent used<sup>37,38</sup>. Partitioning of microbial cells to the hydrocarbon phase indicates the interaction of the cell surface to the mucosa through van der Waals and electrostatic interactions rather than the hydrophobic nature of the cell<sup>39</sup>.

### Utilization of prebiotics

Prebiotics are nondigestible food components that are utilized by the beneficial colonic bacteria such as *Lactobacillus* and *Bifidobacterium*, stimulating their growth and metabolic activity<sup>36,40</sup>. Inulin consists of D-fructose units linked by a  $\beta$  (2-1) bonds with either a glucose or fructose unit at the end of the chain<sup>41</sup>. Humans do not have enzymes to hydrolyze the  $\beta$  (2-1) linkages and thus cannot digest it. However, these prebiotic components can only be utilized by certain bacteria present in the colon<sup>41</sup>, such as by most of the bifidobacteria<sup>42</sup> which can produce  $\beta$ -fructosidase enzyme. While the production of  $\beta$ -fructosidase by *lactobacilli* is rare, nevertheless few isolates of *Lb. paracasei*, *Lb. casei*, *Lb. acidophilus*, and *Lb. delbrueckii* can produce it<sup>43,44</sup>. Genomic and proteomic analysis has revealed a functional inulin operon fosRABCDXE in *Lb.*



**Fig. 4.** Stability of  $\alpha$ -amylase inhibitor from LAB isolates at different storage conditions.



**Fig. 5.** Adherence ability of different LAB isolates to hydrocarbons expressed as hydrophobicity (%). Data represent the means of three independent replicates.

plantarum responsible for coding  $\beta$ -fructosidase and fructose transport<sup>45</sup>. In this study, all the five LAB isolates were able to utilize at least three prebiotic components including inulin (Table 2). Moreover, these isolates are also able to utilize low-calorie polyols such as mannitol and sorbitol<sup>46</sup>. Sorbitol is one of the emerging prebiotic components although its absorption in the upper GI tract is very slow. Thus, a significant proportion of sorbitol can reach the colon to be utilized by the colonic bacteria. *In vivo* studies in rats have shown that feeding with sorbitol increases the colonic and cecal population of *Lactobacilli*<sup>47</sup>. The bifunctional alcohol dehydrogenase *adhE* under the control of transcriptional regulators *AcrR* and *Rex*, is responsible for the utilization of mannitol and sorbitol in *Lb. plantarum*<sup>48</sup>. A study on the utilization of pectin by probiotic lactobacilli is

meager<sup>46</sup> although Bifidobacteria have been shown to utilize pectin in a strain-dependent manner<sup>49</sup>.

### Hemolysis and gelatin hydrolysis

Among the safety aspects, the probiotic microorganisms should neither be hemolytic nor should hydrolyze gelatin. The lactobacilli isolates were screened for hemolysis and gelatin hydrolysis since these are among the important virulence factors present in clinical isolates, and common in *Lactobacilli* isolates from dairy sources<sup>50</sup>. In our study none of the LAB isolates was able to cause  $\alpha$ - or  $\beta$ -hemolysis and gelatin hydrolysis (data not shown) as reported by Halder et al<sup>51</sup>.

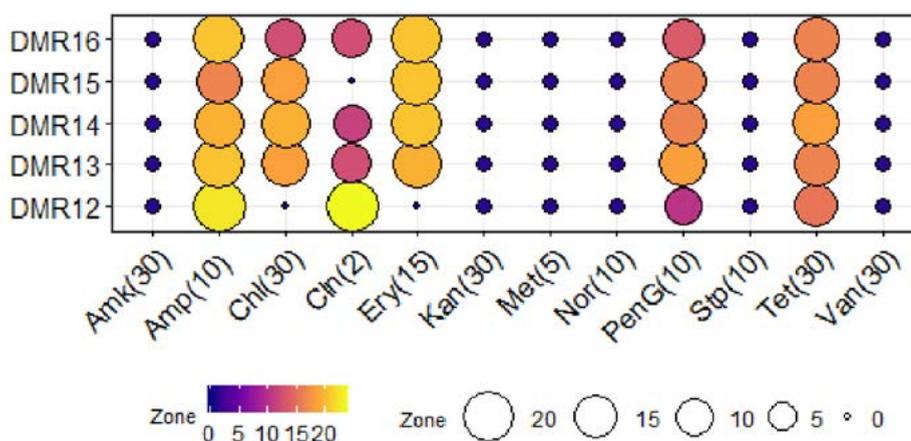
### Antibiotic sensitivity

Probiotic bacteria should also be sensitive to antimicrobial agents or the resistance should not be transferrable to the pathogenic bacteria in

**Table 2.** Utilization of prebiotic components by LAB isolates having AAI activity

Isolate code	Utilization of prebiotics							
	Fruto-oligo-saccharides	Inulin	Mannitol	Raffinose	Sorbitol	Stachyose	Pectin	Xylitol
DMR12	-	+	+	-	w	-	+	-
DMR13	-	+	-	w	-	+	+	-
DMR14	-	+	+	-	+	-	-	-
DMR15	-	+	+	-	+	-	+	-
DMR16	-	+	+	-	+	+	+	-

Growth characteristics are represented by w, weakly positive; +, positive; ++, strongly positive; -, no growth



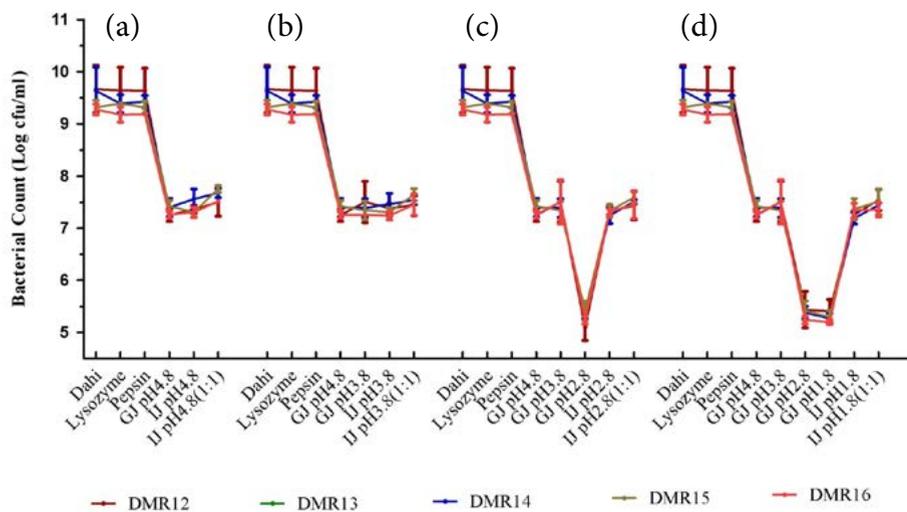
**Fig. 6.** Antibiotic sensitivity of LAB isolates. Blue dark, purple colour circles indicates the resistant characteristics while Yellow and dark colour circles represent the sensitive characteristics. Conc. of the antibiotics is indicated in parenthesis in the unit of mcg.

the same niche<sup>52</sup>. Microbial isolates used in the food and feed having transferable antimicrobial resistance are considered as a hazard by the European Food Safety Authority (EFSA)<sup>53</sup>. All five LAB isolates of homemade *dahi* were completely sensitive to ampicillin while with erythromycin only DMR12 was resistant (Fig. 6). All the isolates showed intermediate resistance to tetracycline. However, these isolates were completely resistant to amikacin, kanamycin, methicillin, norfloxacin, streptomycin, vancomycin, and several authors have reported that lactobacilli are resistant to most aminoglycosides<sup>54</sup>. These isolates need further investigation to determine if these resistance genes are transferable or not. Vancomycin resistance is widespread among lactobacilli, though the resistance is not intrinsic<sup>55</sup>. The presence of multiple drug efflux pumps has been reported in *Lactobacillus plantarum* and *Lactococcus lactis* and it is the frequently employed mechanism of resistance<sup>56</sup>. Aminoglycoside phosphotransferase has been reported from the members of *Lb. delbrueckii* group and *Lb. sakei*<sup>57,58</sup> which confers resistance in these isolates.

**Survival studies during oro-gastric-intestinal transit**

To elicit the full effect in the host, the probiotic candidate microorganism should survive

the harsh conditions encountered in the upper part of the digestive tract<sup>59,60</sup>. Most of the bacteria isolated from food samples are very sensitive to the acidic conditions of the stomach, nevertheless, several members of LAB are acid-tolerant and can grow well in acidic conditions because of the presence of proton extrusion mechanism<sup>61</sup>. Protection of potential probiotic bacterium by the food matrix i.e. *dahi* and the effect of lysozyme, the influence of pepsin and the gastric stress, and further the intestinal stress due to the presence of bile, simulating the successive passage of bacteria to the intestine during digestion was performed<sup>21</sup>. All the isolates showed a very good response to the *in vitro* simulation of oro-gastric-intestinal transit (Fig. 7). The effect of lysosome and pepsin on bacterial survival was almost nil while with the introduction of gastric juice of pH 4.8, the survival capability decreased by 2 log cycles. There was a good number of surviving populations (5.2 log cfu/ml) even at the lowest gastric pH of 1.8 which was similar to that reported for *Lb. acidophilus* under similar conditions<sup>62</sup>. After exposure to gastric juice of pH 1.8, these isolates were subjected to intestinal stress containing pancreatin and bile acids. Nevertheless, these isolates were able to increase its population to 7.5 log cfu/ml as reported by Fernandez et al<sup>62,63</sup>. Probiotic



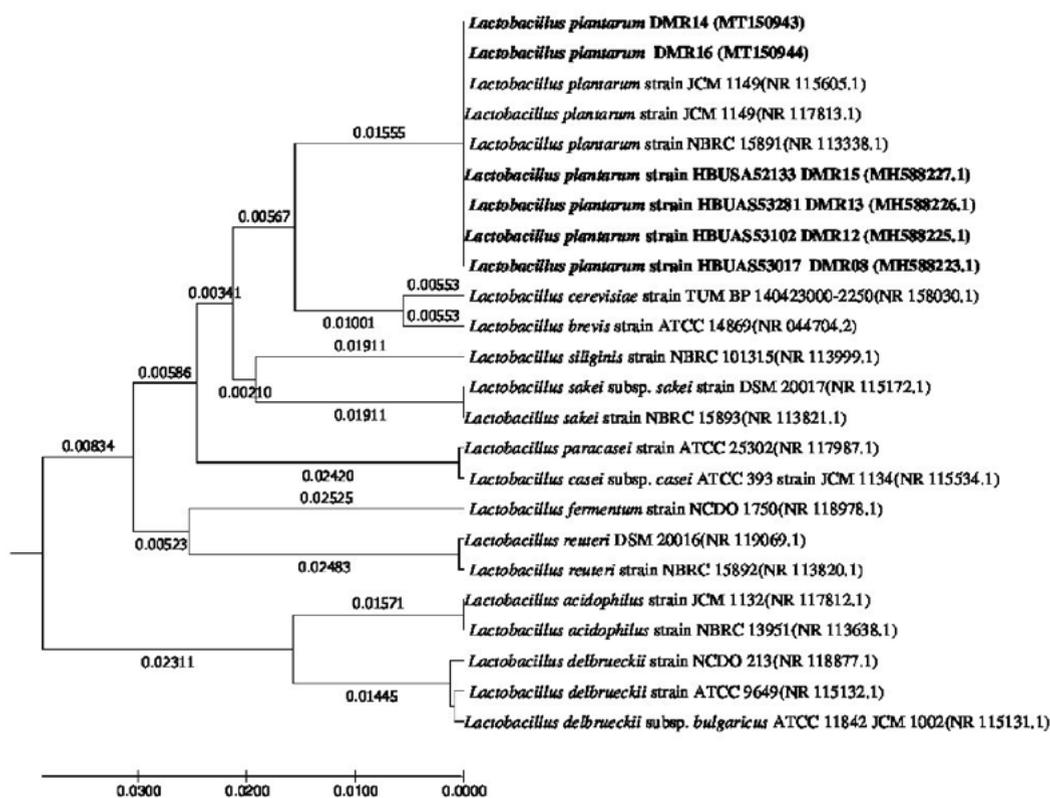
**Fig. 7.** Microbial count (cfu/g) of LAB isolates after exposure to different stress conditions prevailing during oro-gastric intestinal transit. Each figure represents a stress condition up to a particular pH and then exposed to intestinal stress (a) up to pH 4.8 and then exposed to intestinal stress, (b) up to pH 3.8 and then exposed to intestinal stress, (c) up to pH 2.8 and then exposed to intestinal stress, (d) Stress up to pH 1.8 and then exposed to intestinal stress

microorganisms should also tolerate bile salts present in the small intestine and *Lactobacillus* are resistant to intestinal bile contents to varying degrees<sup>64</sup>.

### Bacterial Identification

The characterization of the isolates based on morphology and growth characteristics as per the Bergy's Manual of Systematic Bacteriology<sup>24</sup> allowed all the five isolates of LAB to be identified as *Lactobacillus* sp. (Table 3). Further the species

of the bacterial isolates were confirmed by the 16S rRNA gene sequence analysis. The DNA sequences were aligned by the 'codon code aligner' and the phylogenetic tree (Fig. 8) was prepared by the UPGMA method using MEGA7 software<sup>28</sup>. All the LAB isolates were identified as *Lactobacillus plantarum* upon the comparison of the sequences with the NCBI nr/nt database using the BLAST tool. The sequences were submitted to the GenBank (Table 4).



**Fig. 8.** Evolutionary relationship of taxa. The evolutionary history was inferred using the UPGMA method<sup>1</sup>. The optimal tree with the sum of branch length = 0.25083902 is shown. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method<sup>2</sup> and are in the units of the number of base substitutions per site. The analysis involved 25 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 658 positions in the final dataset. Evolutionary analyses were conducted in MEGA7<sup>3</sup>.

### CONCLUSION

Some of the isolates of *Lactobacillus plantarum* isolated from homemade cow *dahi* were able to produce  $\alpha$ -amylase inhibitors. This is the first report that describes  $\alpha$ -amylase

inhibitor production from *Lactobacillus* isolates of homemade cow *dahi*. Besides, these isolates also had the potential to adhere to the hydrocarbons indicating their potential to attach to the intestinal epithelium. None of the isolates was hemolytic and

**Table 3.** Phenotypic characterization of LAB isolates having AAI activities

Isolate Code	Growth in/at						Acid production from											Genus							
	NaCl (%)		pH		Temp. (°C)		D-Fructose	Galactose	Glycerol	Cellulose	Maltose	D-Mannitol	Melibiose	Lactose	Rhamnose	D-Ribose	D-Salicin		D-Sorbitol	Sucrose	Trehalose	D-Xylose			
	6.5	10	3.6	9.6	10	15																	45		
DMR12	+	-	w	-	-	w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>Lactobacillus sp.</i>	
DMR13	w	-	-	-	+	+	+	-	w	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	<i>Lactobacillus sp.</i>
DMR14	-	-	+	-	-	+	+	-	w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
DMR15	w	-	+	-	-	w	+	+	w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>Lactobacillus sp.</i>
DMR16	+	-	+	-	-	w	+	+	w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>Lactobacillus sp.</i>

Growth characteristics are represented by w, weakly positive; +, positive; ++, strongly positive; -, negative  
 All the isolates were Gram positive, catalase negative and non-spore forming, homofermentative rods (could not produce gas from glucose)  
 Except DMR12, none of the isolates produced ammonia from arginine  
 None of the isolates produced acid from D-arabinose

**Table 4.** Bacterial isolates, their 16S identities and NCBI GenBank accession number

No.	Isolate ID	Bacteria	Accession No.
1	DMR12	<i>Lactobacillus plantarum</i>	MH588225.1
2	DMR13	<i>Lactobacillus plantarum</i>	MH588226.1
3	DMR14	<i>Lactobacillus plantarum</i>	MT150943
4	DMR15	<i>Lactobacillus plantarum</i>	MH588227.1
5	DMR16	<i>Lactobacillus plantarum</i>	MT150944

neither could hydrolyze gelatin. *Lb plantarum* also could utilize some of the prebiotic components efficiently than other isolates. Being able to resist the oro-gastric-intestinal environment, *Lb plantarum* isolates showed the ability to cope with the harsh environment of the GI tract. Therefore, these *Lb. plantarum* isolates can be the potential probiotic candidate which can be used in the control of PPG and the management of diabetes.

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**CONFLICTS OF INTEREST**

The authors declare that there is no conflict of interest.

**AUTHORS' CONTRIBUTION**

RKT has contributed substantially in design, conducting experiments and manuscript preparation while LKC helped in 16S rRNA gene sequencing and its analysis. The overall design of the work and analysis of data, the manuscript correction was done by BT.

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## ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files

## REFERENCES

- Magliano DJ, Islam RM, Barr ELM, et al. Trends in incidence of total or type 2 diabetes: Systematic review. *BMJ*. 2019;366:1-12. <https://doi.org/10.1136/bmj.l5003>
- Saeedi P, Petersohn I, Salpea P, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract*. 2019;157:107843. <https://doi.org/10.1016/j.diabres.2019.107843>
- Maffettone A, Rinaldi M, Fontanella A. Postprandial hyperglycemia: A new frontier in diabetes management? *Ital J Med*. 2018;12:108-115. <https://doi.org/10.4081/itjm.2018.961>
- Kim YM, Jeong YK, Wang MH, Lee WY, Rhee HI. Inhibitory effect of pine extract on  $\alpha$ -glucosidase activity and postprandial hyperglycemia. *Nutrition*. 2005;21(6):756-761. <https://doi.org/10.1016/j.nut.2004.10.014>
- Toeller M.  $\alpha$ -Glucosidase inhibitors in diabetes: efficacy in NIDDM subjects. *Eur J Clin Invest*. 1994;24:31-35. <https://doi.org/10.1111/j.1365-2362.1994.tb02253.x>
- Evans JL, Rushakoff RJ. Oral pharmacological agents for type 2 diabetes: sulfonylureas, meglitinides, metformin, thiazolidinediones,  $\alpha$ -glucosidase inhibitors, and emerging approaches.
- Hill C, Guarner F, Reid G, et al. Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014;11(8):506-514. <https://doi.org/10.1038/nrgastro.2014.66>
- Gueimonde M, Salminen S. New methods for selecting and evaluating probiotics. *Dig Liver Dis*. 2006;38(SUPPL. 2):242-247. [https://doi.org/10.1016/S1590-8658\(07\)60003-6](https://doi.org/10.1016/S1590-8658(07)60003-6)
- van der Aa Kuhle A, Skovgaard K, Jespersen L. *In vitro* screening of probiotic properties of *Saccharomyces cerevisiae* var. *boulardii* and food-borne *Saccharomyces cerevisiae* strains. *Int J Food Microbiol*. 2005;101(1):29-39. <https://doi.org/10.1016/j.ijfoodmicro.2004.10.039>
- Sourabh A, Kanwar SS, Sharma OP. Screening of indigenous yeast isolates obtained from traditional fermented foods of Western Himalayas for probiotic attributes. *J Yeast Fungal Res*. 2011;2(8):117-126.
- Khalesi S, Bellissimo N, Vandelanotte C, Williams S, Stanley D, Irwin C. A review of probiotic supplementation in healthy adults: helpful or hype? *Eur J Clin Nutr*. 2019;73(1):24-37. <https://doi.org/10.1038/s41430-018-0135-9>
- Weichselbaum E. Probiotics and health: A review of the evidence. *Nutr Bull*. 2009;34(4):340-373. <https://doi.org/10.1111/j.1467-3010.2009.01782.x>
- Wessels S, Axelsson L, Bech Hansen E, et al. The lactic acid bacteria, the food chain, and their regulation. *Trends Food Sci Technol*. 2004;15(10):498-505. <https://doi.org/10.1016/j.tifs.2004.03.003>
- Tamang JP, Tamang B, Schillinger U, Franz CMAP, Gores M, Holzapfel WH. Identification of predominant lactic acid bacteria isolated from traditionally fermented vegetable products of the Eastern Himalayas. *Int J Food Microbiol*. 2005;105:347-356. <https://doi.org/10.1016/j.ijfoodmicro.2005.04.024>
- Feng GH, Richardson M, Chen M shun, Kramer KJ, Morgan TD, Reeck GR.  $\alpha$ -Amylase Inhibitors from Wheat : Amino Acid Sequences and Patterns of Inhibition of Insect and Human  $\alpha$ -Amylases. *Insect Biochem Molec Biol*. 1996;26(5):419-426. [https://doi.org/10.1016/0965-1748\(95\)00087-9](https://doi.org/10.1016/0965-1748(95)00087-9)
- Rosenberg M, Gutnick D, Rosenberg E. Adherence of bacteria to hydrocarbons: A simple method for measuring cell-surface hydrophobicity. *FEMS Microbiol Lett*. 1980;9:29-33.
- Isenberg H. Hemolysis on sheep blood agar. In: *Clinical Microbiology Procedures Handbook. Vol. 1*. Washington D.C: American Society of Microbiology. 1992:1.20.16-1.20.17.
- Isenberg H. Gelatin liquefaction test. In: *Clinical Microbiology Procedures Handbook, Vol. 1*. Washington, DC.: American Society of Microbiology; 1992:1.19.42-1.19.43.
- Bauer AW, Kirby WMM, Sherris JC, Turck AM, Graevenitz A Von. 40 Microbiology: A Centenary Perspective 1966 Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Am J Clin Pathol*. 1978;45(3):493-496.
- Charteris WP, Kelly PM, Morelli L, Collins JK. Antibiotic susceptibility of potentially probiotic *Bifidobacterium* isolates from the human gastrointestinal tract. *Lett Appl Microbiol*. 1998;26(5):333-337. <https://doi.org/10.1046/j.1472-765X.1998.00342.x>
- Marteau P, Minekus M, Havenaar R. Survival of Lactic Acid Bacteria in a Dynamic Model of the Stomach and Small Intestine : *J Dairy Sci*. 1997;80(6):1031-1037. [https://doi.org/10.3168/jds.S0022-0302\(97\)76027-2](https://doi.org/10.3168/jds.S0022-0302(97)76027-2)
- Fernandes ML, Perin LM, Todorov SD, Nero LA, De Alencar ER, De Aguiar Ferreira M. In vitro evaluation of the safety and probiotic and technological potential of *pediococcus pentosaceus* isolated from sheep milk. *Semin Agrar*. 2018;39(1):113-131. <https://doi.org/10.5433/1679-0359.2018v39n1p113>
- Marteau P, Minekus M, Havenaar R, Huis in't Veld JH. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of bile. *J Dairy Sci*. 1997;80(6):1031-1037. [https://doi.org/10.3168/jds.S0022-0302\(97\)76027-2](https://doi.org/10.3168/jds.S0022-0302(97)76027-2)
- Vos P De, Garrity GM, Jones D, et al. *Bergey's Manual of Systematic Bacteriology*. 2nd Vol. 3. (Whitman WB, Parte AC, Goodfellow M, et al., eds.). Dordrecht: Springer; 2009.

25. Green M, Sambrook J. *Molecular Cloning A Laboratory Manual*. Vol 1. Forth. (Mamiatis T, Fritsch EF, Sambrook J EJ, ed.). New York: Cold Spring Harbor Laboratory Press; 2012.
26. Sneath P, Sokal R. *Numerical Taxonomy*. San Francisco: Freeman; 1973.
27. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc Natl Acad Sci*. 2004;101:11030-11035. <https://doi.org/10.1073/pnas.0404206101>
28. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol*. 2016;33(7):1870-1874. <https://doi.org/10.1093/molbev/msw054>
29. Ramchandran L, Shah NP. Proteolytic profiles and angiotensin-I converting enzyme and  $\alpha$ -glucosidase inhibitory activities of selected lactic acid bacteria. *J Food Sci*. 2008;73(2):M75-M81. <https://doi.org/10.1111/j.1750-3841.2007.00643.x>
30. Panwar H, Calderwood D, Grant IR, Grover S, Green BD. *Lactobacillus* strains isolated from infant faeces possess potent inhibitory activity against intestinal alpha- and beta-glucosidases suggesting anti-diabetic potential. *Eur J Nutr*. 2014;53(7):1465-1474. <https://doi.org/10.1007/s00394-013-0649-9>
31. Mohamed EAH, Siddiqui MJA, Ang LF, et al. Potent  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from *Orthosiphon stamineus* Benth as anti-diabetic mechanism. *BMC Complement Altern Med*. 2012;12(1):176. <https://doi.org/10.1186/1472-6882-12-176>
32. Li K, Yao F, Xue Q, et al. Inhibitory effects against  $\alpha$ -glucosidase and  $\alpha$ -amylase of the flavonoids-rich extract from *Scutellaria baicalensis* shoots and interpretation of structure–activity relationship of its eight flavonoids by a refined assign-score method. *Chem Cent J*. 2018;12(1):82. <https://doi.org/10.1186/s13065-018-0445-y>
33. DiNicolantonio JJ, Bhutani J, O’Keefe JH. Acarbose: safe and effective for lowering postprandial hyperglycaemia and improving cardiovascular outcomes. *Open Heart*. 2015;2(1):e000327. <https://doi.org/10.1136/openhrt-2015-000327>
34. Safarik I. Rapid Detection of Alpha-Amylase. *J Enzym Inhib*. 1990;3:245-247. <https://doi.org/10.3109/14756369009035843>
35. Iniguez-Palomares C, Perez-Morales R, Acedo-Felix E. Evaluation of probiotic properties in *Lactobacillus* isolated from small intestine of piglets. *Rev Latinoam Microbiol*. 2008;49(3-4):46-54.
36. El-Nagar G, Clowes G, Tudorica C, Kuri V, Brennan C. Rheological quality and stability of yog-ice cream with added inulin. *Int J Dairy Technol*. 2002;55:89-93. <https://doi.org/10.1046/j.1471-0307.2002.00042.x>
37. Shakirova L, Grube M, Gavare M, Auzina L, Zikmanis P. *Bifidobacterium lactis* Bb12 cell surface hydrophobicity and survival of the cells under adverse environmental conditions. *J Nutr*. 2013;140:85-93.
38. Wu. Q, Shah NP. Effects of elaidic acid, a predominant industrial trans fatty acid, on bacterial growth and cell surface hydrophobicity of *lactobacilli*. *J Food Sci*. 2014;79:2485–2490. <https://doi.org/10.1111/1750-3841.12695>
39. Martienssen M, Reichel O, Kohlweyer U. Surface properties of bacteria from different wastewater treatment plants. *Acta Biotechnol*. 2001;21:207-225.
40. Forssten SD, Sindelar CW, Ouweland AC. Probiotics from an industrial perspective. *Anaerobe*. 2011;17(6):410-413. <https://doi.org/10.1016/j.anaerobe.2011.04.014>
41. Roberfroid MB, Delzenne NM. Dietary Fructans. *Annu Rev Nutr*. 1998;18(1):117-143. <https://doi.org/10.1146/annurev.nutr.18.1.117>
42. Roberfroid MB, Van Loo JAE, Gibson GR. The Bifidogenic Nature of Chicory Inulin and Its Hydrolysis Products. *J Nutr*. 1998;128(1):11-19. <https://doi.org/10.1093/jn/128.1.11>
43. Tsujikawa Y, Nomoto R, Osawa R. Difference in Degradation Patterns on Inulin-type Fructans among Strains of *Lactobacillus delbrueckii* and *Lactobacillus paracasei*. *Biosci Microbiota, Food Heal*. 2013;32(4):157-165. <https://doi.org/10.12938/bmfh.32.157>
44. Velikova P V, Gotcheva VG, Petrova PM. Novel Bulgarian *Lactobacillus* strains ferment prebiotic carbohydrates. In: *J BioSci. Biotech.* ; 2014:55-60.
45. Buntin N, Hongpattarakere T, Ritari J, et al. An Inducible Operon Is Involved in Inulin Utilization in *Lactobacillus plantarum* Strains, as Revealed by Comparative Proteogenomics and Metabolic Profiling. *Appl Environ Microbiol*. 2017;83(2). <https://doi.org/10.1128/AEM.02402-16>
46. Yeo SK, Liong MT. Effect of prebiotics on viability and growth characteristics of probiotics in soymilk. *J Sci Food Agric*. 2010;90(2):267-275. <https://doi.org/10.1002/jsfa.3808>
47. Sarmiento-Rubiano LA, Zuniga M, Perez-Martinez G, Yebra MJ. Dietary supplementation with sorbitol results in selective enrichment of lactobacilli in rat intestine. *Res Microbiol*. 2007;158(8-9):694-701. <https://doi.org/10.1016/j.resmic.2007.07.007>
48. Yang X, Teng K, Su R, et al. AcrR and Rex Control Mannitol and Sorbitol Utilization through Their Cross-Regulation of Aldehyde-Alcohol Dehydrogenase (AdhE) in *Lactobacillus plantarum*. *Appl Environ Microbiol*. 2019;85(4). doi:10.1128/AEM.02035-18"
49. Slovakova L, Duskova D, Marounek M. Fermentation of pectin and glucose, and activity of pectin-degrading enzymes in the rabbit caecal bacterium *Bifidobacterium pseudolongum*. *Lett Appl Microbiol*. 2002;35:126-130.
50. Kaktcham NF, Zambou FM, El-Soda TM. Antimicrobial and safety properties of lactobacilli isolated from two Cameroonian traditional fermented foods. *Sci Pharm*. 2012;80:189-204. <https://doi.org/10.3797/scipharm.1107-12>
51. Halder D, Mandal M, Chatterjee SS, Pal NK, Mandal S. Indigenous probiotic *Lactobacillus* isolates presenting antibiotic like activity against human pathogenic bacteria. *Biomedicines*. 2017;5(2):1-11. <https://doi.org/10.3390/biomedicines5020031>
52. Jose NM, Bunt CR, Hussain MA. Implications of Antibiotic Resistance in Probiotics. *Food Rev Int*. 2015;31(1):52-62. <https://doi.org/10.1080/87559129.2014.961075>

53. EFSA/EDC. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2016. *EFSA J.* 2018;16(2):5182.
54. Rozman V, Mohar Lorbeg P, Accetto T, Bogovic Matijasic B. Characterization of antimicrobial resistance in lactobacilli and bifidobacteria used as probiotics or starter cultures based on integration of phenotypic and in silico data. *Int J Food Microbiol.* 2020;314(October 2019):108388. <https://doi.org/10.1016/j.ijfoodmicro.2019.108388>
55. Holliman RE, Bone GP. Vancomycin resistance of clinical isolates of lactobacilli. *J Infect.* 1988;16(3):279-283. [https://doi.org/10.1016/S0163-4453\(88\)97676-1](https://doi.org/10.1016/S0163-4453(88)97676-1)
56. Wachter-Rodarte M del C, Trejo-Munuzuri TP, Montiel-Aguirre JF, et al. Antibiotic resistance and multidrug-resistant efflux pumps expression in lactic acid bacteria isolated from pozol, a nonalcoholic Mayan maize fermented beverage. *Food Sci Nutr.* 2016;4(3):423-430. <https://doi.org/10.1002/fsn3.304>
57. Campedelli I, Mathur H, Salvetti E, et al. crosssm Genus-Wide Assessment of Antibiotic Resistance in *Lactobacillus* spp. p. *Appl Environ Microbiol.* 2019;85(1):e01738-18. <https://doi.org/10.1128/AEM.01738-18>
58. Rodriguez C, Petrelli L, Ramirez MS, Centron D, Hebert EM, Saavedra L. Genomic overview of acquired antibiotic resistance mechanisms in *Lactobacillus*. In: Ruzal SM, ed. *Lactobacillus Genomics and Metabolic Engineering*. Buenos Aires, Argentina: Caister Academic Press; 2019:185-204. <https://doi.org/10.21775/9781910190890.10>
59. Afzaal M, Saeed F, Saeed M, et al. Survival and stability of free and encapsulated probiotic bacteria under simulated gastrointestinal conditions and in pasteurized grape juice. *J Food Process Preserv.* 2019;(July):1-8. <https://doi.org/10.1111/jfpp.14346>
60. Havenaar R, Brink B Ten, Huis In 't Veld JHJ. Selection of strains for probiotic use. In: *Probiotics: The Scientific Basis*. Dordrecht: Springer Netherlands; 1992:209-224. [https://doi.org/10.1007/978-94-011-2364-8\\_9](https://doi.org/10.1007/978-94-011-2364-8_9)
61. Conway P, Gorbach S, Goldin B. Survival of Lactic Acid Bacteria in the Human Stomach and Adhesion to Intestinal Cells. *J Dairy Sci.* 1987;70(1):1-12. [https://doi.org/10.3168/jds.S0022-0302\(87\)79974-3](https://doi.org/10.3168/jds.S0022-0302(87)79974-3)
62. Fernandez MF, Boris S, Barbes C. Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. *J Appl Microbiol.* 2003;94(3):449-455. <https://doi.org/10.1046/j.1365-2672.2003.01850.x>
63. Rolim FRL, dos Santos KMO, de Barcelos SC, et al. Survival of *Lactobacillus rhamnosus* EM1107 in simulated gastrointestinal conditions and its inhibitory effect against pathogenic bacteria in semi-hard goat cheese. *LWT - Food Sci Technol.* 2015;63(2):807-813. <https://doi.org/10.1016/j.lwt.2015.05.004>
64. Tokatl M, Gulgor G, Bagder Elmac S, Arslankoz Isleyen N, Ozcelik F. *In vitro* Properties of Potential Probiotic Indigenous Lactic Acid Bacteria Originating from Traditional Pickles. *Biomed Res Int.* 2015. <https://doi.org/10.1155/2015/315819>