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**Research Paper**

# Probiotic properties of exopolysaccharide producing lactic acid bacteria isolated from vegetables and traditional Indian fermented foods

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**ABSTRACT**

A total of 203 lactic acid bacteria (LAB) isolated from vegetables and traditional fermented food products of India such as idli batter, dhokla batter and dahi were screened for exopolysaccharide (EPS) production. Based on the amount of EPS produced, 17 LAB isolates were selected for biochemical and genetic characterization using 16S rRNA gene sequencing. The isolates were belonging to the genera *Lactobacillus*, *Weissella* and *Pediococcus*. *In vitro* examination was performed to evaluate their probiotic potential. Resistance to low pH and 0.3 % bile salts was studied. Bile salt hydrolase (BSH) activity, susceptibility to various antibiotics and antimicrobial activity against the non pathogenic *E. coli* K12 were also investigated. The results showed that the isolates could not grow in the presence of oxbile however some survived exposure to it for 2.5 h. Some isolates were able to grow in the presence of sodium taurocholate and showed considerable antimicrobial activity against *E. coli* K12. Five isolates were showing BSH activity which is reported for the genus *Weissella* for the first time. The results suggest that traditional fermented products could be an alternative and readily available

**Keywords:** Exopolysaccharides, BSH activity, Weissella, dhokla batter, idli batter, antimicrobial activity

## Introduction

Probiotics are living microorganisms that exert a health benefit to the host when administered in sufficient amounts (FAO/WHO 2002). Lactic acid bacteria (LAB) isolated from animal origin are commonly employed as probiotics (Saad *et al*. 2013), however other isolation sources such as fermented food products where LAB are used as starter cultures or occur spontaneously as the fermentative microflora may

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provide potential probiotic organisms as well (Abriouel *et al*. 2012; Garai-Ibabe *et al*. 2010; Mathara *et al*. 2008). Probiotics are showing a wide array of health benefits including but not limited to cholesterol lowering properties (Bukowska *et al*. 1998), immunomodulation and amelioration of inflammatory bowel disease and diarrhoea as reviewed by Saad *et al*. (2013). The ability to produce exopolysaccharides (EPSs) is a common trait to lactic starters/probiotics. Polysaccharides that impart thickening and gelling properties are invaluable in the food industry formulation and EPSs impose highly desirable rheological changes in the food matrix such as increased viscosity, improved texture and reduced syneresis (Badel *et al*. 2011). EPSs may occur as a capsule or as slime that is loosely attached to the cell surface and they are believed to have a protective function helping the cell to withstand desiccation, osmotic stress, antibiotics, toxic compounds and bacteriophages in their natural habitat (De Vuyst and Degeest 1999). EPS-producing bacteria have a greater ability to withstand technological stresses (Stack *et al*. 2010) and survive the passage through the gastrointestinal tract (Lindström *et al*. 2012) compared to their non-producing counterparts. Further EPSs may induce positive physiological responses including cholesterol lowering effects (Levrat-Verny and Behr 2000; Maeda *et al*. 2004), reduced formation of pathogenic biofilms (Kim *et al*. 2009), modulation of adhesion to epithelial cells (Ruas-Madiedo *et al*. 2006) and increased levels of bifidobacteria showing a prebiotic potential (Bello *et al*. 2001; Hongpattarakere *et al*. 2012). Hence the choice of EPS-producing starter cultures seems to give several advantages over non-producing ones.

With that in mind the present study was aiming at isolating EPS-producing LAB from vegetables and indigenous fermented food products of Gujarat including dhokla batter, idli batter, dahi, and cabbage. Gujarat is an Indian state populated by more than 50 million people, located in the western part of the country where fermented products are commonly consumed as a part of the traditional diet. Idlis are a naturally fermented food made from rice (*Oryza sativa*) and black gram (*Phaseolus mungo*). The ingredients are soaked separately and ground before fermentation and the batter is steamed to produce soft, spongy pancakes often eaten for breakfast (Soni and Sandhu 1989; Thakur *et al*. 1995). Dhoklas are made in a similar way however chickpea (*Cicer arietinum*) and curd are also added to the mixture (Rati Rao *et al*. 2006). Dahi is widely consumed and accounts for about 90 % of the total production of fermented milk products in India (Behare *et al*. 2009). It contains a mixed culture of *Streptococcus* and *Lactobacillus* species in addition to the yoghurt organisms *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* (Behare *et al*. 2009; Vijayendra *et al*. 2008). Further the probiotic potential of the isolated LAB strains was tested including *in vitro* tolerance to low pH and bile. Additionally bile salt hydrolase (BSH) activity, antibiotic susceptibility and antimicrobial activity were investigated.

## Materials and methods

*Screening for EPS producing bacterial strains, growth conditions and media*

Various traditional Indian fermented foods including dhokla batter, idli batter, dahi, cabbage and vegetables such as carrot, cabbage, turmeric, cucumber and tomato were used as sources of LAB. A total of 46 samples were collected from households and local markets into sterile glass bottles, stored at 4°C and processed within two hours.

Samples were homogenized at 10 % (w/v) in phosphate buffer (0.1M, pH 7.2), serially diluted and plated onto De Man, Rogosa and Sharpe agar (MRS agar, Himedia, Banglore) containing either 5 % (w/v) sucrose or glucose by following the pour plate method. Plates were incubated at 37°C for 24 to 48 h anaerobically. The strains producing mucoid or ropy colonies were recorded as capable of producing EPS and sub-cultured on fresh agar plates. Purified isolates were preserved at -20°C on MRS agar slants, in MRS broth containing 10 % glycerol (v/v) and in freeze-dried form. The isolates were propagated twice before further use.

***Phenotypic and biochemical characterization***

All isolates were Gram-stained and tested for catalase activity. Phenotypic properties such as growth at different pH and concentrations of NaCl was studied at 37°C. Growth at different temperatures was studied at 15, 37 and 45°C. Cell morphology and motility were determined using a phase contrast microscope, magnification 1000 times.

The carbohydrate fermentation patterns of the isolates were assayed using API 50 CHL test strips (BioMerieux, France). The strips were incubated at 37°C and reactions were recorded after 24 and 48 h.

***Genotypic characterization***

Genomic DNA from the isolated LAB was extracted (Sambrook and W. Russell 2001) followed by species specific 16S ribosomal RNA (rRNA) gene sequencing. The 16S rRNA coding gene was amplified through PCR using universal primers, 63F (5' -CAGGCCTAACACATGCAAGTC-3' ) and 1389R (5'-ACGGGCGGTGTGTACAAG-3') for all *Lactobacillus* and *Weissella* species*;* and 8F (5'- AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTAGGACTT-3') (Eurofins, Germany) for *Pediococcus.* The PCR reactions contained 4 µl of 5X GC reaction buffer (Finnzymes, Vantaa, Finland), 0.5 µM of each primer, 40 µM dNTP-mix (Biolab, UK), 1 U DNA polymerase (Finnzymes, Vantaa, Finland), and a 50-100 ng template. The final volume of the reaction mixture was adjusted to 40 µl using Milli Q water (Millipore). PCR amplifications were carried out in a thermal cycler (T Gradient model, Biometera, Germany) using the following steps: one cycle of denaturation for 5 min at 94°C followed by thirty cycles of 94°C for 1 min. Annealing was performed at 55°C for 30 s, extension at 72°C for 1.5 min and final extension was done at 72°C for 7 min. The PCR products were kept at 4°C and separated on a 0.8 % (w/v) agarose gel by running them at 100 V for 45 min followed by staining with ethidium bromide.

The amplicons were purified with QIAquick PCR Purification Kit (Qiagen, Germany) according to the instructions provided by the manufacturer and sent for sequencing to GATC Biotech AG (Konstanz, Germany). The 16S rRNA gene sequences were analysed with the Basic Local Alignment Search Tool (www.ncbi.nml.nih.gov 2011) and identification was performed on the basis of 16S rRNA sequence similarity with the type strains.

### *Precipitation and quantification of EPS*

A semi-defined media (Kimmel and Roberts 1998), with low amounts of EPS equivalent ingredients, was used for the production of EPS from the isolated strains. Sucrose (5 % w/v) was used as the carbon source for Lactobacilli and *Weissella* species whereas glucose (5 % w/v) was used for Pediococci. After inoculation (2 % v/v) the samples were incubated at 37°C for 24 h for *Lactobacillus* and *Weissella* isolates and 48 h for Pediococci. Cells were removed by centrifugation (15 000 g, 20 min) and the cell free supernatant was treated with 2.5 % (v/v) of 80 % (w/v) trichloroacetic acid (Merck) and the precipitated proteins were removed by centrifugation (15 000 g, 20 min). The resulting supernatant was mixed with three volumes of 95 % cold ethanol (Solveco AB, Sweden) and incubated overnight at 4°C to precipitate the EPS. The EPS was recovered by centrifugation at 4°C (15 000 g, 20 min), dried at 42°C and resuspended in distilled water. The samples were dialyzed (MW cut off 12-14,000 Da, Spectrum Lab, USA) against distilled water for 48 h changing the water six times to remove low molecular weight contaminants. Finally, the samples were freeze dried and weighed. The same procedure was performed on un-inoculated media and the weight of the resulting precipitate was subtracted from the amount of EPS produced by the LAB.

### *Antibacterial activity*

To screen the LAB isolates for antibacterial activity a well-diffusion method was used. The isolates were tested against the non pathogenic *E. coli* K12. Twenty ml of Luria bertani (LB) agar at 45°C were vigorously mixed with 50 µl overnight culture of the indicator strain and poured into petri plates. After solidification, wells were punched in the agar and filled with 100 µl of cell-free supernatant, obtained by centrifugation of the LAB cultures (4000 *g*, 15 min) followed by filter-sterilization through 0.22 µm filter (Acrodisc, USA). The plates were incubated at 37°C and 30°C for Lactobacilli/*Weissella* and Pediococci respectively for 24 h and subsequently observed for any zone of inhibition. The assay was performed three times in duplicate.

### *Acid and bile tolerance*

To determine the tolerance to acid, the LAB isolates were grown in MRS broth at 37°C overnight and sub-cultured (1.5 %) in 10 ml of fresh MRS broth adjusted to pH 3 with hydrochloric acid (1.0 M). The cultivations were incubated for 2.5 h at

37°C. Samples were withdrawn at 0 h and at the end of 2.5 h of incubation to measure the initial bacterial population and residual cell population by plating suitable dilutions on MRS agar plates. The plates were incubated at 37°C and 30°C for Lactobacilli/*Weissella* and Pediococci respectively for 48 h and the number of colonies was counted.

Oxbile and sodium taurocholate were used to evaluate bile tolerance of the isolated LAB strains. Each strain was inoculated (2 % v/v) into 10 ml MRS broth containing 0.3 % (w/v) of oxbile (Sigma, USA) or sodium taurocholate (Sigma, USA) along with a control (without bile). All the tubes were incubated at 37°C and 30°C for Lactobacilli/*Weissella* and Pediococci respectively. After 24 h of incubation the bacterial concentration was checked by viable count determination on MRS agar by plating suitable dilutions. The assays were performed three times in duplicate.

### *Bile salt hydrolase activity*

The ability of the strains to hydrolyse bile salts was determined according to the method of Dashkevicz and Feighner (1989). Test plates were prepared with 0.5 % (w/v) of taurodeoxycholic acid (TDCA) in MRS agar medium. The strains were streaked on the media and the plates were incubated anaerobically using Anaerocult$^{R}$ A (Merck, Germany) at 37°C and 30°C for Lactobacilli/*Weissella* and Pediococci respectively for 48-72 h. The presence of precipitated bile acid around colonies (opaque halo) was considered as a positive result (Dashkevicz and Feighner 1989). The experiment was performed three times in duplicate.

### *Antibiotic susceptibility*

The antibiotic susceptibility of the LAB isolates was determined using Neo-Sensitabs (Rosco Diagnostica, Taastrup, Denmark) antibiotic discs for ampicillin (10 μg), erythromycin (15 μg), chloramphenicol (30 μg), norfloxacin (10 μg), polymyxin (150 μg), vancomycin (30 μg), gentamicin (10 μg), tetracycline (30 μg), and kanamycin (30 μg). Fifty microliters of the actively grown culture containing about $10^5$-$10^6$ CFU/ml were spread evenly on the surface of the MRS agar plate and kept in static condition for 15 min before placing the diffusion discs. The plates were incubated at 37°C and 30°C for Lactobacilli/*Weissella* and Pediococci respectively for 24-48 h and inhibition zones were measured inclusive of the diameter of the discs and the results were expressed as sensitive, S; intermediate, I and resistant, R according to the instructions given by the manufacturer. The assay was performed three times in duplicate.

## Results and discussion

### *Isolation, screening for EPS production and identification of isolates*

A total of 46 food products such as tomato, cucumber, turmeric, carrot, fresh and

fermented cabbage, dahi, idli batter and dhokla batter were sampled for EPS producing LAB. Out of 203 isolates, a total of 17 isolates were selected for further studies based on their ability to produce mucoid or ropy colonies on MRS agar plates supplemented with either sucrose or glucose as carbon source. All selected isolates (both rods and cocci) were found to be Gram positive, catalase negative bacteria which were non-motile and did not form spores. All isolates were able to grow at 37°C, pH 4 and 6.5 and a concentration of 2 and 4 % NaCl. Growth at 15 and 45°C, pH 2 and 6.5 % NaCl was differing between strains (Table 1). The strains identified as Pediococci were producing EPS when grown on glucose (5 % w/v), while all other LAB isolates were producing EPS on sucrose (5 % w/v). Isolates were identified as *L. fermentum* (7 strains), *L. plantarum* (2 strains), *W. cibaria* (5 strains), *W. confusa* (1 strain) and *P. parvulus* (2 strains) on the basis of 16S rRNA sequence similarity with the type strains (Table 1). However it should be noted that *W. cibaria* and *W. confusa* cannot be distinguished from each other based on only 16S rRNA sequence similarity (> 99 % shared sequences) (Björkroth *et al*. 2002). The sequences determined in this study have been deposited in the GenBank database with accession numbers JN792454-70. The isolation of *Weissella* from idli batter and dahi is to the best of our knowledge reported for the first time and so is the isolation of Pediococci from idli batter.

***EPS Quantification***

Lactobacilli are known to produce both hetero- (De Vuyst and Degeest 1999; Ismail and Nampoothiri 2010) and homopolysaccharides (Kralj *et al*. 2004; Van der Meulen *et al*. 2007). EPS-producing *L. plantarum* has been isolated from various food sources such as starter dough for Chinese steamed buns (Luangsakul *et al*. 2009), corn silage (Tallon *et al*. 2003), curd (Ismail and Nampoothiri 2010), turmeric (Pianpumepong and Noomhorm 2010) and bamboo shots (Chen *et al*. 2010). *Lactobacilli* are reported to produce 1-10 g of EPS per litre growth media (Ruas-Madiedo and de los Reyes-Gavilán 2005) which is more than what was found in the present study (< 1 g, Table 1). The isolated *L. fermentum* strains displayed mucoid colonies on sucrose and it is known that it produces an extracellular glycosyltransferase enzyme that synthesizes large á-glucans from sucrose (Kralj *et al*. 2004).

EPS-producing *Weissella* species are commonly isolated from fermented foods like sour cream (Van der Meulen *et al*. 2007), soya (Malik *et al*. 2009), sourdough (Galle *et al*. 2010) and Thai pork sausage (Wongsuphachat *et al*. 2010). *Weissella* species produce EPS in amounts of a few grams (Galle *et al*. 2010) up to 18 g/l under optimized conditions (Wongsuphachat *et al*. 2010). In the present study, all isolates of *Weissella* spp. produced on an average 500 mg of EPS per litre (dry mass basis) in a semi-defined medium which is less than reported previously; however no optimization of growth conditions were made.

Table 1: Genetic and phenotypic characterization of LAB isolates

| Isolate | Source of isolation | Identity based on 16S rRNA | Number of base pairs | % sequence similarity | Gene accession number | Growth temperature | | Growth at pH 2 | Growth at 6.5 % NaCl | EPS (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 15°C | 45°C | | | |
| AD1 | dahi | *L. fermentum* | 968 | 98 | JN792470 | - | + | + | - | 360 |
| AI2 | dhokla batter | *L. fermentum* | 1017 | 99 | JN792468 | + | + | + | + | 570 |
| AI3 | dhokla batter | *L. fermentum* | 1137 | 99 | JN792457 | + | + | + | + | 280 |
| AV2 | fermented cabbage | *L. fermentum* | 1034 | 98 | JN792461 | - | + | + | - | 250 |
| AV3 | carrot | *L. fermentum* | 1041 | 98 | JN792462 | - | + | + | + | 600 |
| AV4 | cabbage | *L. fermentum* | 1031 | 98 | JN792463 | - | + | + | - | 680 |
| 138 | fresh turmeric | *L. fermentum* | 1134 | 99 | JN792459 | - | + | + | - | 350 |
| AD29 | dahi | *L. plantarum* | 985 | 99 | JN792465 | - | + | + | + | 390 |
| 86 | dahi | *L. plantarum* | 1027 | 98 | JN792454 | - | + | + | + | 960 |
| AI10 | idli batter | *W. confusa* | 1077 | 99 | JN792460 | - | + | + | - | 610 |
| AV1 | fermented cabbage | *W. cibaria* | 1151 | 98 | JN792467 | - | + | + | + | 500 |
| 85 | dahi | *W. cibaria* | 1109 | 99 | JN792458 | - | + | + | - | 570 |
| 92 | idli batter | *W. cibaria* | 1109 | 98 | JN792466 | - | + | + | + | 570 |
| 142 | cucumber | *W. cibaria* | 1141 | 98 | JN792456 | - | + | + | - | 570 |
| 145 | cabbage | *W. cibaria* | 938 | 99 | JN792455 | - | + | + | - | 480 |
| AI1 | idli batter | *P. parvulus* | 1100 | 99 | JN792469 | + | - | - | - | 470 |
| AV5 | tomato | *P. parvulus* | 817 | 99 | JN792464 | + | - | - | - | 410 |

Per cent sequence similarity with type strains *L. fermentum* CIP 102980[T], *L. fermentum* OMZ 1117[T], *L. fermentum* IFO 3956[T], *L. plantarum* CIP 103151[T], *W. confusa* JCM 10931[T], *W. cibaria* NRIC 0136[T] and *P. parvulus* DSM 20331[T]
+, growth observed; -, no growth observed
bp, base pairs

Isolates AI1 and AV5 were displaying a ropy phenotype in agreement with earlier findings about *P. parvulus* (Dueñas *et al*. 1994). Previously it has been reported that *P. parvulus* produces a ropy EPS at a concentration of 100 mg/l after prolonged incubation (Duenas *et al*. 2003) which is less than strain AI1 and AV5 that produced 470 and 410 mg/l of EPS, respectively.

### *Antibacterial activity*

All investigated isolates were showing an antagonistic activity against the non-pathogenic *E. coli* K12 (Table 2). Interestingly the antimicrobial activity was differing within a species where *W. cibaria* AV1 showed the least activity (14.5 mm) and *W. cibaria* 142 the most activity (23.5 mm) of all tested isolates. Antimicrobial activities attributable to the production of bacteriocins by LAB such as *Lactobacillus* (Yanagida *et al*. 2006), *Weissella* (Srionnual *et al*. 2007), and *Pediococcus* (Schneider *et al*. 2006) species have been reported. However, the antimicrobial activity could also be associated with the accumulation of organic acids, fatty acids, diacetyl and hydrogen peroxide (Dunne *et al*. 2001). The substances that are responsible for the antimicrobial activities detected in the present study remains to be identified.

**Table 2:** Investigation of antimicrobial activity against *E. coli* K12

| Isolate | Species | Zone of Inhibition (mm) |
|---|---|---|
| AD1 | *L. fermentum* | 17.2 ± 0.76 |
| AI2 | *L. fermentum* | 20.3 ± 0.58 |
| AI3 | *L. fermentum* | 16.2 ± 1.04 |
| AV2 | *L. fermentum* | 23.2 ± 0.76 |
| AV3 | *L. fermentum* | 22.8 ± 0.76 |
| AV4 | *L. fermentum* | 20.3 ± 0.76 |
| 138 | *L. fermentum* | 20.2 ± 0.76 |
| AD29 | *L. plantarum* | 23.2 ± 0.76 |
| 86 | *L. plantarum* | 22.2 ± 0.76 |
| AI10 | *W. confusa* | 21.8 ± 1.26 |
| AV1 | *W. cibaria* | 14.5 ± 0.50 |
| 85 | *W. cibaria* | 18.8 ± 1.04 |
| 92 | *W. cibaria* | 23.2 ± 0.76 |
| 142 | *W. cibaria* | 23.5 ± 1.32 |
| 145 | *W. cibaria* | 20.5 ± 0.50 |
| AI1 | *P. parvulus* | 19.7 ± 1.04 |
| AV5 | *P. parvulus* | 20.2 ± 0.76 |

Values are presented are means ± standard deviation of three experiments each performed in duplicate

* Isolates chosen for further characterization

### *Acid and bile tolerance*

Eight isolates were showing tolerance to acid and bile and their rate of survival is presented in Table 3. The selected isolates were including two strains of each *L. plantarum* (86 & AD29), *L. fermentum* (AI2 & AI3), and *W. cibaria* (92 & 142) and one strain of *W. confusa* (AI10) and *P. parvulus* AI1. The isolates chosen were compared concerning their carbohydrate fermentation pattern to make sure that they were different (result not shown). The *L. plantarum* isolates showed different performance at low pH. The strain 86 remained unaffected in acidic condition while strain AD29 showed a reduction in the viable count to 49 % of initial numbers after exposure to acid for 2.5 h (Table 3). An even larger difference was found between *Weissella* isolates where *W. cibaria* 92 was very sensitive to acidic conditions (16 % survival) whereas *W. cibaria* 142 showed an increase in the viable count indicating a high tolerance to acid for this isolate.

**Table 3:** Survival of the LAB isolates after exposure to low pH and oxgall, and their BSH activity

| Isolate | Species | Oxgall (% survival) | pH 3 | BSH activity (% survival) |
|---|---|---|---|---|
| 86 | *L. plantarum* | 32 | 98 | - |
| AD29 | *L. plantarum* | 14 | 49 | - |
| AI2 | *L. fermentum* | 22 | 76 | + |
| AI3 | *L. fermentum* | 36 | 84 | + |
| 92 | *W. cibaria* | 33 | 16 | + |
| 142 | *W. cibaria* | 54 | 131 | + |
| AI10 | *W. confusa* | 72 | 90 | ± |
| AI1 | *P. parvulus* | 61 | 76 | + |

The % survival is means of three experiments each performed in duplicate
+, Occurrence of opaque halo; -, no opaque halo

Bile functions as a biological detergent emulsifying lipids when released into the duodenum. It contains among other things bile acids (Begley et al. 2006), and the bile acid resistance is currently studied for potential probiotics (FAO/WHO 2002). It was found that none of the isolates were growing in the presence of 0.3 % oxbile as there was no increase in OD. However all of the isolates were able to survive in the presence of oxbile (0.3 %) as presented in Table 3 where *W. confusa* AI10, *P. parvulus* AI1 and *W. cibaria* 142 were showing the highest survival rates. The lowest rate of survival was shown by *L. plantarum* AD29 (14 %). All isolates except *L. plantarum* AD29 (67 % survival rate) were able to grow in 0.3 % sodium taurocholate (results not shown). AD29 seems to be sensitive to bile salts and low pH hence not showing any probiotic potential. The acid and bile tolerance varied between isolates within the same species which has been observed previously both for Lactobacilli (Gu et al. 2008) and *Weissella* (Lee et al. 2012).

**Table 4:** Antibiotic susceptibility of LAB isolates

| Isolate | Species | Erythromycin (15µg) | Vancomycin (30µg) | Norfloxacin (10µg) | Chloramphenicol (30µg) | Polymyxin (150µg) | Ampicillin (10µg) | Gentamicin (10µg) | Tetracycline (30µg) | Kanamycin (30µg) |
|---|---|---|---|---|---|---|---|---|---|---|
| 86 | *L. plantarum* | S | R | R | S | R | S | R | S | R |
| AD29 | *L. plantarum* | S | R | R | S | I | S | R | S | R |
| AI2 | *L. fermentum* | S | R | R | S | R | S | R | S | R |
| AI3 | *L. fermentum* | S | R | R | S | I | S | R | S | R |
| 92 | *W. cibaria* | S | R | R | S | R | S | R | S | R |
| 142 | *W. cibaria* | S | R | R | S | R | S | R | S | R |
| AI10 | *W. confusa* | S | R | R | S | I | S | I | S | R |
| AI1 | *P. parvulus* | S | R | R | S | S | S | R | S | R |

S, sensitive; I, intermediate; R, resistant

***Bile salt hydrolase activity***

It is believed that BSH activity is a preferable trait for probiotics since the detoxification of bile salts by BSH enzymes may lead to increased survival rates for the microorganism in question (Begley et al. 2006). In the present study *L. fermentum* AI2 and AI3, *P. parvulus* AI1, and *W. cibaria* 142 and 92 were found to be BSH-positive. One of the isolates, *W. confusa* AI10 showed poor BSH activity while both *L. plantarum* isolates (86 and AD29) were showing a BSH negative trait. The BSH activity seems to be species related in this case, however BSH activity for *L. plantarum* has been reported previously as well as for *L. fermentum*. There may be pronounced variations in BSH activity within a genera and between strains (Tanaka et al. 1999).

In 2006 Begley et al. reported that BSH activity had not been found in bacteria isolated from environments without bile salt. However since then, BSH positive isolates have been found in food. Recently it has been reported that 90 % of their isolated Pediococci and all Lactobacilli from fermented olives were BSH positive (Abriouel et al. 2012). In the present study 5 (out of 8 tested) BSH positive strains were isolated from foods such as dhokla- and idli batter and cucumber. It is to the best of our knowledge the first report on BSH activity in the genus *Weissella*.

***Antibiotic susceptibility***

The transmission of antibiotic resistance genes from probiotics to pathogenic bacteria may occur and thus it is important to screen strains for the absence of transferable resistance genes. Levels of susceptibility that cannot be accounted for should be further investigated (Danielsen and Wind 2003). All isolates were sensitive against chloramphenicol, erythromycin, ampicillin and tetracycline and resistant to vancomycin, norfloxacin and kanamycin (Table 4). All were resistant to gentamicin, but AI10 that showed an intermediate response. The susceptibility for polymyxin was different between isolates. The present results for *L. plantarum* are in agreement with Danielsen et al. (2003) except for gentamicin however the concentration of that antibiotic in the disc used in this study was lower than the minimal inhibition concentration reported in the previous study (Danielsen and Wind 2003). Susceptibilities for the *Weissella* isolates are in agreement with previous findings (Lee et al. 2012). *P. parvulus* AI1 was resistant to gentamicin in contrary to earlier findings (Danielsen et al. 2007). Plasmid bound resistance genes may be present in Pediococci and thus strain AI1 should be further investigated in this matter.

## Conclusion

This study demonstrates the diversity of potential probiotic LAB in dairy and non-dairy food products from the western part of India. The antibacterial activity exhibited by these isolates may be useful to control undesirable contaminations in food systems which render them interesting functional starters. Out of the investigated isolates *L. plantarum* 86, *W. confusa* AI10, *P. parvulus* AI1 and especially *W. cibaria* 142 were showing interesting probiotic characteristics such as high tolerance to acid

and bile and antimicrobial activity against *E. coli*. Isolates 142 and AI1 also showed BSH activity. Since results obtained by *in vitro* studies may not reflect probiotic performance *in vivo*, the most potential strains should be subjected to further *in vitro* and *in vivo* investigations before they may be considered probiotics.

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**References**

Abriouel H, Benomar N, Cobo A, Caballero N, Fuentes MAF, Perez-Pulido R, Galvez A (2012). Characterization of lactic acid bacteria from naturally-fermented Manzanilla Alorena green table olives. *Food Microbiology* 32(**2**):308-316

Badel S, Bernardi T, Michaud P 2011. New perspectives for *Lactobacilli* exopolysaccharides. *Biotechnology Advances* **29:**54-66

Begley M, Hill C, Gahan CGM (2006). Bile salt hydrolase activity in probiotics. *Applied and Environmental Microbiology* **72(3)**:1729-1738

Behare P, Singh R, Singh RP (2009). Exopolysaccharide-producing mesophilic lactic cultures for preparation of fat-free Dahi - an Indian fermented milk. *Journal of Dairy Research* **76**(1):90-97

Bello FD, Walter J, Hertel C, Hammes, W.P. 2001. In vitro study of prebiotic properties of levan-type exopolysaccharides from Lactobacilli and non-digestible carbohydrates using denaturing gradient gel electrophoresis. *Systematic and Applied Microbiology* **24(2)**:232-237

Björkroth KJ, Schillinger U, Geisen R, Weiss N, Hoste B, Holzapfel WH, Korkeala HJ, Vandamme P (2002). Taxonomic study of *Weissella confusa* and description of *Weissella cibaria* sp. nov., detected in food and clinical samples. *International Journal of Systematic and Evolutionary Microbiology* **52:**141-148

Bukowska H, Pieczul-Mróz J, Jastrzebska M, Chelstowski K, Naruszewicz M 1998. Decrease in fibrinogen and LDL-cholesterol levels upon supplementation of diet with Lactobacillus plantarum in subjects with moderately elevated cholesterol *Atherosclerosis* **137:**437-438

Chen Y-s, Wu H-c, Liu C-h, Chen H-c, Yanagida F 2010. Isolation and characterization of lactic acid bacteria from jiang-sun (fermented bamboo shoots), a traditional fermented food in Taiwan. *Journal of the Science of Food and Agriculture* 90(**12**):1977-1982

Danielsen M, Simpson PJ, O'Connor EB, Ross RP, Stanton C (2007). Susceptibility of Pediococcus spp. to antimicrobial agents. *Journal of Applied Microbiology* 102(**2**):384-389

Danielsen M, Wind A 2003. Susceptibility of Lactobacillus spp. to antimicrobial agents. *International Journal of Food Microbiology* 82(**1**):1-11

Dashkevicz MP, Feighner SD (1989). Development of a differential medium for bile salt hydrolase-active Lactobacillus spp. *Applied and Environmental Microbiology* 55(**1**):11-16

De Vuyst L, Degeest B 1999. Heterpolysaccharides from lactic acid bacteria. *FEMS Microbiology Reviews* **23:**153-177

Dueñas M, Irastorza A, Fernandez K, Bilbao A. 1994. Heterofermentative *Lactobacilli* causing ropiness in basque country ciders. *Journal of Food Protection,* 58(**1**):76-80.

Duenas, M., Munduate, A., Perea, A., Irastorza, A 2003. Exopolysaccharide production by *Pediococcus damnosus* 2.6 in a semidefined medium under different growth conditions. *International Journal of Food Microbiology* 87(**1-2**):113-120

Dunne, C., O'Mahony, L., Murphy, L., Thornton, G., Morrissey D, O'Halloran S, Feeney M, Flynn S, Fitzgerald G, Daly C, Kiely B, O 'Sullivan GC, Shanahan F, Collins JK (2001). In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. *American Journal of Clinical Nutrition* 73:386S-392S

FAO/WHO 2002. Guidelines for the evaluation of probiotics in food. London, Ontario

Galle, S., Schwab, C., Arendt, E., Ganzle, M. 2010. Exopolysaccharide-forming *Weissella* strains as starter cultures for sorghum and wheat sourdoughs. *Journal of Agricultural and Food Chemistry* **58**(**9**):5834-5841

Garai-Ibabe G, Duenas MT, Irastorza A, Sierra-Filardi E, Werning ML, Lopez P, Corbi AL, Fernandez de Palencia, P. 2010. Naturally occurring 2-substituted (1,3)-â-D-glucan producing *Lactobacillus suebicus* and *Pediococcus parvulus* strains with potential utility in the production of functional foods. *Bioresource Technology,* **101**(23):9254-9263.

Gu R.X., Yang Z.Q., Li Z.H., Chen S.L., Luo Z.L. (2008). Probiotic properties of lactic acid bacteria isolated from stool samples of longevous people in regions of Hotan, Xinjiang and Bama, Guangxi, China. *Anaerobe* **14**(6):313-317.

Hongpattarakere, T., Cherntong, N., Wichienchot, S., Kolida, S., Rastall, R.A. 2012. *In vitro* prebiotic evaluation of exopolysaccharides produced by marine isolated lactic acid bacteria. *Carbohydrate Polymers* **87:**846-852.

Ismail, B., Nampoothiri, K.M. 2010. Production, purification and structural characterization of an exopolysaccharide produced by a probiotic *Lactobacillus plantarum* MTCC 9510. *Archives of Microbiology* 192(**12**):1049-1057.

Kim Y, Oh, S., Kim, S.H. 2009. Released exopolysaccharide (r-EPS) produced from probiotic bacteria reduce biofilm formation of enterohemorrhagic *Escherichia coli* O157:H7. *Biochemical and Biophysical Research Communications* 379:324-329.

Kimmel, S.A., Roberts, R.F. 1998. Development of a growth medium suitable for exopolysaccharide production by *Lactobacillus delbrueckii* ssp. *bulgaricus* RR. *International Journal of Food Microbiology* 40(**1-2**):87-92.

Kralj S, van Geel-Schutten GH, Dondorff MMG, Kirsanovs S, van der Maarel MJEC, Dijkhuizen L 2004. Glucan synthesis in the genus *Lactobacillus*: isolation and characterization of glucansucrase genes, enzymes and glucan products from six different strains. *Microbiology (Reading)* 150(**11**):3681-3690.

Lee, K.W., Park, J.Y., Jeong, H.R., Heo, H.J., Han, N.S., Kim, J.H. 2012. Probiotic properties of *Weissella* strains isolated from human faeces. *Anaerobe* 18(**1**):96-102.

Levrat-Verny, M.A., Behr, S. 2000. Low levels of viscous hydrocolloids lower plasma cholesterol in rats primarily by impairing cholesterol absorption. *Journal of Nutrition* 130(**2**):243-249.

Lindström, C., Holst, O., Nilsson, L., Öste, R., Andersson, K.E. 2012. Effects of *Pediococcus parvulus* 2.6 and its exopolysaccharide on plasma cholesterol levels and inflammatory markers in mice. *AMB Express* 2(**66**).

Luangsakul, N., Keeratipibul, S., Jindamorakot, S., Tanasupawat, S. 2009. Lactic acid bacteria and yeasts isolated from the starter doughs for Chinese steamed buns in Thailand. *LWT - Food Science and Technology,* 42(**8**):1404-1412.

Maeda, H., Xia, Z., Omura, K. 2004. Effects of an exopolysaccharide (kefiran) on lipids, blood pressure, blood glucose, and constipation. *Biofactors* 22(**1-4**):197-201.

Malik A., Radji, M., Kralj, S., Dijkhuizen, L. 2009. Screening of lactic acid bacteria from

Indonesia reveals glucansucrase and fructansucrase genes in two different *Weissella confusa* strains from soya. *FEMS Microbiology Letters* 300(**1**):131-138

Mathara, J.M., Schillinger, U., Kutima, P.M., Mbugua, S.K., Guigas, C., Franz, C., Holzapfel WH 2008. Functional properties of *Lactobacillus plantarum* strains isolated from Maasai traditional fermented milk products in Kenya. *Current Microbiology* 56(**4**):315-321.

Pianpumepong, P., Noomhorm, A. 2010. Isolation of probiotic bacteria from turmeric (*Curcuma longa* Linn.) and its application in enriched beverages. *International Journal of Food Science & Technology* 45(**12**):2456-2462.

Rati, Ra.o E., Vijayendra, S.V.N., Varadaraj, (eds) 2006. Fermentation biotechnology of traditional foods of the Indian subcontinent. Taylor and Francis Group, LCC

Ruas-Madiedo, P., de los Reyes-Gavilán, C.G. 2005. Invited Review: methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria. *Journal of Dairy Science,* **88:**843-856.

Ruas-Madiedo, P., Gueimonde, M., Margolles, A., De los Reyes-Gavilán Clara, G., Salminen S (2006). Exopolysaccharides produced by probiotic strains modify the adhesion of probiotics and enteropathogens to human intestinal mucus. *Journal of Food Protection* 69(**8**):2011-2015.

Saad, N., Delattre, C., Urdaci, M, Schmitter, J.M., Bressollier, P. 2013. An overview of the last advances in probiotic and prebiotic field. *LWT - Food Science and Technology* 50(**1**):1-16.

Sambrook, J., W. Russell, D. 2001. Molecular cloning: a laboratory manual. *Quarterly Review of Biology* 76(**3**):348-349.

Schneider, R., Fernández FJ, Aguilar MB, Guerrero-Legarreta I, Alpuche-Solís A, Ponce-Alquicira, E. 2006. Partial characterization of a class IIa pediocin produced by *Pediococcus parvulus* 133 strain isolated from meat (Mexican "chorizo"). *Food Control* 17:909-915

Soni, S.K., Sandhu, D.K. 1989. Fermentation of Idli: Effects of changes in raw material and physico-chemical conditions. *Journal of Cereal Science* 10(**3**):227-238

Srionnual, S., Yanagida, F., Lin L-H, Hsiao K.N., Chen, Y.S. 2007. Weissellicin 110, a newly discovered bacteriocin from *Weissella cibaria* 110, isolated from Plaa-Som, a fermented fish product from Thailand. *Applied and Environmental Microbiology* 73(**7**):2247-2250.

Stack, H.M., Kearney, N., Stanton, C., Fitzgerald Gerald, F., Ross, R.P. 2010. Association of beta-glucan endogenous production with increased stress tolerance of intestinal Lactobacilli. *Applied and Environmental Microbiology,* **76**(**2**):500-507.

Tallon,R., Bressollier, P., Urdaci, M.C. 2003. Isolation and characterization of two exopolysaccharides produced by *Lactobacillus plantarum* EP56. *Research in Microbiology* 154(**10**):705-712.

Tanaka, H., Doesburg, K., Iwasaki, T., Mierau, I. 1999. Screening of lactic acid bacteria for bile salt hydrolase activity. *Journal of Dairy Science,* **82**(**12**):2530-2535.

Thakur S, Prasad MS, Rastogi NK 1995. Effect of xanthan on textural properties of idli (traditional south Indian food). *Food Hydrocolloids,* 9(**2**):141-145.

Van der Meulen, R., Grosu-Tudor, S., Mozzi, F., Vaningelgem, F., Zamfir, M., Font de Valdez G, De Vuyst, L. 2007. Screening of lactic acid bacteria isolates from dairy and cereal products for exopolysaccharide production and genes involved. *International Journal of Food Microbiology,* 118(**3**):250-258.

Vijayendra, S.V.N., Palanivel, G., Mahadevamma, S., Tharanathan, R.N. 2008. Physico-chemical characterization of an exopolysaccharide produced by a non-ropy strain of *Leuconostoc* sp. CFR 2181 isolated from dahi, an Indian traditional lactic fermented milk product. *Carbohydrate Polymers,* **72**(**2**):300-307.

Wongsuphachat W, Kittikun A, H., Maneerat S 2010. Optimization of exopolysaccharides production by *Weissella confusa* NH 02 isolated from Thai fermented sausages. *Songklanakarin Journal of Science and Technology* 32(**1**):27-35

www.ncbi.nml.nih.gov (2011) BLAST. PUblisher.

Yanagida F, Chen YS, Shinohara T 2006. Searching for bacteriocin-producing lactic acid bacteria in soil. *Journal of General and Applied Microbiology* 52(**1**):21-28.