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Microbial diversity in ngari, hentak and tungtap, fermented fish products of North-East India

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Summary

Ngari, hentak and tungtap are traditional fermented fish products of North-East India. Eighteen samples of ngari, hentak and tungtap were collected and were analysed for microbial load. Lactic acid bacteria, endospore-forming rods, yeasts and aerobic mesophilic counts ranged from 4.0 to 7.2, 3.3–4.6, <1–3.5 and 4.3–7.3 log c.f.u./g, respectively. Lactic acid bacteria were identified as *Lactococcus lactis* subsp. *cremoris, Lactococcus plantarum*, *Enterococcus faecium, Lactobacillus fructosus*, *Lactobacillus amylophilus*, *Lactobacillus coryniformis* subsp. *torquens* and *Lactobacillus plantarum*. Endospore-forming rods were identified as *Bacillus subtilis* and *Bacillus pumilus*, aerobic coccal strains were identified as *Micrococcus*. Yeasts were identified as species of *Candida* and *Saccharomycopsis*. Pathogenic contaminants were detected in all samples, however, none of the sample contained more than 10^2 c.f.u./g of *Bacillus cereus*, 10^3 c.f.u./g of *Staphylococcus aureus* and enterobacteriaceae population, respectively. Enzymatic and antimicrobial activities of the isolates were tested. None of the strains produced biogenic amines in the method applied. Most strains of LAB had a high degree of hydrophobicity, indicating their 'probiotic' characters. This study has demonstrated the microbial diversity within the species of lactic acid bacteria, *Bacillus* and yeasts.

Introduction

Traditional processing of fish such as fermentation, salting, drying and smoking are the principal methods of fish preservation in South-East Asia (Cooke *et al.* 1993). Ethnic people of North-East India catch fishes from the rivers and lakes, some of these are traditionally fermented (Tamang 2001). Ngari is a fermented fish product of Manipur in North-East India. During its preparation, the fish (*Puntius sophore*) is rubbed with salt, dried in the sun for 3–4 days, pressed tightly in an earthen pot, sealed airtight and then stored at room temperature for 4–6 months (Thapa 2002). Ngari is eaten as a side-dish with cooked rice. Hentak is a ball-like thick paste prepared by fermentation of a mixture of sun-dried fish (*Esomus danricus*) powder and petioles of aroid plants (*Alocasia macrorhiza*) in Manipur (Thapa 2002). Dry fish is crushed to powder, an equal amount of petioles of aroid plants is mixed and a ball-like thick paste is made. The mixture is kept in an earthen pot and is fermented for 7–9 days. Hentak is consumed as curry as well as a condiment with boiled rice. Sometimes, it is given to mothers in confinement and patients in convalescence (Sarojnalini & Singh 1988). Tungtap is a fermented fish paste, commonly consumed by the Khasia tribes of Meghalaya in North-East state of India (Thapa 2002). Dry fish (*Danio* spp.) is mixed with salt, kept in an earthen pot and fermented for 4–7 days. It is consumed as a pickle.

There appears to be no information on the microbiology of these traditionally processed fish products of North-East India. The aim of the present study was to examine the composition of microorganisms, mainly the lactic acid bacteria of ngari, hentak and tungtap. Enzymatic activities, antimicrobial properties, ability to produce biogenic amines and degree of hydrophobicity of isolates were also examined.

Materials and methods

Collection of samples

Six samples of each ngari and hentak were purchased from different shops in Ima market of Imphal in Manipur, respectively, and six samples of tungtap were collected from Shillong of Meghalaya. Samples were

collected aseptically in pre-sterile poly-bags kept in an ice-box, and transported to laboratory for analyses.

Microbial analysis

Ten gram of sample was suspended in 90 ml of 0.85% (w/v) sterile physiological saline and homogenized in a stomacher lab-blender 400 (Seward, UK) for 1 min. Decimal dilution series were prepared in sterile diluent and diluted suspension of sample was mixed with the molten media and poured into plates. Lactic acid bacteria (LAB) were selectively isolated on MRS agar (HiMedia M641, India) plates supplemented with 1% $CaCO_3$ and incubated under anaerobic conditions in an Anaerobic Gas-Pack system (HiMedia LE002, India) at 30 °C for 3 days. The predominant LAB were obtained from MRS plates with the highest sample dilutions (10^{-5} or 10^{-6}). For aerobic endospore bacterial counts, a diluted suspension of sample was heated for 2 min in continuously boiling water (Tamang & Nikkuni 1996). Bacterial endospores were enumerated in pour-plates of nutrient agar (HiMedia MM012, India) and incubated aerobically at 37 °C for 1 day. Aerobic mesophilic counts (AMC) were determined using plate count agar (HiMedia M091A, India) incubated at 30 °C for 2 days. Moulds and yeasts were isolated on potato dextrose agar (HiMedia M096, India) and yeast extract-malt extract agar (HiMedia M424, India), respectively supplemented with 10 IU/ml benzylpenicillin and 12 μg/ml streptomycin sulphate and incubated aerobically at 28 °C for 3 days. Colonies were selected randomly or all sampled if the plate contained less than 10 colonies, according to Leisner *et al.* (1997). Purity of the isolates was checked by streaking again on fresh agar plates of the isolation media, followed by microscopic examinations. Identified strains of microorganisms were preserved in 15% glycerol at −20 °C and deposited at Culture Collection Centre of Food Microbiology Laboratory, Sikkim Government College, India.

Pathogenic contaminants

Ten gram of sample was blended with in 90 ml of peptone–physiological saline (0.1% neutral peptone, 0.85% NaCl) in a stomacher lab-blender 400 (Seward, UK) for 1 min. Serial dilution series were prepared in the same diluent in duplicates. Samples were tested for enumeration of pathogenic contaminants such as *Bacillus cereus* using selective *Bacillus cereus* agar base (HiMedia M833, India), *Staphylococcus aureus* using Baird Parker agar base (HiMedia M043, India) and enterobacteriaceae using Violet Red Bile Glucose agar (HiMedia M581, India) (Han *et al.* 2001).

Characterization and identification

Characterization and identification of yeasts were carried out following the method described by Kurtzman & Fell (1998). Cell morphology and motility test of bacterial strains were observed in a phase contrast microscope (Olympus CH3-BH-PC, Japan) following the method of Harrigan (1998). Gram staining of isolates was performed following the method of Bartholomew (1962). Catalase test was studied using 0.5 ml of 10% hydrogen peroxide solution and observed for the production of gas bubbles. Isolates were identified by phenotypic properties using CO_2 production from glucose, growth at different temperatures, pH and ability to grow in varying concentrations of NaCl in MRS broth as described by Schillinger & Lücke (1987) and Dykes *et al.* (1994). The configuration of lactic acid produced was determined enzymatically using D-lactate and L-lactate dehydrogenase kits (Roche Diagnostic, France). The presence of *meso*-diaminopimelic acid (*m*-Dpm) in the cell walls of lactic acid bacteria was determined using thin chromatography on cellulose plate (Schillinger & Lücke 1987). Ability of the isolates to ferment carbohydrates was studied using API 50 CHL (bioMérieux, France) system. Bacterial strains were identified following the taxonomic keys laid down in *Bergey's Manual of Systematic Bacteriology*, volume 2 (Sneath *et al.* 1986) and Wood & Holzapfel (1995). Endospore-forming bacteria were identified according to the keys based on Claus & Berkeley (1986) and Slepecky & Hemphill (1992).

Proteolytic activity

Surface-dried plates of milk agar (Gordon *et al.* 1973) were streaked with 24-h-old cultures, incubated at 30 °C for 4 days (lactic acid bacteria) and 37 °C for 2 days (endospore-forming bacteria), and examined for any clearing of casein around and underneath the growth for assessment of proteolytic activity.

Amylolytic activity

Surface-dried plates of starch agar (Gordon *et al.* 1973) were streaked with 24-h-old cultures, incubated at 30 °C for 4 days (lactic acid bacteria) and 37 °C for 2 days (endospore-forming bacteria). After incubation the plates were flooded with iodine solution for 15–30 min and examined the clear zone underneath (after the growth was scrapped off) for amylolytic activity.

Lipolytic activity

Surface-dried plates of tributyrin agar (Leuschner *et al.* 1997) were streaked with 24-h-old culture and incubated at 30 °C for 4 days (lactic acid bacteria) and 37 °C for 2 days (endospore-forming bacteria). Lipolytic activity was detected by a clear zone surrounding the culture in the turbid tributyrin agar.

Protease activity assay

Protease activity was measured by a modification of the method of Maeda *et al.* (1993). Cultures were grown in

phytone broth (Nagai *et al*. 1994) on a rotary shaking incubator at 30 °C at 180 rev/min for 72 h. Cultures were immediately centrifuged at 17,000 rev/min for 10 min. The enzyme solution was diluted to an appropriate concentration. The enzyme solution and the substrate solution containing 1% Azocasein (Sigma Chemical Co., USA) was dissolved in 100 mM phosphate buffer, (pH 6.8) were pre-incubated separately at 37 °C for 5 min in a water-bath incubator (RSB-12, Remi, India). The enzyme reaction was started by adding 2 ml of 1% Azocasein to 1 ml of enzyme solution and incubated at 37 °C for 20 min. The reaction was quenched by the addition of 2.5 ml of 10% (w/v) trichloroacetic acid. After centrifugation at 15,000 rev/min for 10 min, 2 ml of supernatant was neutralized with equal amount of 1 N NaOH and the absorbance was measured at 450 nm in UV–VIS spectrophotometer (Specord 200, Analytik Jena, Germany). One unit of protease activity was defined as the quantity required to increase the absorbance by 0.1 under the above conditions.

α-*Amylase activity assay*

The blue value method of Fuwa (1954) as modified by Kawaguchi *et al*. (1992) was followed for determination of α-amylase activity. Cultures were grown on broth medium (1.0% soluble starch, 1.0% beef extract, 1.0% peptone, and 0.3% NaCl, pH 7.0) on a rotary shaking incubator at 30 °C at 180 rev/min for 48 h. The cultures were immediately centrifuged at 17,000 rev/min for 10 min. The enzyme solution was diluted to an appropriate concentration. The enzyme solution and 1.5% soluble starch dissolved in 100 mM Tris–HCl buffer (pH 7.0) were pre-incubated separately at 37 °C for 5 min in water-bath shaker (RSB-12, Remi, India). Then, the reaction mixture was started by adding 1 ml of 1.5% soluble starch (HiMedia RM089, India) to 0.5 ml enzyme solution and incubated at 37 °C for 10 min. The reaction was stopped by the addition of 2.5 ml of stop solution (0.5 M acetic acid–0.5 M HCl, 5:1). Then 100 ml of the reaction mixture was added to potassium iodide solution, left at room temperature for 20 min and the absorbance at 660 nm of the resulting solution was measured in UV–VIS spectrophotometer (Specord 200, Analytik Jena, Germany). One unit of α-amylase activity was defined as the amount of α-amylase which produced 10% reduction in the intensity of blue colour under the above conditions.

Antagonism and bacteriocin activity

Isolates were screened for antagonistic activity by the deferred inhibition test described by Schillinger & Lücke (1989), using the bacteriocin screening medium (Tichaczek *et al*. 1992). The indicator strains used for antagonist as well as bacteriocin screening included: *Listeria monocytogenes* DSM 20600, *Bacillus cereus* CCM 2010, *Enterococcus faecium* DSM 20477 and *Streptococcus mutans* DSM 6178. The bacteriocin activity of the isolates was determined using cell-free neutralized supernatants (Uhlman *et al*. 1992).

Biogenic amine

The ability of functional LAB to produce biogenic amines was determined qualitatively on an improved screening medium as described by Bover-Cid & Holzapfel (1999) using a 'cocktail' of four precursor amino acids (histidine, lysine, ornithine and tyrosine).

Hydrophobicity

The degree of hydrophobicity of the strains was determined by employing the methods described by Rosenberg (1984) and Ding & Lämmler (1992). These methods were based on adhesion of cells to hexadecane droplets. Cultures were grown in 5 ml of MRS broth, centrifuged at 7500 rev/min for 5 min and the cell pellet was washed with 9 ml of Ringer solution (Merck), resuspended in a cylomixer and repeated twice. The 1 ml of the suspension was taken and the absorbance at 580 nm was measured in UV–VIS spectrophotometer (Specord 200, Analytik Jena, Germany). Then 1.5 ml of the suspension was mixed with 1.5 ml of *n*-hexadecane (HiMedia RM 2238, India) in duplicate and mixed thoroughly in a cylomixer for 2 min. The two phases were allowed to separate for 30 min. The 1 ml of the lower phase was taken and the absorbance at 580 nm was measured. The percentage hydrophobicity of strain adhering to hexadecane was calculated using the equation:

$$\text{Hydrophobicity (\%)} = \frac{\text{OD}_{580}(\text{initial}) - \text{OD}_{580}(\text{with hexadecane}) \times 100}{\text{OD}_{580}(\text{initial})}$$

Replications

All samples were analysed using duplicate subsamples. Microbiological data were calculated from two to four counting plates for each subsample and microorganism, and reported as mean values.

Results

Microorganisms

Six samples of each ngari, hentak and tungtap were analysed for microbial load, respectively (Table 1). The load of lactic acid bacteria (LAB) was higher than that of other microorganisms in all three fermented fish products. Mould was not recovered in any sample analysed. Out of 121 LAB strains isolated from ngari, hentak and tungtap, 66 strains were cocci and 55 strains were non-sporeforming rods. All strains of LAB were Gram-positive, non-sporeformers, non-motile, catalase-

Table 1. Microbial counts of fermented fish products.

Product	Log c.f.u./g sample			
	LAB	Bacterial endospores	Yeast	AMC
Ngari[a]	6.8 (5.8–7.2)	4.2 (3.3–4.6)	3.1 (2.8–3.3)	7.0 (6.3–7.2)
Hentak[a]	4.6 (4.0–5.8)	3.8 (3.3–4.2)	<1	4.7 (4.3–5.9)
Tungtap[a]	5.9 (5.2–6.2)	3.2 (2.5–3.7)	3.0 (2.6–3.5)	6.1 (5.3–6.3)

c.f.u. – Colony forming unit; LAB – lactic acid bacteria; AMC – aerobic mesophilic count.
[a] Data represent the means of six samples. Ranges are given in parentheses.

negative and facultative anaerobes; they did not hydrolyse casein, gelatin and starch. Gas production from glucose was used as a first step in the differentiation of lactic rods (Kandler 1983). Heterofermentative LAB was identified as *Lactobacillus fructosus* and homofermentative LABs were identified as *Lactobacillus amylophilus*, *Lactobacillus coryniformis* subsp. *torquens* and *Lactobacillus plantarum* (Table 2) on the basis of sugar fermentation using the API system, lactic acid isomer and *meso*-diaminopimelic acid determination, and also based on the taxonomical keys of Sneath *et al*. (1986) and Wood & Holzapfel (1995). Coccal LABs were identified as *Lactococcus plantarum*, *Lactococcus lactis* subsp. *cremoris* and *Enterococcus faecium* (Table 2). Forty-six strains of endospore-forming rods were isolated from fermented fish samples. Based on key of Slepecky & Hemphill (1992) representing all species of *Bacillus* described by Claus & Berkeley (1986), representative strains of endospore-forming rods were identified as *Bacillus subtilis* and *Bacillus pumilus*. Sixteen strains of aerobic cocci were isolated from fermented fish products. All strains were Gram-positive, cocci in tetrads and clusters, non-spore-formers, non-motile and catalase-positive. These strains were identified as *Micrococcus*. However, the species could not be identified due to limited tests. Sixteen strains of yeasts were isolated from ngari and tungtap. On the basis of the taxonomical keys of Kurtzman & Fell (1998), yeasts strains were identified as *Candida* and *Saccharomycopsis* (Table 3). Yeasts were not recovered from hentak samples.

Pathogenic contaminants

Pathogenic contaminants *Bacillus cereus, Staphylococcus aureus* and enterobacteriaceae were detected in ngari, hentak and tungtap (Table 4). Count of *Bacillus cereus* was $<10^2$ c.f.u./g whereas the number of enterobacteriaceae and *Staphylococcus aureus* were $<10^3$ c.f.u./g.

Enzymatic activity and antimicrobial activity

Representative strains of LAB and *Bacillus*, isolated from ngari, hentak and tungtap were tested for protease, α-amylase and lipolytic activities, respectively (Table 5). LAB showed low protease activity whereas *Bacillus subtilis* showed remarkable high activity. Antagonistic properties of LAB and *Bacillus* isolates were tested against the indicator strains (*Listeria monocytogenes* DSM 20600), *Bacillus cereus* CCM 2010, *Enterococcus faecium* DSM 20477 and *Streptococcus mutans* DSM 6178). *Lactobacillus coryniformis* subsp. *torquens* T2:L1 (isolated from tungtap) showed inhibition zone against *Enterococcus faecium* DSM 20477 and *Bacillus subtilis* T1:S1 (isolated from tungtap) against *Streptococcus mutans* DSM 6178. None of the strains were found to produce any bacteriocin with the methods applied (data not shown).

Biogenic amines

Isolates of LAB and *Bacillus* spp. were screened for their ability to produce biogenic amines with the surface plate method as described by Bover-Cid & Holzapfel (1999). Interestingly, none of the strains produced tyramine, cadaverine, histidine and putrescine in the method applied.

Hydrophobicity

Table 6 shows the percentage hydrophobicity of the LAB isolates. Two strains of LAB showed high degrees of hydrophobicity (>75%), among which *Lactobacillus fructosus* HL1 (isolated from hentak) showed the highest percentage of hydrophobicity of 84.3%. All tested strains had more than 30% hydrophobicity.

Discussion

Ngari and hentak are unique fermented fish cuisine of Manipur. Tungtap is a traditional fermented fish product consumed by the Khasia tribes in Meghalaya in North-East India. Lactic acid bacteria were pre-dominant in ngari, hentak and tungtap. LAB species were also reported from other Asian fermented fish products such as species of *Lactobacillus* from nam-plaa and kapi, fermented fish products of Thailand (Tanasupawat *et al*. 1992). Though the load of *Bacillus* spp. was around 10^4 c.f.u./g, their presence shows the dominance in fish products next to LAB. *Bacillus* species were found in the fish products due to their ability as endospore formers to survive under the prevailing conditions (Crisan & Sands 1975). Species of *Micrococcus* were also recovered in a few samples of ngari, hentak and tungtap. Species of *Micrococcus* have also been reported from some fermented fish products of Thailand (Phithakpol 1993) and Japan (Wu *et al*. 2000). Yeasts such as *Candida* and *Saccharomycopsis* were also present in ngari, hentak and tungtap. Species of *Candida* and *Saccharomyces* were also reported from nam-plaa and kapi (Watanaputi *et al*. 1983).

Table 2. Characteristics of lactic acid bacteria strains isolated from fermented fish products of Northeast India.

Product	Strain code	Cell morphology	Cell size (um)	Gram-stain	Catalase	Arginine hydrolysis	CO_2 from glucose	Growth at/in								m-Dpm	Lactic acid isomer	Sugars fermented																	Identity
								10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
Ngari	Ng2:L4	Coccus	D= 0.9 (0.8-1.2)	+	–	–	–	–	–	–	+	–	+	–	–	–	L	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–	*Lactococcus plantarum*
	Ng2:L5	Coccus	D= 1.0 (0.8-1.2)	+	–	–	–	–	–	–	+	–	+	–	–	–	L	–	–	–	–	–	+	–	+	–	–	+	–	–	+	–	–	–	*Lactococcus plantarum*
	Ng1:R2	Rod	L= 1.6 (1.4-1.7) B= 0.8 (0.7-0.9)	+	–	–	–	–	–	–	+	–	+	–	–	+	DL	–	+	–	–	–	–	–	–	–	–	+	–	+	–	+	–	–	*Lactobacillus plantarum*
Hentak	HL1	Rod	L= 1.6 (1.2-2.0) B= 0.9 (0.8-1.2)	+	–	–	+	+	+	–	+	–	+	–	–	–	D(L)	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	*Lactobacillus fructosus*
	H1:B1	Rod	L= 1.8 (1.6-2.0) B= 0.8 (0.4-1.2)	+	–	–	–	+	+	–	+	–	+	–	–	–	L	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	*Lactobacillus amylophilus*
	H2:B3	Coccus	D= 1.1 (0.8-1.2)	+	–	–	–	+	+	+	+	–	+	–	–	–	L	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	–	–	*Enterococcus faecium*
Tungtap	T2:L1	Rod	L= 1.7 (1.6-2.0) B= 0.9 (0.8-1.2)	+	–	–	–	–	–	–	+	–	–	–	–	–	D	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	–	+	*Lactobacillus coryniformis* subsp. *torquens*
	T2:L2	Coccus	D= 0.8 (0.4-1.2)	+	–	–	–	–	–	–	+	–	+	–	–	–	L	–	–	–	+	+	+	–	+	–	–	+	+	+	+	–	–	–	*Lactococcus lactis* subsp. *cremoris*
	T2:L5	Rod	L= 1.3 (1.2-1.6) B= 0.8 (0.7-0.9)	+	–	–	+	–	–	–	+	–	–	–	–	–	D(L)	–	–	–	–	–	–	–	–	–	–	+	–	–	+	w	–	–	*Lactobacillus fructosus*

D – diameter; L – length; B – breadth.

Table 3. Characteristics of yeasts isolated from fermented fish products of Northeast India.

Product	Strain code	Cell morphology	Cell size (µm)	Mycelium	Nitrate reduction	Growth at 37°C	Sugars fermented								Sugars assimilated															Identity
							Glucose	Galactose	Lactose	Maltose	Raffinose	Sucrose	Starch	Trehalose	Arabinose	Cellobiose	Galactose	Glycerol	Inositol	Lactose	Maltose	Melibiose	Mannitol	Rhamnose	Raffinose	Sucrose	Starch	Trehalose	Xylose	
Ngari	Ng:Y1	Oval	L= 5.1 (5.0-5.3) B= 3.1 (3.0-3.2)	Pseudo	–	+	–	–	–	–	–	–	–	–	+	+	+	+	–	–	+	+	+	–	+	+	–	+	+	*Candida sp.*
	Ng:Y2	Oval	L= 6.2 (6.1-6.4) B= 3.2 (2.2-4.5)	Absent	–	–	–	–	–	–	–	–	–	–	+	+	+	+	–	+	+	–	+	–	+	+	–	+	+	*Candida* sp.
Tungtap	T1:Y1	Oval	L= 7.1 (5.7-7.7) B= 4.7 (3.5-4.9)	Pseudo	–	+	+	–	–	–	–	+	–	–	+	–	+	+	–	–	+	–	+	–	–	+	–	–	+	*Candida* sp.
	T1:Y2	Cylindrical	L= 23.6 (20.1-25.0) B= 5.6 (4.1-7.1)	TRUE	–	–	–	–	–	–	–	–	–	–	+	–	+	+	–	–	+	–	+	–	–	+	–	–	+	*Saccharomycopsis* sp.

L – length; B – breadth. Ascus and ascopsores are absent in all strains.

Table 4. Microbial counts of pathogenic contaminants in fermented fish products.

Product	Log c.f.u./g sample		
	Bacillus cereus	*Staphylococcus aureus*	Enterobacteriaceae
Ngari[a]	2.3 (1.8–2.6)	3.0 (2.5–3.5)	3.3 (3.1–3.5)
Hentak[a]	2.2 (1.7–2.4)	2.8 (2.4–3.0)	3.0 (2.7–3.3)
Tungtap[a]	2.3 (1.7–2.5)	<1	3.5 (2.9–3.9)

[a] Data represent the means of six samples. Ranges are given in parentheses.

Table 6. Percentage hydrophobicity of LAB strains isolated from fermented fish products.

Product	Strain	% Hydrophobicity
Ngari	*Lactococcus plantarum* Ng1:R2	60.0 (+)
	Lactococcus plantarum Ng2:L4	67.2 (+)
	Lactococcus plantarum Ng2:L5	53.0 (+)
Hentak	*Lactobacillus fructosus* HL1	84.3 (++)
	Lactobacillus amylophilus H1:B1	55.3 (+)
	Enterococcus faecium H2:B3	45.6 (+)
Tungtap	*Lactobacillus coryniformis* subsp. *torquens* T2:L1	63.7 (+)
	Lactococcus lactis subsp. *cremoris* T2:L2	47.1 (+)
	Lactobacillus fructosus T2:L5	81.0 (++)

++ – Hexadecane adherence ≥75% (hydrophobic); + – hexadecane adherence 26–74% (intermediate).

Population of *Bacillus cereus*, *Staphylococcus aureus* and enterobacteriaceae was not more than 10^3 c.f.u./g in samples of ngari, hentak and tungtap, which would be the impact of competition and/or antagonistic reaction of predominant lactic acid bacteria that have prevented the proliferation (Adams & Nicolaides 1997). However, the presence of *Bacillus cereus*, *Staphylococcus aureus* and enterobacteriaceae in fermented fish products was due to contamination during processing. A small number of *Bacillus cereus* counts in foods is not considered significant (Beumer 2001). *Staphylococcus aureus* is regarded as a poor competitor and its growth in fermented foods is generally associated with a failure of the normal microflora (Nychas & Arkoudelos 1990). Though there has been no reported case of toxicity or illness due to consumption of fermented fish products in North-East India, gross contamination of fermented fish products in the region suggests that the products need to be investigated clinically.

All isolates of LAB showed low protease activity, whereas isolates of *Bacillus* strains showed remarkable proteolytic activities. Some isolates of LAB showed high amylolytic activity. Proteolysis and liquefaction that occur during fish production has been reported to be largely the result of autolytic breakdown of the fish tissues (Backhoff 1976). *Lactobacillus coryniformis* subsp. *torquens* T2:L1, isolated from tungtap, showed antagonistic properties against the indicator strains, which can reduce the number of other undesired microorganisms in the fish products as well as help in the preservation of fish (Einarsson & Lauzon 1995). Biogenic amines have been reported in fish products (Ten Brink *et al*. 1990). None of the isolates tested was found to decarboxylase the amino acids used tyrosine, lysine, histidine and ornithine. However, the lack of histamine, tyramine, cadaverine and putrescine producers isolated from traditionally fermented fish products in our study could possible be explained by the lack of free amino acids within the samples. The concentration of amino acids in food is important for biogenic amines formation (Joosten & Northolt 1989). Before confirming the non-production of biogenic amines in ngari, hentak and tungtap, qualitative and quantitative analysis of biogenic amine is necessary.

Adherence is one of the most important selection criteria for probiotic bacteria (Shah 2001). Strains of LAB isolated from ngari, hentak and tungtap showed high degrees of hydrophobicity indicating that the

Table 5. Enzymatic activity of the selected strains, isolated from fermented fish products.

Product	Strain	Protease[a] (U/ml)	α-Amylase[a] (U/ml)	Lipolytic activity
Ngari	*Lb. plantarum* Ng1:R2	0.5	1.1	–
	Lactococcus plantarum Ng2:L4	0.5	1.0	–
	Lactococcus plantarum Ng2:L5	0.4	4.4	+
	Bacillus subtilis Ng1:S1	4.3	1.2	+
	Bacillus pumilus Ng1:S2	1.0	0	–
Hentak	*Lb. fructosus* HL1	0.3	1.0	–
	Lb. amylophilus H1:B1	0.3	0.9	–
	Enterococcus faecium H2:B3	0.8	1.2	+
	Bacillus subtilis H1:S1	4.5	2.3	+
Tungtap	*Lb. coryniformis* subsp. *torquens* T2:L1	1.0	1.1	–
	Lactococcus lactis subsp. *cremoris* T2:L2	0.4	3.1	–
	Lb. fructosus T2:L5	0.3	1.1	+
	Bacillus subtilis T1:S1	2.7	3.2	+

[a] Strains showing positive hydrolysis test (>2.0 mm) were assayed. Data represent the means of three sets. Lb – *Lactobacillus*.

strains were not hydrophilic in nature. Functional effects of probiotic bacteria include adherence to the intestinal cell wall for colonization in the gastrointestinal tract with capacity to prevent pathogenic adherence or pathogen activation (Salminen *et al.* 1996). The high degree of hydrophobicity by some LAB strains isolated from ngari, hentak and tungtap indicates the potential of adhesion to gut epithelial cells of human intestine, advocating their 'probiotic' character. Lactic acid bacteria are normal residents of the complex ecosystem of the gastrointestinal tract (Holzapfel *et al.* 1998).

This study has demonstrated the microbial diversity ranging from species of lactic acid bacteria belonging to coccal-lactics (*Lactococcus*, *Enterococcus*) to species of homofermentative and heterofermentative rods (*Lactobacillus*), endospore-forming rods (*Bacillus*), aerobic coccus (*Micrococcus*) to species of yeasts (*Candida*, *Saccharomycopsis*) along with pathogenic contaminants such as *Bacillus cereus*, *Staphylococcus aureus* and enterobacteriaceae in indigenous fermented fish products of North-East India. Some strains of LAB possess the protective and functional properties which can be used as starter culture(s) for controlled optimized production of fish preservation. The isolated, identified and preserved microorganisms from lesser-known fish products may contribute significant information on the unknown microbial gene pool as genetic resources of the Himalayan regions.

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