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Functional properties of lactic acid bacteria isolated from ethnic fermented vegetables of the Himalayas

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ABSTRACT

A total of 94 strains of Lactic acid bacteria (LAB), previously isolated from ethnic fermented vegetables and tender bamboo shoots of the Himalayas, were screened for functional properties such as acidification capacity, enzymatic activities, degradation of antinutritive factors and oligosaccharides, production of biogenic amines, hydrophobicity and adherence to mucus secreting HT29 MTX cells. Strong acidification and coagulation activities of LAB strains were recorded. Most of the LAB strains showed antimicrobial activities against the used indicator strains; however, only *Lb. plantarum* IB2 (BFE 948) isolated from inziangsang, a fermented leafy vegetable product, produced a bacteriocin against *Staphylococcus aureus* S1. LAB strains showed enzymatic activities and also degraded oligosaccharides. Almost all the strains of LAB were non-producers of biogenic amines except few strains. Some strains of *Lb. plantarum* showed more than 70% hydrophobicity. Adherence to the mucus secreting HT29 MTX cells was also shown by seven strains indicating their probiotic nature.

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1. Introduction

Lactic acid bacteria (LAB) are the dominant microorganisms in ethnic fermented vegetables and bamboo shoot products of the Himalayas (Tamang, 2009). Gundruk, sinki and khalpi are lactic fermented vegetable products of Nepal, Darjeeling hills, Sikkim and Bhutan (Tamang et al., 1988), and inziangsang is a fermented leafy vegetable product of Nagaland and Manipur (Tamang and Tamang, 2009). The LAB involved in these ethnic fermented vegetables were identified as *Lactobacillus brevis*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Pediococcus acidilactici* and *Leuconostoc fallax* (Tamang et al., 2005). Predominant functional LAB strains associated with the ethnic fermented tender bamboo shoot products, mesu, soidon, soibum and soijim of the Himalayas, were identified as *Lb. brevis*, *Lb. plantarum*, *Lb. curvatus*, *P. pentosaceus*, *Leuc. mesenteroides* subsp. *mesenteroides*, *Leuc. fallax*, *Leuc. lactis*, *Leuc. citreum* and *Enterococcus durans* (Tamang et al., 2008). Some of the LAB strains may also possess protective and functional properties, which render them interesting candidates for use as starter culture(s) for controlled and optimised production of fermented vegetable products. Though ethnic fermented foods are widely prepared and consumed in the Himalayas, application of standard starter culture is not a tradition except for production of alcoholic beverages (Thapa and Tamang, 2004). The functional properties of LAB are important for selection of appropriate strains to be used as starter culture (Durlu-Ozkaya et al., 2001; Badis et al., 2004). Industrialised countries have benefited from a century of experience with starter cultures, particularly selected for large scale food fermentation (Holzapfel et al., 1998). The present paper aims to study the functional properties of LAB from ethnic fermented vegetables and tender bamboo shoots which include acidifying and coagulating capacity, antimicrobial activities, degradation of phytic acid, utilization of oligosaccharides, and adherence to hydrocarbons and intestinal mucosa.

2. Materials and methods

2.1. LAB strains studied

A total of 94 strains of LAB were studied for various functional properties. Out of which, 38 strains were previously isolated from ethnic fermented vegetables of the Himalayas: gundruk (12 strains), sinki (10), khalpi (12) and inziangsang (4); and 56 strains were isolated from the Himalayan fermented bamboo shoot products: mesu (35 strains), soibum (7), soidon (7), and soijim (7). These LAB strains were phenotypically and genotypically identified as *Lb. brevis*, *Lb. plantarum*, *Lb. curvatus*, *P. pentosaceus*, *P. acidilactici*, *Leuc. mesenteroides* subsp. *mesenteroides*, *Leuc. fallax*, *Leuc. lactis*, *Leuc. citreum* and *E. durans* (Tamang et al., 2005, 2008).

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2.2. Acidification and coagulation

Acidification and coagulation properties were assayed by inoculating 10% skim milk (RM1254, HiMedia, India) with LAB strains. Observation was made for commencement of clotting, and the pH was measured after 72 h of incubation at 30 °C (Olasupo et al., 2001).

2.3. Antimicrobial activity

The LAB strains were screened for antimicrobial activity by the agar spot method of Schillinger and Lücke (1989). The indicator strains used for antagonisms included: *Listeria innocua* DSM 20649, *L. monocytogenes* DSM 20600, *Bacillus cereus* CCM 2010, *Staphylococcus aureus* S1, *Enterococcus faecium* DSM 20477, *Streptococcus mutans* DSM 6178, *Klebsiella pneumoniae* subsp. *pneumoniae* BFE 147, *Enterobacter cloacae* BFE 282, *E. agglomerans* BFE 154 and *Pseudomonas aeruginosa* BFE 162. Cell-free neutralised supernatant fluids of LAB strains were screened for antimicrobial activity by the agar spot test (Uhlman et al., 1992), using the bacteriocin screening medium (MRS agar containing only 0.2% glucose) as described by Tichaczek et al. (1992).

Bacteriocin activity was quantified based on an agar diffusion assay as described by Cabo et al. (1999). The twofold serial dilutions of the neutralised supernatants are spotted (10 µl) onto the surface of MRS or Standard I Nutrient agar containing the required indicator bacteria. Bacteriocin titres were expressed as the reciprocal of the highest dilution exhibiting a zone of inhibition and were reported in activity units (AU) per ml.

2.4. Enzymatic activities

The enzymatic activities of LAB strains were assayed using the commercial API-zym (bioMérieux, France) galleries. Cultures were grown on MRS agar and cells were harvested in 2 ml sterile normal saline which was used to prepare a suspension of 10^7 cells/ml. The strip was unpacked and 2 drops of cell suspensions were inoculated in each cupule of the strip containing ready-made enzyme substrates and incubated at 30 °C for 6 h. After incubation, 1 drop of ready-made zym-A and zym-B reagents was added and observed for colour development based on the manufacturer's colour chart. A value ranging from 0–5 were assigned, corresponding to the colours developed: 0 corresponds to a negative reaction, 5 (= 40 nanomoles) to a reaction of maximum intensity, and values 4, 3, 2 and 1 were intermediate reactions corresponding to 30, 20, 10 and 5 nanomoles, respectively.

2.5. Phytic acid degradation

Ability of LAB strains to degrade phytic acid was determined on a synthetic phytic acid screening medium (Holzapfel, 1997), containing calcium phytate (Sigma, USA) as sole phosphate source. Control was prepared without calcium phytate. In preparing the medium, phytate and salts are added separately. After adding glucose, sodium-citrate, magnesium sulfate, manganese sulfate and ferrous sulfate to the phytate solution, the pH was adjusted to 6.0 and the medium was autoclaved. Vitamins, amino acids and nucleotides were filter sterilised and added to the medium before plating. The pH of the medium was finally adjusted around 5.8–6.0. The plates were streaked with 24 h-old broth culture and incubated aerobically at 30 °C for 5 days. A clear zone around the colony of the test organism indicated a positive reaction.

2.6. Degradation of oligosaccharides

Abilities of LAB strains to degrade stachyose and raffinose were performed in MRS broth without beef extract (pH 6.4) containing 2% stachyose and 1% raffinose (instead of glucose), respectively, and 0.004% chlorophenol red as indicator. Inoculation was followed by incubation at 30 °C for 3 days (Holzapfel, 1997).

2.7. Biogenic amines

The ability of LAB strains to produce biogenic amines was determined qualitatively in screening medium (Bover-Cid and Holzapfel, 1999) using a ‘cocktail’ of four precursor amino acids (histidine, lysine, ornithine and tyrosine). Change of the bromocresol purple indicator to purple was considered as index of significant amino acid decarboxylase activity, corresponding to >350 mg of a particular amino acid/litre (Olasupo et al., 2001).

2.8. Hydrophobicity assay

Bacterial adhesion to hydrocarbons was determined and results were expressed according to Rosenberg (1984), modified as follows. Fresh cultures were grown in MRS broth at 30 °C for 24 h and centrifuged at 8000 ×g for 5 min. The pellet was washed three times with 9 ml of quarter strength Ringer's Solution (QSRS) (Merck, Germany), and thoroughly mixed in a vortex. 1 ml of the suspension was taken and the absorbance at 580 nm was measured. Then, 1.5 ml of the suspension was mixed with equal volume of n-hexadecane (RM 2238, HiMedia, India) in duplicates and mixed thoroughly in a vortex. The phases were allowed to separate for 1 h at room temperature, after which the aqueous phase was carefully removed and absorbance at 580 nm was measured. The percentage hydrophobicity was expressed as follows: hydrophobicity $\% = [A_0 - A / A] \times 100$, where A_0 and A are the absorbance values of the aqueous phase before and after contact with n-hexadecane hydrophobicity determinations were done in three replicates. The percent hydrophobic index greater than 70% was arbitrarily classified as hydrophobic (Martin et al., 1989; Nostro et al., 2004).

2.9. In vitro adherence assay

Mucus secreting HT29 MTX cells (clone 5M21) obtained from Dr. Thecla Lessuffleur (INSERM U178, Villejuif, France) were used. Cells were routinely grown in Dulbecco's modified Eagles minimal essential medium supplemented with 10% fetal calf serum and 100 µg streptomycin/ml and 100 U penicillin/ml at 37 °C in a 10% CO_2 atmosphere. For adhesion assay, HT29 monolayers were prepared in 24-well tissue plates. Cells were inoculated at a concentration of 7×10^4 cells per well to obtain confluence and cultured for 21 days prior to the adhesion assay. Cell culture medium was changed on alternate days, and the last two media changes were without penicillin/streptomycin. Overnight cultures of lactobacilli grown in Dulbecco's modified Eagles medium (DMEM) supplemented with 2% FCS were centrifuged, washed with Ringer's Solution (see above) and resuspended in DMEM. Viable counts were determined by plating on MRS agar. A 1 ml aliquot of the bacterial solution (adjusted to 1×10^8 cfu/ml) was added to each well of the tissue culture plate. The plates were centrifuged at 2000 ×g for 2 min and incubated in a 10% CO_2 atmosphere. After 1 h of incubation, viable counts of the supernatants were determined by plating serial dilutions on MRS agar. Cells were lysed by addition of Triton X 100 (0.05% solution) and appropriate dilutions were again plated on MRS agar. Adhesion was calculated from the initial viable counts, those of the supernatants and those of the cell lysates. Each determination was done in triplicates. The known probiotic strain *Lactobacillus rhamnosus* GG was used as control.

3. Results

3.1. Acidification

About 38% of LAB strains showed strong acidification properties by lowering the pH to less than 5 (Fig. 1). The coagulation time ranged from 19 to 44 h at 30 °C. The fastest coagulation time of 19–24 h was shown by several strains of *Lb. plantarum*.

3.2. Antimicrobial activities

Table 1 shows the antagonistic activities of LAB strains, isolated from ethnic fermented vegetable and tender bamboo shoot products against *L. innocua* DSM20649, *L. monocytogenes* DSM20600, *B. cereus* CCM2010, *S. aureus* S1, *E. faecium* DSM20477, *S. mutans* DSM6178, *K. pneumoniae* subsp. *pneumoniae* BFE147, *E. cloacae* BFE282, *E. agglomerans* BFE154 and *P. aeruginosa* BFE162. Most of the LAB strains showed clear inhibition zones in agar-spot plates showing antagonisms. However, only 47 strains tested for antagonism showed clear inhibition zones of more than 4 mm in agar-spot plates. Out of these which, 17 strains were *Lb. plantarum*, 11 were *Lb. brevis*, 10 were pediococci, 5 were *Leuc. fallax*, 3 were *Leuc. lactis* and 1 was *Leuc. mesenteroides*.

LAB strains showing inhibition zones of >4 mm against indicator strains in agar-spot plates, were selected for the bacteriocin assay using cell-free culture supernatants. Among the 47 tested organisms only *Lb. plantarum* IB2 (BFE948) isolated from inziangsang showed a clear zone of >10.5 mm in agar spot assay against *S. aureus* S1 indicating bacteriocin activity (data not shown). However, no bacteriocin activity of this *Lb. plantarum* strain against any other indicator strains was detected. Quantification of bacteriocin activity shown by *Lb. plantarum* IB2 (BFE948) was calculated 32 AU/ml.

3.3. Enzymatic activities

Twenty one representative genera and species of LAB strains from each product were selected randomly for enzymatic studies (Tables 2 and 3). These strains showed relatively moderate esterase (C4) and strong arylamidase and phosphatase activities. However, they showed no detectable proteinase activity with the methods applied. Acid phosphatase activity was detected in all strains. Phosphohydrolase activity was shown by all strains tested except *Leuc. fallax* SjC1 (BFE931), isolated from soijim. The majority of strains showed α-glucosidase and glucosaminidase activities.

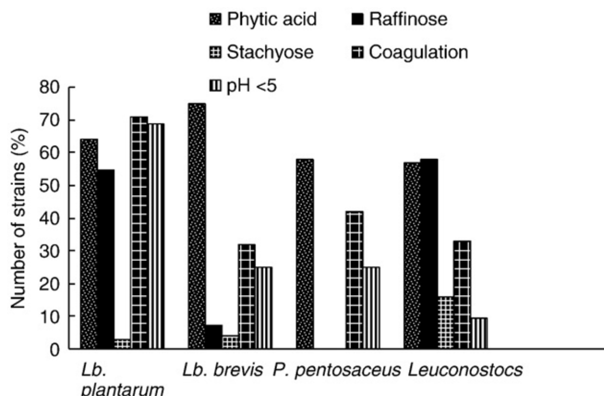

Fig. 1. LAB strains showing functional properties {degradation of phytic acid, fermentation of raffinose and stachyose, coagulation and acidification (pH<5)}.

3.4. Degradation of antinutritive factors

LAB strains, isolated from fermented vegetable products and tender bamboo shoot products, were screened for their ability to degrade antinutritive factors (data not shown). About 59% of LAB strains degraded phytic acids, 16.6% degraded raffinose and 3% degraded stachyose in the applied method (Fig. 1). *Lb. plantarum* showed the highest percentage of degradation of antinutritive factors. Among the remaining non-*Lb. plantarum* strains, *Lb. brevis*, *Leuconostoc* spp. and *Enterococcus durans* degraded phytic acid and oligosaccharides. However, no effect on degradation of antinutritive factors was shown by *Lb. curvatus* and *Leuc. citreum* both isolated from mesu, a fermented bamboo shoot.

3.5. Screening of LAB for production of biogenic amines

Most of the LAB strains did not produce biogenic amines in the method applied. Almost all strains of *Lb. plantarum*, except *Lb. plantarum* MeL3 (BFE944) from mesu, were tested negative for amino acid decarboxylase activity. Biogenic amine mainly ornithine was detected in 8 strains of *Lb. brevis* (5 strains from sinki, 2 from mesu and 1 from soijim) and in *Leuc. lactis* SdR2 (BFE926) from soidon.

3.6. Hydrophobicity of the LAB strains

Out of 94 strains tested, 77 had less than 30% hydrophobicity; 10 strains had in between 30 and 70%, and 7 strains had more than 70% hydrophobicity. The strains showing more than 70% hydrophobicity were *Lb. brevis* strains MeN7 (BFE942) (94.5%), MeR6 (BFE938) (91.5%), SL:B7 (BFE2889) (84%), KG:B2 (BFE952) (81%), MeTR (BFE941) (72%) and *Lb. plantarum* strains MeL2 (BFE934) (94%), MeL3 (BFE944) (75%). None of the pediococci showed more than 14% hydrophobicity percentage.

3.7. In vitro adherence

Seven LAB strains showing more than 70% hydrophobicity were able to adhere to mucus secreting HT29 MTX cells, adhesion of the most strains being in the same range as that of the reference strain *Lb. rhamnosus* GG (data not shown).

4. Discussion

Acidification is an important functional logical property in relevance of selection for starter culture among the LAB (de Vuyst, 2000). The ability of some species of LAB particularly *Lb. plantarum* in acidification of the substrates is significant in food preservation (Ammor and Mayo, 2007). It was found that some LAB strains isolated from ethnic fermented vegetable and bamboo products (Tamang et al., 2005, 2008) were able to lower the pH to 4.0. Interestingly, these strains although originating from plant sources and not from milk, appeared to be adapted to the milk ecology, since they coagulated and acidified the skim milk used in the applied method. Justifying milk coagulation by the LAB strains isolated from fermented vegetables, the enzymatic activity of the LAB (Tables 2 and 3) clearly shows the absence of proteinase but showed high activity of peptidases, which, may be responsible for coagulation of skim milk in the applied method.

Strains of different species of *Lactobacillus*, *Pediococcus* and *Leuconostoc*, isolated from all fermented vegetable and bamboo shoot products, showed antimicrobial activities against a number of potentially pathogenic Gram-negative and Gram-positive bacteria (Table 1). This indicates that functional LAB can reduce the number of undesired microorganisms in vegetable products and simultaneously perform an essential role in the preservation of a food product for human consumption, by fermentation. However, only one strain of

Table 1
LAB strains from fermented vegetable products showing spot antagonism on MRS-0.2 agar.

LAB strains[a]	Indicator strains									
	Listeria innocua DSM 20649	*Listeria monocytogenes* DSM 20600	*Bacillus cereus* CCM 2010	*Staphylococcus aureus* S1	*Enterococcus faecium* DSM 20477	*Streptococcus mutans* DSM 6178	*Klebsiella pneumoniae* subsp. *pneumoniae* BFE 147	*Enterobacter cloacae* BFE 282	*Enterobacter agglomerans* BFE 154	*Pseudomonas aeruginosa* BFE 162
Lb. plantarum (30)	7	0	2	12	0	5	3	1	7	7
Lb. brevis (28)	1	0	0	1	0	0	1	0	4	4
Lb. curvatus (1)	1	0	0	1	0	0	1	0	0	1
P. pentosaceus (12)	2	0	2	7	0	6	2	2	4	2
P. acidilactici (1)	1	0	1	1	0	1	1	1	1	1
Leuconostoc spp. (21)	0	0	0	5	0	2	3	3	3	5
Enterococcus durans (1)	1	0	0	1	0	1	1	1	1	0

[a] LAB strains which produced >4 mm inhibition zone against the indicator strains. Total number of strains tested is given in parentheses.

Lb. plantarum IB2 (BFE948) (inziangsang) was found to produce a bacteriocin against *S. aureus* (S1). A number of Gram-positive pathogenic bacteria including *S. aureus* have been found sensitive to bacteriocins of *Lactobacillus* (Tichaczek et al., 1992; Sudiraman et al., 1993; Niku-Paavola et al., 1999). There are reports on the inhibitory effect of bacteriocins produced by *Lb. plantarum* in foods against *S. aureus* and other Gram-negative bacteria (Jamuna et al., 2005; Lash et al., 2005). Strains of LAB species isolated from several fermented vegetable products were reported to have antimicrobial activities including production of bacteriocins in fermented olives (Rubia-Soria et al., 2006), sauerkraut (Tolonen et al., 2004), fermented carrots (Uhlman et al., 1992), fermented cucumbers (Daeschel and Fleming, 1987) and organic leafy vegetables (Ponce et al., 2008).

The commercial API-zym kit is of relevance for selection of strains as potential starter cultures based on superior enzyme profiles, especially peptidases and esterases, for accelerated maturation and flavour development of fermented products (Tamang et al., 2000). Absence of proteinases (trypsin and chymotrypsin) and presence of strong peptidase (leucine-, valine-, and cystine-arylamidase) and esterase-lipase (C4 and C8) activities produced by the LAB strains isolated from the Himalayan fermented vegetable products are possible traits of desirable quality for their use in the production of typical flavour.

High activity of phosphatase by LAB strains indicates their possible role in phytic acid degradation in fermented vegetable products. Many of the strains seem to be able to reduce phytic acid in a screening test medium. It was also shown that strains of *Lb. plantarum*, *Lb. brevis* and *Leuc. lactis* had moderate to high α-galactosidase activity. This indicated their ability to hydrolyse oligosaccharides of raffinose family (Holzapfel, 2002). About 55% of *Lb. plantarum* strains isolated from the Himalayan fermented vegetable and tender bamboo shoot products degraded raffinose, while none of the *P. pentosaceus* strains and *P. acidilactici* was able to degrade raffinose. Holzapfel (2002) reported that *Lb. plantarum* strains isolated from fermented Ghanaian maize products were able to ferment raffinose, while strains of *P. pentosaceus* and *P. acidilactici* were unable to do so.

Biogenic amines are organic basic compounds which occur in different kinds of foods such as sauerkraut (Taylor et al., 1978), fishery products, cheese, wine, beer, dry sausages and other fermented foods (ten Brink et al., 1990; Halász et al., 1994), fruits and vegetables (Suzzi and Gardini, 2003). In foods, biogenic amines are mainly generated by decarboxylation of the corresponding amino acids through substrate specific enzymes of the microorganisms present in foods (ten Brink et al., 1990; Straub et al., 1995). The inability of LAB strains from the Himalayan fermented vegetables to produce biogenic amines is a good indication of their acceptability and their potential for the possible development as starter culture.

A few strains of LAB showed more than 75% hydrophobicity, indicating the hydrophobic nature of strains in general. A percentage of hydrophobicity greater than 70% was arbitrarily classified as hydrophobic (Nostro et al., 2004). The high degree of hydrophobicity of some of these LAB strains probably indicates the potential of adhesion

Table 2
Enzymatic profiles of the LAB strains from fermented vegetable products using API-zym system.

Enzyme	LAB strains (activity in nanomoles[a])									
	GK:C1 (963)	GLn:C1 (965)	IB1 (947)	IB2 (948)	IB5 (950)	KG:B2 (952)	KG:B1 (951)	KB:C1 (959)	SL:B1 (2887)	SL:B3 (2885)*
Alkaline phosphatase	0	0	10	5	5	0	5	0	0	0
Esterase (C4)	0	0	5	0	0	5	5	5	10	5
Esterase lipase (C8)	0	0	5	5	0	5	5	0	10	5
Lipase (C14)	5	5	5	5	5	5	5	0	5	0
Leucine arylamidase	>40	>40	>40	>40	>40	>40	>40	5	30	5
Valine arylamidase	>40	>40	>40	>40	>40	>40	>40	0	>40	0
Cystine arylamidase	5	5	20	20	30	10	10	0	>40	0
Acid phosphatase	20	20	>40	20	20	>40	10	10	30	30
Napthol-AS-BI-phosphohydrolase	20	>40	20	20	30	20	10	5	20	10
α-galactosidase	0	0	20	5	0	30	5	0	10	0
β-galactosidase	5	5	>40	>40	20	30	>40	0	30	0
β-glucuronidase	0	0	10	0	0	20	0	0	5	0
α-glucosidase	0	0	>40	>40	0	>40	>40	>40	>40	>40
β-glucosidase	10	30	>40	30	20	30	30	0	>40	5
N-acetyl-β-glucosaminidase	20	>40	0	30	0	0	>40	0	0	0

Data represents the means ± SD of 3 replicates.
Trypsin, α-chymotrypsin, α-mannosidase and α-fucosidase were not hydrolysed by any LAB strain.
GK:C1 *Lb. plantarum* (gundruk); GLn:C1 *Pediococcus pentosaceus* (gundruk); SL:B1 *Lb. brevis* (sinki); SL:B3 *Leuconostoc fallax* (sinki); KG:B2 *Lb. brevis* (khalpi); KG:B1 *Lb. plantarum* (khalpi); KB:C1 *Leuc. fallax* (khalpi); IB1 *Lb. brevis* (inziangsang); IB2 *Lb. plantarum* (inziangsang); IB5 *P. acidilactici* (inziangsang).
*BFE.
[a] 0, no enzyme activity; 5, 10, 20, 30, >40 indicates nanomoles of hydrolysed substrate after 6 h of incubation at 30 °C.

Table 3
Enzymatic profiles of the LAB strains from fermented bamboo shoot products using API-zym system.

Enzyme	LAB strains (Activity in nanomoles[a])										
	MeB4 (935)	MeL2 (934)	MeB:C3 (945)	SbC1 (929)	SbB1 (928)	SjR7 (933)	SdR3 (927)	SdC1 (925)	SdR2 (926)	SjC1 (931)	SjR3 (932)
Alkaline phosphatase	10	5	0	0	5	5	10	0	5	0	5
Esterase (C4)	5	0	0	5	0	5	0	5	5	5	0
Esterase lipase (C8)	5	5	5	0	5	5	5	0	10	0	5
Lipase (C14)	0	5	5	0	0	5	0	0	5	0	0
Leucine arylamidase	>40	>40	>40	5	5	>40	10	5	>40	5	5
Valine arylamidase	>40	>40	>40	0	0	>40	0	0	>40	0	0
Cystine arylamidase	10	20	20	5	0	10	0	5	30	0	0
Acid phosphatase	>40	30	20	10	>40	>40	20	10	>40	20	10
Napthol-AS-BI-phosphohydrolase	>40	20	30	5	5	5	5	5	10	0	5
α-galactosidase	20	5	0	0	5	10	5	0	20	0	5
β-galactosidase	>40	>40	5	0	5	>40	10	0	>40	0	5
β-glucuronidase	0	0	0	0	0	0	0	0	0	0	0
α-glucosidase	30	>40	0	>40	20	30	20	>40	>40	>40	5
β-glucosidase	20	>40	5	0	0	>40	0	0	>40	0	0
N-acetyl-β-glucosaminidase	0	>40	5	0	0	0	0	0	0	0	0

Data represents the means ± SD of 3 replicates.
Trypsin, α-chymotrypsin, α-mannosidase and α-fucosidase were not hydrolysed by any LAB strain.
MeB4 *Lb. brevis* (mesu); MeL2 *Lb. plantarum* (mesu); MeB:C3 *Pediococcus pentosaceus* (mesu); SdR3 *Lb. brevis* (soidon); SdC1 *Leuconostoc fallax* (soidon); SdR2 *Leuc. lactis* (soidon); SbC1 *Leuc. fallax* (soibum); SbB1 *Leuc. lactis* (soibum); SjR7 *Lb. brevis* (soijim); SjC1 *Leuc. fallax* (soijim); SjR3 *Leuc. lactis* (soijim).
[a] 0, no enzyme activity; 5, 10, 20, 30, >40 indicates nanomoles of hydrolysed substrate after 6 h of incubation at 30 °C.

to human gut epithelial cells, advocating their probiotic character (Holzapfel et al., 1998), provided these strains are consumed in a viable state. The ability to adhere to the intestinal mucosa is considered one of the main criteria in the selection of potential probiotic cultures (Apostolou et al., 2001; Shah, 2001; Holzapfel and Schillinger, 2002). Functional effects of probiotic bacteria include adherence to the intestinal cell wall for colonization in the gastro intestinal tract (GIT) with capacity to prevent pathogenic adherence or pathogen activation (Spinler et al., 2008). As reported by Ouwehand et al. (1999), hydrophobicity did not correlate with adhesiveness, indicating that adhesion to the host cell is a complex process mediated by many different mechanisms. The behaviour of LAB could be dependent on interfacial processes and thus on cell surfaces physicochemical properties and chemical composition (Gatti et al., 1997; Boonaert and Rouxhet, 2000; Gómez-Zavaglia et al., 2002).

5. Conclusion

Scientific knowledge on the Himalayan ethnic fermented vegetables is sparse outside the region. Strains of LAB play complex role in the traditional fermentation by their functional properties related to a specific enzyme spectrum, acidifying capacity, degradation of antinutritive factors, antimicrobial activities, probiotic properties, and even as non-producers of biogenic amines. Some strains of LAB having superior functional properties isolated from the ethnic fermented vegetable and bamboo shoot products of the Himalayas may be developed as starter culture(s) for controlled and optimised production of the fermented vegetable products.

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