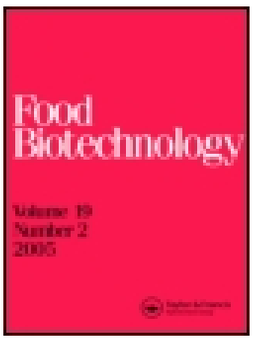

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Lactic Acid Bacteria Isolated from Indigenous Fermented Bamboo Products of Arunachal Pradesh in India and Their Functionality

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Ekung, *eup* and *hirring* are some common indigenous fermented bamboo products of Northeast India. We have isolated, characterized, and identified the predominant lactic acid bacteria (LAB) from 44 samples of *ekung, eup,* and *hirring* and studied their technological properties. The phenotypic characterizations of LAB isolates were based on physiological, biochemical tests and API kits, and were identified as *Lactobacillus plantarum, L. brevis, L. casei*, *L. fermentum, Lactococcus lactis, and Tetragenococcus halophilus*. Technological properties of LAB such as acidifying capacity, antimicrobial activities, degradation of phytic acid and oligosaccharides, bile-salt tolerance, enzymatic activities, biogenic amines production, and degree of hydrophobicity also were studied. This study showed that strains of LAB played important roles by their functional properties related to acidifying capacity, degradation of antinutritive factors, tolerance to bile-salt, wide enzymatic activities, and nonproducers of biogenic amines. Understanding the biological and biochemical basis of indigenous knowledge of the ethnic people of Northeast India for production of nonperishable bamboo shoots by lactic acid fermentation has merit. It helps to develop both low-cost functional foods, and understand the functionality of microbial diversity. Some of the LAB strains possess functional properties, which render them interesting candidates for use as LAB starter cultures.

Key Words: ekung; *eup*; fermented bamboo products; *hirring*; LAB

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INTRODUCTION

Young succulent shoot of the bamboo is mostly used as an edible delicacy by the ethnic people of Northeast India (Tamang, 2005). Arunachal Pradesh is in Northeast India with an area of 83,743 sq. km, with more than 500 types of plants, including indigenous bamboo species, and is populated by many indigenous tribes (Nandy et al., 2006). Three common indigenous fermented bamboo shoot products of Arunachal Pradesh are *ekung, eup,* and *hirring*. During preparation of *ekung*, young bamboo tender shoots (*Dendrocalamus hamiltonii* Nees. et Arn. ex Munro, *D. giganteus* Munro, *Bambusa balcooa* Roxb., *B. tulda* Roxb., *Phyllostachys assamica* Gamble ex Brandis) of about 8–10 feet high are collected, their outer leaf sheaths are removed, and edible portions are chopped into small pieces. The bamboo baskets are laid into the pit, lined with leaves; chopped bamboo shoot pieces are put into the basket, covered with leaves and then sealed. Heavy stones are kept to give weight to drain excess water from the bamboo shoots and fermented for one to three months. *Ekung* can be kept for a year at room temperature. It is consumed raw or cooked with meat, fish, or vegetables. *Eup* is a dry fermented bamboo tender shoot product. Preparation of *eup* is similar to *ekung*. Unlike *ekung*, *eup* is cut into smaller pieces after fermentation and then sun dried for 5–10 d until its color changes to chocolate brown. It is eaten as curry along with meat, fish, or vegetables. *Eup* can be kept up to two years at room temperature. During preparation of *hirring*, the topmost tender edible portions of young bamboo shoots (*D. giganteus*, *P. assamica*, *B. tulda*) are collected, outer leaf sheaths are removed, and shoots are either cut longitudinally into 2 to 3 pieces of size 1–2 inches × 9–15 inches, or whole shoots are flattened by crushing and are put into bamboo baskets lined with leaves. The baskets are placed into the pit, covered with leaves, sealed and weighted down with heavy stones, and fermented for one to three months. After fermentation, baskets are taken out from pits; *hirring* is ready for consumption as side-dish. The shelf-life of *hirring* is about two to three months. Like *mesu*, a traditional fermented bamboo shoot product of Sikkim and Darjeeling hills (Tamang, 2000), salt is not added during production of all *ekung*, *eup,* and *hirring*.

There are few reports on the taxonomical and ecological aspects of bamboos of Northeast India (Giri and Janmejay, 1987, 2000; Basar and Bisht, 2002; Bhatt et al., 2003). Tamang et al. (2008) reported the phenotypic and genotypic identification of lactic acid bacteria isolated from some ethnic fermented bamboo tender shoots of Northeast India such as *mesu, soibum, soidon,* and *soijim*. The present paper is aimed to isolate and identify the predominant lactic acid bacteria from the ethnic fermented bamboo tender shoot products of Arunachal Pradesh and to assess their technological and functional properties of the product, which in turn may help to explore the benefits of lesser-known food bioresources of Northeast India.

MATERIALS AND METHODS

Samples

In total, 44 samples of *ekung* (16), *eup* (13), and *hirring* (15) were collected from different places of Arunachal Pradesh in India. All samples were collected aseptically in sterile bottles and were stored at 4ºC for analyses.

Microbiological Examination

Samples (10 g) of each product were mixed with 90 ml of 0.85% (w/v) sterile physiological saline and homogenized in a Stomacher lab-blender (400, Seward, UK) for 1 min. A serial dilution in the same diluents was made. Lactic acid bacteria (LAB) were enumerated on MRS agar (M641, HiMedia, India) plates supplemented with 1% $CaCO_3$ under anaerobic condition kept in an Anaerobic Gas-Pack system (LE002, HiMedia, India) and incubated at 30°C for 48–72 h. Representative LAB strains were isolated randomly from MRS plates of the highest sample dilutions. Purity of the isolates was checked by streaking again and subculturing on fresh agar plates of the isolation media, followed by microscopic examinations. Identified isolates were preserved at –20°C in respective media with 15% (v/v) glycerol.

The presence of yeasts and molds in the samples were also examined on yeast-malt agar (M424, HiMedia, India) and potato dextrose agar (M096, HiMedia) supplemented with 10 IU ml^{-1} benzylpenicillin and 12 μg ml^{-1} streptomycin sulphate, respectively, and were incubated at 28°C for 72 h. Samples were tested for enumeration of *Bacillus cereus* using selective *B. cereus* agar base (M833, HiMedia), *Staphylococcus aureus* using Baird Parker agar base (M043, HiMedia), enterobacteriaceae in violet red bile glucose agar (M581, HiMedia), *Salmonella* and *Shigella* in *Salmonella-Shigella* Agar (M108, HiMedia), and *Listeria* in *Listeria* identification agar base (M1064, HiMedia) according to the method described by Metaxopolous et al. (2001).

Characterization and Identification

Cell morphology of all bacterial isolates and their motility were determined using a phase contrast microscope (Olympus CH3-BH-PC, Japan). Bacterial isolates were Gram-stained and tested for catalase production by placing a drop of 10% hydrogen peroxide solution on isolates and were preliminarily identified on the basis of carbon dioxide production from glucose, ammonia production from arginine, growth at different temperatures (10°C, 15°C, 45°C), the ability to grow in different concentrations of sodium chloride (6.5%, 10%, 18%) and pH (3.9, 9.6) in MRS broth (M369, HiMedia, India), following the methods of Schillinger and Lücke (1987) and Dykes et al. (1994).

The configuration of lactic acid produced from glucose was determined enzymatically using D-lactate and L-lactate dehydrogenase test kits (Tamang et al., 2005). The presence of *meso*-diaminopimelic acid (DAP) in the cell walls of LAB was determined on cellulose plates using a thin layer-chromatography (Tamang et al., 2000). Sugar fermentation of LAB isolates were determined by the API 50 CHL test strips (bioMérieux, France), and the identifications were interpreted using APILAB PLUS software (bioMérieux, France). Taxonomical keys of Simpson and Taguchi (1995) and Wood and Holzapfel (1995) were followed for identification of LAB isolates.

Acidification and Coagulation

The acidification and coagulating abilities of the LAB isolates was assayed by inoculating 10% skim milk with 24 h old cultures (RM1254, HiMedia, India) at the 1% level and incubated at 30°C. Observation was made for commencement of clotting, and pH was measured after 72 h of incubation (Olasupo et al., 2001).

Bile Salt Tolerance

The ability of LAB strains to grow in presence of bile salt was performed by spotting about 10 µl of the overnight culture directly on the assay medium consisted of MRS agar supplemented with 0.5% bile salt (Merck, Germany) (du Toit et al., 1998).

Phytic Acid and Oligosaccharide Degradation

The ability of LAB isolates to degrade phytic acid was determined on a synthetic phytic acid screening medium containing calcium phytate (Sigma, St. Louis, Mo., USA), and the plates were incubated at 30°C for 5 d (Holzapfel, 1997). Clear zone around the colony of the test organism indicated a positive reaction. Screening of LAB for degradation of stachyose and raffinose were performed in MRS broth (pH 6.4) containing 2% stachyose and 1% raffinose (instead of glucose), respectively, at 30°C for 3 d (Holzapfel, 1997).

Hydrophobicity Assay

Bacterial adhesion to hydrocarbons was determined using n-hexadecane (RM 2238, HiMedia, India) as described by Dewan and Tamang (2007). Fresh cultures were grown in MRS broth at 30°C for 24 h and centrifuged at 8,000 g for 5 min. The pellet was washed with 9 ml of Ringer solution (Merck, Germany), and 1 ml of the suspension was taken for measurement of absorbance at 580 nm. Then 1.5 ml of the suspension was mixed with equal volume of n-hexadecane (RM 2238, HiMedia, Mumbai, India) and mixed thoroughly in a

vortex. The phases were allowed to separate for 30 min at room temperature, after which aqueous phase was carefully removed and absorbance at 580 nm was measured. The percentage hydrophobicity was expressed as follows: hydrophobicity % = $[A_0\text{-}A/A] \times 100$, where A_0 and A are the absorbance values of the aqueous phase before and after contact with n-hexadecane**.** The percent hydrophobic index greater than 70% was arbitrarily classified as hydrophobic (Nostro et al., 2004).

Antimicrobial Activity

The LAB isolates were screened for antimicrobial activity by the agar spot method (Schillinger and Lücke, 1989). The indicator strains used for antagonism were *Listeria innocua* DSM 20649, *L. monocytogenes* DSM 20600, *Bacillus cereus* CCM 2010, *Staphylococcus aureus* S1, *Pseudomonas aeruginosa* BFE 162, *Enterobacter agglomerans* BFE 154, *E. cloacae* BFE 282, and *Klebsiella pneumoniae* subsp. *pneumoniae* BFE 147. Cell-free neutralized supernatants of LAB isolates were screened for bacteriocin activity by the agar spot test method (Uhlman et al., 1992) using the bacteriocin screening medium (BSM) of Tichaczek et al. (1992). One-day old LAB cultures grown in MRS broth were centrifuged followed by filtration of the supernatant through a 0.2 μm pore-size cellulose acetate filter. The cell-free supernatant was adjusted to pH 6.5 by addition of 1N NaOH and stored frozen until tested. The cell-free extracts were spotted onto soft MRS agar (containing 0.7% agar) plates, inoculated with indicator strains and were incubated at 30°C for 24 h, and subsequently examined for zone of inhibition.

Enzymatic Profile

A rapid enzymatic profile of LAB isolates was assayed using commercial API-zym galleries (bioMérieux, France).

Screening for Biogenic Amine Production

Biogenic amine-forming capacity of LAB isolates was determined qualitatively in medium (Joosten and Northold, 1989) containing histidine, lysine, ornithine, and tyrosine. Change of the bromocresol purple used as indicator to purple color was considered as positive reactions of amino acid decarboxylase activity (Bover-Cid and Holzapfel, 1999).

pH of the Samples

The pH of the samples (10 g) was determined directly using a digital pH meter (Type 361, Systronics, India) calibrated with standard buffer solutions (Merck, Germany).

RESULTS

Microbiology

Forty-four market samples of *ekung, eup* and *hirring* were tested for presence of LAB, yeasts, molds, and pathogenic bacteria. Data of microbiological analysis of market samples showed the dominance of LAB ranging from 1.8×10^7 to 1.3×10^8 cfu g^{-1}. The yeast, mold, and pathogens were not detected. The average pH of market sample of *ekung* was 3.9 (3.7–4.1); *eup,* 4.1 (3.9–4.4); and *hirring,* 4.0 (3.8–4.2). A total of 128 bacterial isolates were isolated from *ekung* (43), *eup* (40), and *hirring* (45) and were purified in MRS broth. Their cell morphology and preliminary taxonomical tests were performed. All bacterial isolates were considered LAB because they grew well in anaerobic agar and formed clear halo in $CaCO_3$ supplemented MRS agar plates, were Gram-positive, catalase-negative bacteria, nonmotile, and nonspore formers. The representative strains of LAB were randomly selected from each grouped strains having similar morphology, the ability to produce gas from glucose and hydrolyze arginine, and isolated from the respective sample (Table 1). A total of 76 representative strains of LAB were grouped and phenotypically characterized including determination of the sugar fermentation by API kit, lactic acid configuration, determination of DAP, growth at different temperatures, pH, and NaCl. Two tetrad-forming cocci strains (Ek22, Ek28) from *ekung* grew well at 15ºC in 6.5% and 18% NaCl, but did not grow at 45ºC. Extreme salt tolerance (growth in 18% NaCl) differentiated Ek22 and Ek28 strains from other tetrad-forming strains and was identified as *Tetragenococcus halophilus* (Table 2) based on the taxonomical keys of Simpson and Taguchi (1995). Heterofermentative strain (Ek8) from *ekung* was identified as *Lactobacillus brevis* (Table 2). Strains Ep85, Ep92, and Ep93 (*eup*), unlike *L. brevis,* did not grow in 6.5% NaCl and at 15ºC but grew well at 45ºC. Using API system and also keys of Wood and Holzapfel (1995), these strains were identified as *L. fermentum* (Table 2).

Of the 68 homofermentative rods isolated from fermented bamboo shoots (*ekung* and *eup*), 66 strains were arginine negative and shared the ability to grow in pH 3.9, 6.5% NaCl, at 10ºC and 15ºC but not at 45ºC, produced both isomers of lactate from glucose, and contained DAP in their cell walls. Sugar fermentation profile confirmed their identity as *L. plantarum* (Table 2). The remaining two homofermentative rods (Ek34, Ek35) from *ekung* had similar growth behavior at different temperatures, pH and NaCl, with that of *L. plantarum* but differed due to lack of DAP in their cell wall, produced L(+)– isomer of lactate from glucose and few sugar fermentation profiles, and were identified as *L. casei* (Table 2). Two homofermentative coccoid rod strains (Hr56, Hr62) from *hirring* were identified following the API system as *L. lactis* (Table 2).

Table 1: Grouping of representative strains of the LAB isolated from fermented bamboo products.

Product[a]	Cell shape	Gas from glucose	Arginine hydrolysis	Grouped Strains	Representative strains	
					Total no.	Strain code
Ekung (43)	Rod	–	–	36	22	Ek1, Ek4, Ek5, Ek6, Ek7, Ek10, Ek11, Ek12, Ek15, Ek18, Ek19, Ek20, Ek21, Ek23, Ek24, Ek25, Ek29, Ek34, Ek35, Ek38, Ek39, Ek40
	Rod	+	+	3	1	Ek8
	Coccus/ tetrad	–	+	4	2	Ek28, Ek22
Eup (40)	Rod	–	–	35	22	Ep81, Ep82, Ep84, Ep86, Ep87, Ep89, Ep90, Ep91, Ep94, Ep95, Ep96, Ep97, Ep98, Ep99, Ep1, Ep102, Ep103, Ep106, Ep110, Ep111, Ep112, Ep115
	Rod	+	+	5	3	Ep92, Ep93, Ep85
Hirring (45)	Rod	–	–	41	24	Hr41, Hr42, Hr45, Hr47, Hr48, Hr49, Hr50, Hr55, Hr57, Hr58, Hr59, Hr60, Hr64, Hr65, Hr66, Hr67, Hr68, Hr70, Hr71, Hr74, Hr75, Hr76, Hr77, Hr80
	Coccoid rod	–	+	4	2	Hr56, Hr62

[a]Total number of isolates in each product are given in parenthesis. +, positive; –, negative. All strains of LAB were Gram-positive, catalase-negative, nonmotile, and nonsporing.

Technological Properties

Effect of acidification and coagulation by the LAB strains isolated from fermented bamboo shoots were tested (Table 3). *L. plantarum* Ek6 (*ekung*) showed the lowest acidification value of pH 3.9 among all tested strains of LAB. About 76% of LAB strains caused coagulation of milk at 30ºC with a significant drop in pH (Table 3). Of 76 LAB strains screened for antinutritive factors, 47.4% degraded phytic acids, 65.8% degraded raffinose, and 11.8% degraded stachyose in the applied method (Table 3). *L. plantarum* showed the highest percentage of degradation of antinutritive factors. However, no effect

Table 2: Phenotypic characteristics of the LAB isolated from fermented bamboo products.

LAB species	[1]Origin of strains	Growth in pH 3.9	Growth in pH 9.6	Growth at 45°C	DAP	Isomer of lactate	Sugars fermented													
							Arabinose	Cellobiose	Esculin	Lactose	Maltose	Melezitose	Melibiose	Raffinose	Ribose	Salicin	Sorbitol	Sucrose	Trehalose	Xylose
L. plantarum	Ek (20); Ep (22); Hr (24)	+	–	–	+	DL	52/14	+	+	+	+	+	+	45/21	+	+	48/18	+	+	–
L. brevis	Ek (1)	+	–	–	–	DL	+	+	–	–	+	–	–	–	+	–	+	+	–	–
Lact. casei	Ek (2)	+	–	–	–	L	–	1/1	1/1	+	1/1	+	–	–	–	–	–	1/1	–	–
L. fermentum	Ep (3)	+	–	+	–	DL	–	+	+	+	+	–	+	+	–	–	–	+	+	+
L. lactis	Hr (2)	–	+	+	–	L	+	+	+	+	+	+	+	+	–	+	+	+	+	+
[2]*T. halophilus*	Ek (2)	+	–	–	–	L	–	–	–	+	+	+	–	–	–	–	–	+	–	–

[1] Origin of strains: Ek, *ekung*; Ep, *eup*; Hr, *hirring*; number of strains isolated from the respective product is given in parentheses; +, all strains positive; –, all strains negative; (../..), number of positive/negative strains. All strains grew at 10°C and 15°C and in 6.5% NaCl;
[2] All strains did not grow in 10% and 18% NaCl except *T. halophilus*.
DAP, *meso*-diaminopimelic acid.
All strains fermented galactose and mannose, and did not ferment rhamnose.

Table 3: Effect of LAB strains isolated from fermented bamboo products on acidification, growth in bile salt, anti-nutritive factors, and hydrophobicity.

				Antinutritive factors			
Product	**Strains**	**pH**	**Growth in bile salt**	**Phytic acid**	**Raffinose**	**Stachyose**	**% Hydrophobicity**
Ekung	Ek1	4.2 (+)	+	–	+	–	4.4 ± 0.3
	Ek4	4.2 (+)	+	+	+	–	1.6 ± 0.5
	Ek5	4.4 (+)	+	–	+	–	2.5 ± 0.5
	Ek6	3.9 (+)	+	+	–	–	1.9 ± 0.6
	Ek7	4.0 (+)	+	–	+	–	2.1 ± 0.3
	Ek8	4.2 (+)	+	–	–	–	2.3 ± 0.3
	Ek10	5.5 (+)	+	–	+	–	7.2 ± 0.2
	Ek11	4.8 (+)	+	+	+	+	9.2 ± 0.3
	Ek12	4.4 (+)	+	+	+	–	6.3 ± 0.6
	Ek15	4.5 (+)	+	–	–	–	16.2 ± 0.8
	Ek18	4.2 (+)	+	–	–	–	12.3 ± 0.3
	Ek19	4.4 (+)	+	–	–	–	11.2 ± 0.7
	Ek20	4.3 (+)	+	–	+	+	6.1 ± 0.3
	Ek21	4.2 (+)	+	–	–	–	2.5 ± 0.4
	Ek 22	4.4 (+)	+	–	–	–	23.7 ± 0.5
	Ek23	5.9 (+)	+	–	–	–	43.7 ± 5.8
	Ek24	4.5 (+)	+	–	–	–	23.1 ± 2.1
	Ek25	4.4 (+)	+	–	+	–	13.4 ± 0.3
	Ek28	4.7 (+)	+	–	–	–	**67.7 ± 5.0**
	Ek29	4.3 (+)	+	–	+	–	4.2 ± 0.1
	Ek34	5.9 (+)	+	–	–	–	20.7 ± 4.2
	Ek35	5.7 (+)	+	–	–	–	27.2 ± 3.2
	Ek38	5.1 (–)	+	–	+	–	8.9 ± 0.2
	Ek39	4.6 (+)	+	–	+	–	7.4 ± 0.2
	Ek40	4.6 (+)	+	–	+	–	6.4 ± 0.3
Eup	Ep85	4.6 (+)	+	+	+	–	4.3 ± 0.2
	Ep81	4.1 (+)	+	–	+	–	**58.3 ± 1.7**
	Ep82	4.2 (+)	+	+	+	–	37.4 ± 1.2
	Ep84	5.6 (–)	+	–	+	–	25.2 ± 1.1
	Ep86	4.6 (+)	+	+	+	–	14.2 ± 1.2
	Ep87	4.1 (+)	+	+	+	–	24.8 ± 0.7
	Ep89	4.1 (+)	+	–	+	–	31.6 ± 2.1
	Ep90	4.1 (+)	+	–	+	–	32.4 ± 1.9
	Ep91	5.8 (–)	+	+	+	–	1.3 ± 0.3
	Ep92	4.7 (+)	+	+	+	+	2.6 ± 1.9
	Ep93	5.7 (+)	+	+	+	–	4.2 ± 0.4
	Ep94	5.8 (–)	+	–	+	–	7.8 ± 0.8
	Ep95	4.6 (+)	+	–	+	–	15.7 ± 1.9
	Ep96	4.4 (+)	+	–	+	–	14.2 ± 2.0
	Ep97	4.4 (+)	+	+	+	–	6.4 ± 0.7
	Ep98	4.1 (+)	+	–	+	–	4.3 ± 0.3
	Ep99	4.3 (+)	+	+	+	–	7.2 ± 0.4
	Ep101	4.2 (+)	+	+	+	–	18.3 ± 0.8
	Ep102	4.2 (+)	+	+	+	–	14.4 ± 1.0
	Ep103	4.0 (+)	+	+	+	–	23.9 ± 0.9
	Ep106	4.4 (+)	+	+	+	–	23.5 ± 1.3
	Ep110	4.6 (+)	+	+	+	+	12.7 ± 1.3
	Ep111	4.3 (+)	+	+	+	–	14.2 ± 0.7
	Ep112	4.3 (+)	+	–	+	–	23.6 ± 0.8
	Ep115	4.3 (+)	+	–	+	+	4.2 ± 0.2

(*Continued*)

Table 3: (*Continued*).

Product	Strains	pH	Growth in bile salt	Antinutritive factors: Phytic acid	Antinutritive factors: Raffinose	Antinutritive factors: Stachyose	% Hydrophobicity
Hirring	Hr55	4.4 (+)	+	+	–	–	12.3 ± 0.3
	Hr41	4.2 (+)	+	+	+	+	1.0 ± 0.0
	Hr42	4.4 (+)	+	+	+	+	1.5 ± 0.1
	Hr45	4.2 (+)	+	–	–	–	3.2 ± 0.2
	Hr47	4.3 (+)	+	–	–	–	2.2 ± 0.2
	Hr48	4.2 (+)	+	+	+	–	5.4 ± 0.2
	Hr49	4.6 (+)	+	+	–	–	8.2 ± 0.6
	Hr50	4.6 (+)	+	+	+	+	31.8 ± 0.9
	Hr56	5.1 (+)	+	+	+	–	**59.5 ± 2.5**
	Hr57	5.0 (+)	+	+	–	–	32.4 ± 0.8
	Hr58	4.8 (+)	+	+	–	–	15.3 ± 0.3
	Hr59	4.1 (+)	+	+	+	–	3.2 ± 0.1
	Hr60	4.5 (+)	+	+	–	–	6.2 ± 0.1
	Hr62	5.6 (+)	+	+	+	–	7.8 ± 0.1
	Hr64	4.6 (+)	+	+	–	–	4.2 ± 0.1
	Hr65	4.2 (+)	+	–	–	–	5.3 ± 0.1
	Hr66	4.4 (+)	+	+	+	–	18.0 ± 0.2
	Hr67	4.4 (+)	+	+	–	–	3.7 ± 0.9
	Hr68	4.3 (+)	+	+	+	+	9.2 ± 0.2
	Hr70	4.6 (+)	+	+	+	–	7.4 ± 0.9
	Hr71	4.5 (+)	+	–	–	–	23.2 ± 0.4
	Hr74	4.6 (+)	+	–	+	–	5.3 ± 0.4
	Hr75	4.7 (+)	+	–	–	–	7.2 ± 0.9
	Hr76	4.3 (+)	+	–	–	–	12.7 ± 0.3
	Hr77	4.8 (–)	+	–	+	–	3.2 ± 0.8
	Hr80	4.6 (+)	+	–	–	–	43.5 ± 3.2

Data represent the means ± SD of 3 replicates.
The pH value represents the pH of skim milk recorded after 72 h of incubation and doesn't represent the final pH of bamboo fermentation.
Data on effect of LAB strains on coagulation are shown in parenthesis (+, indicates coagulation; –, indicates noncoagulation).

on degradation of antinutritive factors was shown by *T. halophilus*, *L. brevis*, and *L. casei*. All strains of LAB grew well in bile salt showing their tolerance to bile except *T. halophilus* strains Ek22 and Ek28, *L. casei* strains Ek34 and Ek35, and *L. lactis* strains Hr56 and Hr62 (Table 3). None of the strains showed more than 70% hydrophobicity (Table 3). Strains of LAB isolated from fermented bamboo shoot products were screened for their ability to produce biogenic amines. None of the LAB strains produced biogenic amines in the applied method (data not shown). The antimicrobial activities of LAB strains were tested against different bacteria. Most of the strains showed antagonisms; however, only 16 of (*L. plantarum*) out of the total 76 LAB strains showed the inhibition zones of more than 4 mm by scale in agar-spot plates (data not shown). None of the LAB strains showed bacteriocin producing activity in the applied method.

Table 4: API-ZYM profiles of LAB strains from fermented bamboo products.

	Strain (activity in nanomoles[a])					
Enzyme	**Ek4**	**Ek23**	**Ek28**	**Ek34**	**Ep92**	**Hr42**
Control (without enzyme)	0	0	0	0	0	0
Alkaline phosphatase	5	10	2	0	0	5
Esterase (C4)	0	0	10	0	0	0
Esterase lipase (C8)	5	0	5	0	0	0
Lipase (C14)	5	0	0	5	0	0
Leucine arylamidase	>40	30	0	30	20	30
Valine arylamidase	30	20	0	30	5	30
Cystine arylamidase	20	0	0	5	0	5
Trypsin	0	0	0	0	0	0
α-chymotrypsin	0	0	0	0	0	0
Acid phosphatase	20	20	>40	20	5	10
Napthol-AS-BI-phosphohydrolase	20	10	5	20	5	10
α-galactosidase	10	0	0	0	5	5
β-galactosidase	>40	0	0	0	10	>40
β-glucuronidase	0	0	0	0	0	0
α-glucosidase	20	0	0	0	20	20
β-glucosidase	30	0	0	30	20	20
N-acetyl-β- glucosaminidase	>40	0	0	30	0	>40
α-mannosidase	0	0	0	0	0	0
α-fucosidase	0	0	0	0	0	0<

Data represent the means ± SD of 3 replicates.
[a]0, no enzymatic activity; 5, 10, 20, 30, >40 indicates nanomoles of hydrolyzed substrate after 6 h incubation at 30°C.
Ek4 *Lactobacillus. plantarum* (*ekung*); Ek23 *L. plantarum* (*ekung*); Ek28 *T. halophilus* (*ekung*); Ek34 *Lact. casei* (*ekung*); Ep92 *L. fermentum* (*eup*); Hr42 *L. plantarum* (*hirring*).

Enzymatic profiles of LAB strains were assayed using the API-zym (bioMérieux, France) galleries (Table 4). Each of the LAB strain produced wide enzymatic activities. These strains showed relatively moderate esterase (C4) and strong arylamidase and phosphatase activities. However, they showed no detectable proteinase activity in the methods applied. Acid phosphatase activity was detected in all the strains tested among which >40 nanomole activities was showed by *T. halophilus* Ek28 (*ekung*).

DISCUSSION

Based on the characterizations and identification profiles of LAB strains from indigenous fermented bamboo products of Northeast India, species of LAB were summarized as follows: *L. plantarum, L. brevis, L. casei*, *T. halophilus* (*ekung*); *L. plantarum, L. fermentum* (*eup*); and *L. plantarum*, *L. lactis* (*hirring*). The identity of the LAB was similar to other fermented vegetable products of different regions (Steinkraus, 1996; Lee, 1997). *L. casei* has been reported to be dominant species in naturally fermented Sicilian green olives (Randazzo et al., 2004). Similar fermented bamboo

products called *naw-mai-dong* or *nor-mai-dorng* of Thailand also contained lactobacilli, leuconostocs, and pediococci (Dhavises, 1972; Phithakpol et al., 1995). About 90.7% of LAB was represented by lactobacilli in *ekung* and 9.3% by *Tetragenococcus*. Microorganisms from lesser-known fermented bamboo products may contribute significant information on unknown microbial diversity of Northeast India.

No pathogenic bacteria were detected in samples due to acidic nature of the products. Lactic acid produced by LAB may reduce pH to a level where pathogenic bacteria may be inhibited or killed (Holzapfel et al., 1995).

Technological properties of LAB are necessary criteria for selection of potent starter culture among the LAB for upgradation of traditional process and development of functional foods (Badis et al., 2004). 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. The ability of some species of LAB particularly *L. plantarum* in lowering pH of the substrates is significant for food preservation (Brown and Booth, 1991). Due to these nutritional consequences, the degradation of antinutritive factors in food products by fermentation is desirable as reported for a number of foods of plant origin (Holzapfel, 2002). The majority of strains of LAB grew well in bile salt, showing their ability to tolerate bile. Bile salt tolerance is considered an important colonization factor for probiotic bacteria (du Toit et al., 1998). Percent of hydrophobicity greater than 70% has been arbitrarily classified as hydrophobic (Nostro et al., 2004), which would advocate possible probiotic property (Holzapfel et al., 1998). However, such hydrophobic nature was not shown by any strain of LAB from *ekung, eup,* and *hirring*. The results indicate that within the same species each strain has few different characteristics as shown by *L. plantarum* with respect to the degree of hydrophobicity, degradation of stachyose, raffinose, and phytic acid. This differentiation and further study of these strains could lead to identification of different strains within the same species that would potentially have different technological or health-related values. Biogenic amines are organic basic compounds that occur in many fermented vegetable products (Suzzi and Gardini, 2003). However, in our study none of the strain of LAB produced biogenic amines in the applied method. This is a good indication of their acceptability and possible development as starter culture. The production of biogenic amines is not a desirable property for LAB strains to be selected as starter cultures (Buchenhüskes, 1993). A rapid enzymatic profile method is also of relevance for selection of strains as potential starter cultures based on superior enzyme profiles in fermented products (Tamang et al., 2000). Justifying milk coagulation by the LAB strains isolated from fermented bamboo shoots, the enzymatic activity of the LAB (Table 4) clearly shows the absence of proteinase but showed high activity of peptidases.

CONCLUSION

Documenting the biochemical basis of indigenous knowledge of the ethnic people of India in production of perishable bamboo shoots by lactic acid fermentation has merit, both as low-cost functional foods and for understanding microbial diversity. It is worth noticing that no preservative is added during storage in all of the above-mentioned fermented bamboo products. This study showed that strains of LAB play important roles in the traditional fermentation processes by their functional properties related to acidifying and coagulating capacity, degradation of phytic acid and oligosaccharides, tolerance to bile salt, wide enzymatic activities, nonproducers of biogenic amines, and bacterial pathogen antagonistic activities. Some of the LAB strains possess functional properties, which render them interesting candidates for use as starter cultures for production of fermented bamboo products in Arunachal Pradesh.

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