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Microbiological studies of ethnic meat products of the Eastern Himalayas

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ABSTRACT

Native microorganisms from some ethnic meat products of the Eastern Himalayas such as *lang kargyong*, *yak kargyong*, *faak kargyong*, *lang satchu*, *yak satchu* and *suka ko masu* were isolated and characterized. The bacterial isolates included *Lactobacillus sake*, *Lactobacillus curvatus*, *Lactobacillus divergens*, *Lactobacillus carnis*, *Lactobacillus sanfrancisco*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus brevis*, *Enterococcus faecium*, *Leuconostoc mesenteroides*, *Pediococcus pentosaceous*, *Bacillus subtilis*, *Bacillus mycoides*, *Bacillus thuringiensis*, *Bacillus lentus* and *Bacillus licheniformis*, *Micrococcus* and *Staphylococcus*. Yeast isolates included *Debaryomyces hansenii*, *Debaryomyces polymorphus*, *Debaryomyces pseudopolymorphus*, *Pichia burtonii*, *Pichia anomala*, *Candida famata* and the mould *Rhizopus* was also identified. Many of the LAB isolates demonstrated some antimicrobial activity, enzymatic activity and a few showed a high degree of hydrophobicity. None of the strains produced biogenic amines.

1. Introduction

Meat is a part of the daily diet for many people living in the Himalayan regions of India, Nepal, Bhutan and China (Tamang, 2010). The domestic livestock species of the Himalayan regions are mostly cattle, sheep, goats, pigs and yaks which are mainly used for meat and milk products. Ethnic food has always been important to the people of the Eastern Himalayas. About 11.7% of people in Sikkim are vegetarian and 88.3% are non-vegetarians (Tamang et al., 2007). Most of the ethnic people in the Himalayas slaughter domestic animals, consume the fresh meat and the rest is preserved by smoking, drying or fermenting. *Kargyong* is an ethnic sausage-like product prepared from yak, beef and pork meat, and is mostly consumed by the Bhutia, Tibetans, Drupka, Lepcha, and Sherpa in the Himalayas (Rai, Palni, & Tamang, 2009). Three varieties of *kargyong* are prepared and consumed: *yak kargyong* (prepared from yak meat), *lang kargyong* (prepared from beef), and *faak kargyong* (prepared from pork). *Yak kargyong* is a popular fermented sausage in Sikkim, Ladak, Tibet, Arunachal Pradesh and Bhutan in the Himalayas. During the preparation of *kargyong*, meat and its fat are finely chopped and combined with crushed garlic, ginger, and salt and mixed with water. The mixture is stuffed into the segment of gastro-intestinal tract called *gyuma*, used as natural casings, 3–4 cm in diameter and 40–60 cm long. One end of the casing is tied up with rope, and other end is sealed after stuffing and boiled for 20–30 min. Cooked sausages are hung in the bamboo strips above the kitchen oven for smoking and drying for 10–15 days or more to make *kargyong*. It is sliced and fried in edible oil with onion, tomato, powdered or ground chillies, and salt and is made into curry.

Satchu is an air dried or smoked meat product of the Eastern Himalayas, mostly prepared from yak and beef meat. During the preparation of *satchu*, red meat is cut into several strands about 60–90 cm long 2–5 cm thick and then mixed thoroughly with turmeric powder, edible oil or butter and salt. The meat strands are hung in the bamboo strips and are kept in open air in the house or are smoked above the kitchen oven for 10–15 days. Deep fried *satchu* is a popular side-dish which is eaten with traditional alcoholic beverages. *Suka ko masu* is an air dried or smoked chevon or buffalo meat product, and is traditionally consumed by the Gorkhas of the Himalayas. Its preparation is similar to *satchu*.

The aim of the present work was to isolate, characterize and identify the predominant native microorganisms associated with the ethnic meat products of the Eastern Himalayas, and to understand some of their functional activities such as antimicrobial and enzymatic activities, production of biogenic amines and degree of hydrophobicity. This is the first report of the microbiology of Himalayan ethnic meat products.

2. Materials and methods

2.1. Samples

A total of 52 samples of traditionally prepared meat products were collected directly from different places of Sikkim, Darjeeling

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hills in India and Bhutan. Out of 52 samples collected, 17 were *lang kargyong*, 7 *yak kargyong*, 8 *faak kargyong*, 8 *lang satchu*, 6 *yak satchu* and 6 samples were *suka ko masu*. Samples were collected aseptically in polythene bags as well as sterile bottles and were, sealed and labelled. Samples were stored at −20 °C for microbial and biochemical analyses.

2.2. Microbiological analysis

Samples (10 g) were mixed with 90 ml of 0.85% (w/v) sterile physiological saline and thoroughly homogenized using a lab-blender stomacher (400, Seward, UK) for 1 min. A serial dilution in the same diluent was made. Lactic acid bacteria (LAB) were isolated on MRS agar (M641, HiMedia, India) plates supplemented with 1% $CaCO_3$, and were incubated at 30 °C under anaerobic condition in an Anaerobic Gas-Pack container (LE002, HiMedia, India) for 48 h. Mannitol-salt phenol-red agar (Merck, Germany) media were used for the detection of micrococcaceae following the method of Papamanoli, Kotzekidou, Tzanetakis, and Tzanetaki (2002). Inoculated plates were incubated at 30 °C for 48 h. Identification of micrococci was further confirmed by growing in FTO (furazolidone) agar (von Rheinbaben & Hadlok, 1981). Spread plates of Baird Parker agar base (M043, HiMedia, India) with appropriate additions of egg yolk tellurite emulsion (FD046, HiMedia, India) was used for enumeration of staphylococci. Spore-forming bacilli were isolated on nutrient agar (MM012, HiMedia, India), after inactivation of vegetative cells by heating at 100 °C for 2 min (Tamang & Nikkuni, 1996) and then incubated at 37 °C for 24 h. Moulds and yeasts were isolated on potato dextrose agar (M096, HiMedia, India) and yeast–malt extract (YM) agar (M424, HiMedia, India), supplemented with 10 IU/ml benzylpenicillin and 12 μg/ml streptomycin sulphate, respectively; and plates were incubated aerobically at 28 °C for 72 h. Total viable count (TVC) was determined in the plate count agar (M091A, HiMedia, India) plates which were incubated at 30 °C for 48–72 h. Colonies of all microorganisms were selected randomly. Purity of the isolates was checked by streaking again on fresh agar plates of the isolation media and sub-culturing on corresponding broths/agar, followed by microscopic examinations. Identified strains of microorganisms were preserved in respective media using 15% (v/v) glycerol at −20 °C. Samples were also tested for pathogenic bacteria using selective media *Bacillus cereus* agar base (M833, HiMedia, India) for *B. cereus*, violet red bile glucose agar w/o lactose (M581, HiMedia, India) for enterobacteriaceae (Han, Beumer, Rombouts, & Nout, 2001). Salmonella–Shigella agar (M108, HiMedia, India) was used for the detection of *Salmonella* and *Shigella* and *Listeria* identification agar base (M1064, HiMedia, India) with *Listeria* selective supplement (FD 061, HiMedia, India) for *Listeria* in the samples following the standard method of Metaxopoulos, Samelis, & Papadelli (2001).

2.3. Phenotypic characterization and identification

Cell morphology of isolates as well as their motility was determined using a phase contrast microscope (Olympus CH3-BH-PC, Japan). LAB isolates were Gram-stained and tested for catalase production, and were preliminarily identified based on their phenotypic properties such as gas production from glucose, ammonia from arginine, production of dextran from sucrose, growth at different temperatures (8, 10, 15 and 45 °C) and pH (3.9 and 9.6) as well as their ability to grow in different concentrations of sodium chloride (6.5%, 10% and 18%) in MRS broth, following the methods of Dykes, Britz, & von Holy (1994) and Schillinger & Lücke (1987). The configuration of lactic acid produced from glucose was determined enzymatically using commercial D-lactate and L-lactate dehydrogenase test kits (Boehringer-Mannheim GmbH, Cat. No. 1112821, Germany). Carbohydrate fermentation patterns of LAB isolates were determined using the API 50 CHL test strips (bioMérieux, France) and the APILAB PLUS database identification software (bioMérieux, France). The preliminary differential characteristics of micrococci and staphylococci were performed on the basis of cell morphology, tetrad formation observed microscopically, production of catalase, arginine hydrolysis, and growth at NaCl 10%. The strains were further confirmed using FTO agar (von Rheinbaben & Hadlok, 1981). Endospore-forming rods were Gram-stained and tested for catalase production, acid from glucose and were preliminarily identified based on phenotypic properties such as gas production from glucose, arginine hydrolysis, nitrate reduction, growth at different temperatures and at pH 6.8 as well as the ability to grow in different concentrations of sodium chloride. Based on the taxonomical keys of Claus and Berkeley (1986) and Slepecky and Hemphill (1992), all spore-forming rods were Gram-positive, catalase-positive, aerobic and motile. Voges-Proskauer reaction, pH in VP broth, starch and casein hydrolysis was performed on the basis of methods proposed by Gordon, Haynes, & Pang (1973). Anaerobic growth (Claus & Berkeley, 1986), bile salt tolerance (Duc, Hong, Barbosa, Henriques, & Cutting, 2004) and citrate utilization test (Osborne & Stokes, 1955) were also used to identify bacilli. Yeast isolates were identified according to taxonomical keys described by Kurtzman and Fell (1998).

Table 1
Microbiological populations of ethnic meat products collected from different parts of the Eastern Himalayas.

Product	pH	Log cfu/g sample					
		Bacteria			Yeast	Moulds	TVC
		LAB	Bacilli	Micrococcaceae			
Lang kargyong (n = 17)	6.7 ± 0.3	6.5 ± 0.6	1.8 ± 0.4	5.6 ± 0.4	5.6 ± 0.3	1.2 ± 0.2	8.3 ± 0.3
Yak kargyong (n = 7)	6.9 ± 0.2	6.4 ± 0.6	1.7 ± 0.6	5.6 ± 0.2	4.9 ± 0.4	2.1 ± 0.3	8.0 ± 0.7
Faak kargyong (n = 8)	6.5 ± 0.3	7.3 ± 0.2	1.5 ± 0.3	6.0 ± 0.3	5.3 ± 0.3	DL	8.2 ± 0.2
Lang satchu (n = 8)	6.1 ± 0.2	7.0 ± 0.4	1.7 ± 0.5	6.5 ± 0.3	5.1 ± 0.2	0.3 ± 0.1	8.1 ± 0.3
Yak satchu (n = 6)	5.7 ± 0.2	6.4 ± 0.3	1.7 ± 0.5	5.8 ± 0.6	4.6 ± 0.4	0.2 ± 0.1	8.2 ± 0.3
Suka ko masu (n = 6)	5.3 ± 0.3	6.0 ± 0.1	2.5 ± 0.1	4.9 ± 0.1	4.7 ± 0.1	1.0 ± 0.1	5.4 ± 0.2

cfu, Colony forming unit; n, number of samples; DL, detection limit less than 10 cfu and TVC, total viable count.
Data are the means (±SD) of triplicate sets of experiments.

Table 2
Phenotypic characteristics of the LAB isolated from the Himalayan ethnic meat products.

Product	No. of strains	Cell shape	Gas from glucose	NH_3 from arginine	Lactate isomer	Growth at 8 °C	Growth at 45 °C	Sugars fermented																		
								Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	Mannitol	Identity
Lang kargyong (24)	3	Rod	–	–	DL	+	–	–	+	1/2	+	+	+	–	+	–	+	+	+	–	–	+	2/1	–	–	*Lb. sake*
	1	Rod	–	–	DL	+	+	–	+	–	+	+	–	–	+	–	+	+	+	+	+	–	–	–	–	*Lb. curvatus*
	2	Rod	+	+	L	+	–	–	+	+	+	+	+	–	+	–	1/1	+	+	+	+	+	+	–	–	*Lb. divergens*
	5	Rod	–	+	L	+	–	–	+	1/4	+	+	+	–	+	1/4	+	+	+	+	+	+	+	–	+	*Lb. carnis*
	6	Rod	+	–	DL	4/2	–	–	5/1	4/2	+	+	+	–	+	1/5	4/2	+	5/1	+	+	5/1	4/2	1/5	–	*Lb. sanfransisco*
	3	Coccus	–	+	L	–	+	–	1/2	+	2/1	+	+	–	+	–	1/2	1/2	1/2	+	+	+	+	–	+	*Enterococcus faecium*
	4	Coccoid	+	–	D	–	1/3	+	3/1	+	+	+	3/1	–	+	–	3/1	+	+	+	+	+	+	–	3/1	*Leuconostoc mesenteroides*
Faak kargyong (18)	1	Rod	+	+	DL	+	–	–	+	+	+	+	+	–	+	–	+	+	–	+	+	+	+	–	–	*Lb. brevis*
	2	Rod	–	–	DL	+	+	–	+	–	+	+	+	–	+	1/1	+	+	+	+	+	+	+	–	+	*Lb. plantarum*
	3	Rod	–	+	L	+	–	1/2	2/1	–	+	+	+	1/2	+	1/2	+	+	+	+	+	+	+	–	+	*Lb. carnis*
	8	Coccoid	+	–	D	–	–	7/1	6/2	+	+	+	+	–	+	2/6	+	6/2	6/2	+	+	+	+	–	7/1	*Leuc. mesenteroides*
	4	Coccus	–	+	L	–	+	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	*E. faecium*
Yak kargyong (18)	3	Rod	–	1/2	DL	+	–	–	+	–	+	+	+	–	+	–	+	+	+	1/2	1/2	+	–	–	–	*Lb. sake*
	3	Rod	–	–	DL	+	–	–	+	–	+	+	2/1	–	+	–	2/1	2/1	2/1	+	2/1	–	+	–	+	*Lb. plantarum*
	2	Rod	–	–	L	+	1/1	–	+	–	+	1/1	–	–	+	–	+	+	+	+	+	+	–	–	+	*Lb. casei*
	1	Rod	–	–	DL	+	–	–	+	–	+	–	–	–	+	–	+	+	+	+	+	+	–	–	–	*Lb. curvatus*
	2	Rod	–	+	L	+	–	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	–	–	+	*Lb. carnis*
	1	Rod	+	+	L	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	–	–	–	–	+	*Lb. divergens*
	2	Rod	+	–	DL	1/1	–	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	–	–	–	*Lb. sanfransisco*
	2	Coccoid	+	–	D	–	+	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	*Leuc. mesenteroides*
	2	Coccus	–	+	L	1/1	+	–	+	1/1	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	*E. faecium*
Lang satchu (19)	1	Rod	–	–	L	+	–	–	+	–	+	+	+	–	+	+	+	+	+	+	–	–	–	+	+	*Lb. casei*
	1	Rod	–	+	L	+	–	–	+	+	+	+	+	–	+	+	+	+	+	+	–	–	–	+	+	*Lb. carnis*
	5	Coccus (tetrad)	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	3/2	–	–	–	–	*Pediococcus pentosaceous*
	12	Coccus	–	+	L	–	+	–	+	9/3	+	+	+	7/5	9/3	+	11/1	+	+	+	+	10/2	9/3	+	+	*E. faecium*
Yak satchu (14)	14	Coccus (tetrad)	–	+	DL	–	+	–	+	+	+	4/10	+	–	+	–	11/3	+	+	+	+	10/4	5/9	–	–	*P. pentosaceous*
Suka ko masu (15)	3	Rod	–	–	DL	+	–	–	+	1/2	+	+	+	+	+	–	+	+	+	+	+	+	+	1/2	+	*Lb. plantarum*
	2	Rod	–	+	L	–	–	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	–	+	*Lb. carnis*
	10	Coccus	–	+	L	–	+	2/8	+	+	+	+	+	5/5	+	4/6	+	+	+	+	+	+	9/1	–	+	*E. faecium*

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.
Number of isolates is given in parenthesis; +, all strains positive; –, all strains negative; (../..), number of positive/negative strains.

Table 3
Phenotypic characteristics of the bacilli isolated from the Himalayan ethnic meat products.

Product	No. of strains	Cell morphology	Gram-stain	Catalase	Gas from glucose	Acid from glucose	Nitrate reduction	Arginine hydrolysis	Growth at pH 6.8	Growth at NaCl 7.0%	Growth at NaCl 10%	Anaerobic growth	Growth at 5 °C	Growth at 40 °C	Growth at 50 °C	Growth at 65 °C	Starch hydrolysis	Casein hydrolysis	Voges-Proskauer reaction	pH in VP broth	Acid from arabinose	Acid from mannitol	Acid from xylose	Citrate test	Bile test	Identity
Lang	2	Rod	+	+	−	1/1	+	+	+	1/1	+	−	−	+	+	−	+	+	+	5.8	−	+	+	+	−	*B. subtilis*
kargyong	1	Rod	+	+	−	−	+	+	+	−	+	+	−	+	−	−	+	+	+	5.4	−	−	−	−	−	*B. thuringiensis*
(4)	1	Rod	+	+	−	−−	+	+	+	−	+	+	−	+	−	−	+	+	+	5.4	−	−	−	+	−	*B. mycoides*
Faak	1	Rod	+	+	−	−	−	+	+	−	+	+	−	+	+	−	+	+	+	6.1	−	+	+	+	−	*B. subtilis*
kargyong	2	Rod	+	+	−	−	+	+	+	+	+	+	−	+	+	−	+	+	+	5.8	−	1/1	−	1/1	−	*B. mycoides*
(4)	1	Rod	+	+	−	−	−	+	+	−	+	+	−	+	+	−	+	+	+	6	−	+	−	+	−	*B. licheniformis*
Yak	2	Rod	+	+	−	1/1	+	+	+	+	+	1/1	−	+	1/1	−	+	+	+	6.4	−	+	1/1	+	−	*B. subtilis*
kargyong	1	Rod	+	+	−	−	+	+	+	+	+	+	−	+	+	−	+	+	+	6	−	−	−	+	+	*B. mycoides*
(3)																										
Lang satchu	1	Rod	+	+	−	+	−	+	+	−	+	−	−	+	+	−	+	+	+	6.1	−	+	+	+	−	*B. subtilis*
(4)	1	Rod	+	+	−	−	+	+	+	+	−	−	−	+	+	−	+	+	−	7.9	−	+	+	+	−	*B. lentus*
	2	Rod	+	+	−	−	+	+	+	−	1/1	+	−	+	−	−	+	+	+	5.4	−	−	−	+	−	*B. mycoides*
Yak satchu	1	Rod	+	+	−	+	+	+	+	+	+	−	−	+	+	−	+	+	+	6.1	−	+	+	+	−	*B. subtilis*
(4)	1	Rod	+	+	−	−	+	+	+	+	+	+	−	+	−	−	+	+	+	6	−	+	−	+	+	*B. licheniformis*
	2	Rod	+	+	−	−	+	+	+	1/1	+	1/1	−	+	1/1	−	+	+	+	6.2	−	−	−	+	−	*B. mycoides*
Suka ko masu (3)	1	Rod	+	+	−	−	+	+	+	+	+	+	−	+	+	−	+	+	−	6.9	−	−	−	+	+	*B. thuringiensis*
	1	Rod	+	+	−	+	+	+	+	+	+	−	−	+	−	−	+	+	+	6.4	−	+	+	−	+	*B. subtilis*
	1	Rod	+	+	−	−	+	+	+	+	+	+	−	+	+	−	+	+	+	5.7	−	−	−	+	−	*B. mycoides*

All isolates were Gram-positive, catalase-positive, aerobic, motile and sporeformers.
Number of isolates is given in parenthesis; +, all strains positive; −, all strains negative; (../..), number of positive/negative strains.

2.4. pH

The pH of the samples (10 g) was determined using a digital pH meter (Type 361, Systronics, India) calibrated with standard buffer solutions (Merck, Germany), after homogenization in 20 ml of carbon dioxide free distilled water.

2.5. Antimicrobial activity

A total of 92 isolates of LAB were screened for antimicrobial activity by the agar spot method of Schillinger and Lücke (1989). The indicator strains used for antagonisms were *Listeria innocua* DSM 20649, *Listeria monocytogenes* DSM 20600, *B. cereus* CCM 2010, *Klebsiella pneumoniae* subsp. *pneumoniae* BFE 147, *Enterobacter 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, Schillinger, Rupnow, & Holzapfel, 1992), using the bacteriocin screening medium (MRS agar containing only 0.2% glucose) as described by Tichaczek, Nissen-Meyer, Nes, Vogel, and Hammes (1992).

2.6. Enzymatic activities

Enzymatic activities of LAB isolates were assayed using the commercial API-zym galleries (bioMérieux, France). Cultures were grown on MRS agar for 48 h, and were then centrifuged, the supernatant was discarded and the precipitate (cells) mixed aseptically with 2 ml sterile saline which was used to prepare suspension of 10^7 cells/ml. The strip was unpacked and two drops of cell suspension was inoculated in each cup of the strip containing ready-made enzyme substrates and incubated at 30 °C for 6 h. After incubation, one drop of zym-A and zym-B reagents were added and colour development, based on the manufacturer's colour chart, monitored. Values ranging from 0 to 5 were assigned, corresponding to the colours developed: 0 corresponds to a negative reaction, 5 (=40 nmol) to a reaction of maximum intensity, and values 4, 3, 2 and 1 were intermediate corresponding to 30, 20, 10 and 5 nmol, respectively.

2.7. Biogenic amines

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

2.8. Hydrophobicity assay

Hydrophobicity of the isolates was determined as described by Tamang, Tamang, Schillinger, Guigas, and Holzapfel (2009). 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. About 1 ml of the suspension was taken and the absorbance at 580 nm measured. Then, 1.5 ml of the suspension was mixed with an equal volume of *n*-hexadecane (RM 2238, HiMedia, India), in duplicate 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 triplicates. A hydrophobic index greater than 70% was arbitrarily classified as hydrophobic (Nostro et al., 2004).

3. Results and discussion

3.1. Microbial counts and pH

A total of 52 samples of ethnic meat products were collected and analyzed (Table 1). In all traditionally prepared meat products, lactic acid bacteria (LAB) were found at 10^6–10^8 cfu/g. Yeasts were also recovered in all samples at 10^4–10^6 cfu/g. The counts of bacilli were $<10^3$ cfu/g. Filamentous moulds were also detected in a few samples at less than 10^3 cfu/g. The occurrence of micrococcaceae was found at 10^4–10^7 cfu/g. The total viable count in the samples, collected from different places of the Himalayas, varied between 10^5 and 10^9 cfu/g. The mean pH of the samples ranged from 5.3 to 6.9, with *suka ko masu* having the lowest pH (Table 1).

3.2. Identification

The bacterial strains isolated from *lang kargyong* (92), *yak kargyong* (53), *faak kargyong* (78), *lang satchu* (93), *yak satchu* (58), *suka ko masu* (66) were initially considered lactic acid bacteria due to their Gram-positive reaction, no hydrolysis of catalase, were non-sporing and non-motile. Out of 440 isolates of LAB isolated from the Himalayan ethnic meat products, a total of 108 representative strains were grouped on the basis of cell morphology, carbon dioxide production from glucose and hydrolysis of arginine gas production and were phenotypically characterized on the basis of growth temperatures, pH and salt tolerance, and lactate configuration (Table 2). In addition, carbohydrate fermentation patterns using API system were performed. Rod strains of LAB isolated from traditionally prepared meat products of the Himalayas were identified as *Lactobacillus curvatus*, *Lactobacillus sake*, *Lactobacillus divergens*, *Lactobacillus carnis*, *Lactobacillus casei*, *Lactobacillus sanfrancisco*, *Lactobacillus brevis* and *Lactobacillus plantarum*. Phenotypic characterization including carbohydrate fermentation pattern, arginine hydrolysis and growth behaviour at 50 °C confirmed that all tetrad-forming cocci were *Pediococcus* strains as *Pediococcus pentosaceous*. The sugar fermentation profiles using API identification profile and APILAB PLUS database software, also confirmed the identity of *P. pentosaceous*. Coccoid strains were identified as *Enterococcus faecium*. All coccoid strains produced dextran when grown on 5% sucrose agar, and fermented sucrose, galactose, maltose, mannose and xylose, and were identified as *Leuconostoc mesenteroides*.

Based on the taxonomical keys of Claus and Berkeley (1986) and Slepecky and Hemphill (1992), out of 172 isolates of endospore-forming rods, 22 representative strains of bacilli isolates were identified as *Bacillus subtilis*, *Bacillus mycoides*, *Bacillus thuringiensis*, *Bacillus lentus* and *Bacillus licheniformis* (Table 3).

The differential characteristics of micrococci and staphylococci belonging to the family micrococcaceae, following the taxonomical keys described by von Rheinbaben and Hadlok (1981), showed the predominance of *Staphylococcus* sp. (91%) over *Micrococcus* sp. (9%) (Table 4). Predominance of staphylococci over other micrococcaceae in fermented meat products is commonly observed (Papamanoli et al., 2002; Samelis, Metaxopoulos, Vlassi, & Pappa, 1998). The strains were further confirmed as *Micrococcus* sp. showing growth in FTO agar and *Staphylococcus* sp. did not grow in FTO agar.

A total of 215 yeast isolates were isolated from the products. The 19 representative strains of yeast were selected randomly from grouped strains having similar colony appearance, cell shape, type of mycelia and ascospore for detailed identification. Following the taxonomical keys of Kurtzman and Fell (1998), carbohydrate fermentation and assimilation tests of randomly selected representative strains of yeasts were carried out (Table 5). Six species belonging to three yeast genera were identified as follows – *Debaryomyces hansenii* showing smooth surfaced colonies with spheroidal ascospores and fermented glucose weakly, *Pichia anomala* on the basis of sugar fermentation and assimilation tests, they

Table 4
Differential characteristics of *Micrococcus* and *Staphylococcus* isolated from the Himalayan ethnic meat products.

Product	Cell size	Gram-stain	Catalase	Arginine hydrolysis	Tetrads	Growth on NaCl 10%	Growth on FTO agar	Grouped strains	% of Prevalence	Identity
Lang kargyong (45)	1.5 (0.5–2.4)	+	+	–	+	–	+	4	8.9	*Micrococcus*
	1.1 (0.5–1.6)	+	+	+	–	+	–	41	91.1	*Staphylococcus*
Yak kargyong (30)	1.5 (0.5–2.4)	+	+	–	+	–	+	3	10.0	*Micrococcus*
	1.6 (0.5–1.6)	+	+	+	–	+	–	27	90.0	*Staphylococcus*
Faak kargyong (35)	1.0 (0.5–2.4)	+	+	–	+	–	+	3	8.6	*Micrococcus*
	1.2 (0.5–1.6)	+	+	+	–	+	–	32	91.4	*Staphylococcus*
Lang satchu (28)	1.6 (0.5–2.4)	+	+	–	+	–	+	2	7.1	*Micrococcus*
	1.0 (0.5–1.6)	+	+	+	–	+	–	26	92.9	*Staphylococcus*
Yak satchu (40)	1.6 (0.5–2.4)	+	+	–	+	–	+	3	7.5	*Micrococcus*
	1.2 (0.5–1.6)	+	+	+	–	+	–	37	92.5	*Staphylococcus*
Suka ko masu (25)	1.6 (0.5–2.4)	+	+	–	+	–	+	2	8.0	*Micrococcus*
	1.2 (0.5–1.6)	+	+	+	–	+	–	23	92.0	*Staphylococcus*

Number of isolates is given in parenthesis; +, all strains positive; –, all strains negative.
FTO, furazolidone agar.

Table 5
Phenotypic characteristics of the yeasts isolated from the Himalayan ethnic meat products.

Product	No. of strains	Cell shape	Mycelium	Ascospore	Nitrate reduction	Growth at 37 °C	Sugars fermented								Sugars assimilated										Identity
							Glucose	Galactose	Lactose	Maltose	Raffinose	Sucrose	Starch	Trehalose	Arabinose	Cellobiose	Inositol	Lactose	Maltose	Melibiose	Mannitol	Raffinose	Rhamnose	Sucrose	
Lang kargyong (4)	2	S–O	Pseudo	Spheroidal	–	+	+	–	–	–	–	–	–	–	–	+	–	1/1	+	–	+	–	–	+	*Debaryomyces hansenii*
	2	O–E	Pseudo	Hat	–	+	+	+	–	+	+	+	–	–	–	+	–	–	+	–	+	+	–	+	*Pichia anomala*
Faak kargyong (4)	2	S–O	Pseudo	Spheroidal	–	+	+	+	–	+	–	+	–	–	+	+	–	1/1	+	+	+	+	–	+	*D. polymorphus*
	2	O–E	Pseudo	Hat	–	–	+	1/1	–	–	–	+	+	–	+	+	–	+	+	–	+	+	+	–	*Candida famata*
Yak kargyong (2)	2	S–O	Pseudo	Spheroidal	–	–	+	+	–	+	–	+	+	–	–	+	–	1/1	+	+	+	+	+	+	*D. pseudopolymorphus*
Lang satchu (3)	1	S–O	Pseudo	Spheroidal	–	+	+	–	–	–	–	–	–	–	–	–	+	–	–	+	+	–	–	+	*D. hansenii*
	2	O–E	Pseudo	Hat	–	+	+	1/1	–	+	1/1	+	–	–	–	+	–	–	+	–	+	+	–	+	*P. anomala*
Yak satchu (2)	2	S–O	Pseudo	Spheroidal	–	+	+	+	–	+	–	1/1	1/1	–	+	+	–	1/1	+	+	+	+	–	+	*D. polymorphus*
Suka ko masu (4)	2	S–O	Pseudo	Spheroidal	–	+	–	–	–	–	–	–	–	–	–	1/1	–	–	1/1	1/1	+	–	–	+	*D. hansenii*
	2	O–C	True, pseudo	Hat	–	+	+	–	–	+	+	+	+	+	1/1	+	–	–	+	+	+	+	–	+	*P. burtonii*

Number of isolates is given in parenthesis; +, all strains positive; –, all strains negative; (../..), number of positive/negative strains.
O–E, oval to ellipsoidal; S–O, spherical to oval; O–C, oval to cylindrical. All the strains assimilated, xylose, trehalose, starch, galactose, and glycerol.

Table 6
Enzymatic profiles of LAB strains from the Himalayan ethnic meat products.

Enzyme	Strain (activity in nanomoles)																	
	KP:L8	KP:L13	ZK:L5	ZK:L7	LK:L2	KM:L19	KP:L11	YKK:L2	YKK:L3	YKg:L8	FK:L5	FK:L3	KS:L6	SR:L4	YS:L13	YS:L20	BS:L17	BS:L16
Alkaline phosphatase	5	5	5	0	5	5	0	5	10	5	0	0	0	5	0	0	0	0
Esterase (C4)	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
Esterase lipase (C8)	5	5	0	5	5	0	0	0	0	0	0	0	0	5	0	5	5	5
Lipase (C14)	0	0	0	0	0	0	0	5	0	0	0	0	0	5	5	5	0	0
Leucine arylamidase	5	5	5	5	5	5	30	>40	>40	30	10	>40	>40	>40	>40	30	30	30
Valine arylamidase	0	0	0	0	0	0	5	30	>40	>40	0	>40	>40	>40	>40	5	5	5
Cystine-arylamidase	0	0	0	0	0	0	5	>40	5	5	0	5	30	5	10	5	5	5
Acid phosphatase	5	10	10	5	20	20	20	>40	>40	30	0	10	5	10	10	20	30	10
Napthol-AS-BI-phosphohydrolase	10	10	10	20	10	10	10	10	30	5	10	10	10	20	30	20	10	10
α-Galactosidase	0	0	5	0	20	5	30	0	0	0	20	0	0	0	0	0	0	0
β-Galactosidase	0	0	30	5	0	10	>40	30	5	>40	>40	>40	20	10	>40	0	0	0
α-Glucosidase	30	>40	0	5	>40	0	30	0	0	0	>40	0	0	10	0	0	0	0
β-Glucosidase	5	5	0	0	0	0	>40	0	0	0	30	0	10	10	5	0	0	0
N-acetyl-β-glucosaminidase	0	0	0	0	0	0	0	0	0	0	0	10	20	30	5	0	0	0

Data represents the means of three sets of experiment.
Trypsin, α-chymotrypsin, β-glucuronidase, α-mannosidase and α-fucosidase were not hydrolysed by any LAB strain.
KP:L8, *Lb. sake*; KP:L13, *Lb. curvatus*; ZK:L5 *Lb. divergens*; ZK:L7, *Lb. carnis*; LK:L2, *Lb. sanfrancisco*; KM:L19, *E. faecium*; KP:L11, *Leuc. mesenterioides* (*Lang kargyong*); YKK:L2, *Lb. plantarum*; YKK:L3, *Lb. casei*; Ykg:L8, *Lb. sake*; FK:L5 *Lb. brevis*, FK:L3, *Leuc. mesenterioides*; KS:L6, *P. pentosaceous*; SR:L4, *Lb. casei*; YS:L13, *P. pentosaceous*; YS:L20, *P. pentosaceous*; BS:L17, *Lb. plantarum*; BS:L16, *E. faecium.*
0, no enzyme activity; 5, 10, 20, 30, >40 indicates nanomoles of hydrolysed substrate after 6 h of incubation at 30 °C.

Table 7
Antimicrobial activity of the LAB strains isolated from the Himalayan ethnic meat products.

Meat product	Strain	Indicator strains	
		K. pneumoniae subsp. *pneumoniae* BFE 147	*Enterobacter agglomerans* BFE 154
Lang kargyong	ZK:L5	+	+++
	ZK:L7	–	++
	LK:L2	++	+
	KP:L5	++	+
	KP:L14	+	++
	ZK:L4	+	+++
	KM:L19	+	+++
	KM:L35	++	+++
	KP:L7	+	+++
	KP:L11	+++	+
	KP:L18	+	+++
	LK:L4	+	+++
	ZK:L3	+++	++
	ZK:L6	++	++
Yak kargyong	YKK:L2	+	+++
	YKK:L3	–	+++
	YKK:L4	++	+++
	YKK:L8	–	+++
	YKK:L11	–	+++
	YKg:L3	–	+++
	YKg:L7	+	+++
	YKg:L8	+	+++
	YK:L11	+	+++
	YKK:L1	+	+++
	YKK:L5	++	+++
	YKg:L9	+	++
	YK:L1	++	+
	YK:L2	+	++
	YK:L15	+	++
Faak kargyong	SR:L4	++	–
	KS:L18	+	++
	KS:L26	+	++
	TS:L23	++	–
	TS:L7	++	–
	TS:L6	++	–
Yak satchu	YS:L2	++	–
	YS:L7	++	–
	YS:L20	++	–
Suka ko masu	BS:L18	++	–
	BS:L17	++	–
	BS:L21	+++	–

Data shows three sets of experiments.
–, no zone of inhibition; +, diameter of inhibition zone is 2–3 mm; ++, diameter of inhibition zone is 4–6 mm; +++, diameter of inhibition zone is >7 mm.
None of the LAB showed antagonism against *L. innocua* DSM 20649, *Listeria monocytogenes* DSM, *Bacillus cereus* CCM 2010, and *Pseudomonas aeruginosa* BFE 162, except *Lb. sanfransisco* (*lang kargyong*) KP:L14, *Lb. sake* YKg:L7 and YKg:L8, and *Lb. carnis* YKK:L5 (*yak kargyong*) showed antagonism against *L. innocua* DSM 20649.

formed 1–4 hat-shaped ascospores per ascus, *Pichia burtonii* showing dusty, dry and a powdery surface colony, fringed with many strands of mycelia when grown on agar plates. They formed expanding septate hyphae with conidia borne on denticles. There were 1–4 hat-shaped ascospores per ascus. All of them fermented glucose, maltose, raffinose, trehalose and sucrose. They were able to grow in 10% NaCl and 5% glucose in yeast nitrogen base as well as *Candida famata*, *Debaryomyces hansenii*, *Debaryomyces polymorphus* and *Debaryomyces pseudopolymorphus* were identified on the basis of sugar fermentation and assimilation tests. Filamentous moulds were found in a few samples, and were mostly species of *Rhizopus*.

Samples were examined for *Listeria* sp., *Salmonella* sp., and *Shigella* sp. using the selective media. None of these pathogenic bacteria were detected in any sample, indicating the ethnic meat products of the Himalayas are safe to eat.

Table 8
Percentage hydrophobicity of LAB strains from the Himalayan ethnic meat products.

Product	Strain	% Hydrophobic*
Lang kargyong	*Lb. sake* KP:L3	50.0 ± 1.23
	Lb. sake KP:L8	34.2 ± 1.21
	Lb. curvatus KP:L13	43.4 ± 1.32
	Lb. sake KP:L30	38.0 ± 1.83
Yak kargyong	*Lb. plantarum* YKK:L2	70.3 ± 1.03
	Lb. plantarum YKK:L11	62.8 ± 1.25
	Lb. plantarum YKg:L3	36.4 ± 1.22
	Lb. sake YKg:L8	42.5 ± 1.09
	Lb. brevis YK:L11	61.9 ± 0.54
	Lb. sake YKg:L9	46.5 ± 1.61
Faak kargyong	*Lb. brevis* FK:L5	71.7 ± 1.48
	Lb. plantarum FK:L15	31.1 ± 1.7
	Lb. plantarum BS:L9	32.2 ± 0.86

Data represents the means (±SD) of four sets of experiment.
* LAB strains which showed more than 30% hydrophobicity.

LAB strains isolated from traditional meat products showed very weak lipolytic activity in the API-zym test (Table 6). Weak lipolytic activity of LAB strains has been observed during *nham* fermentation (Montel, Masson, & Talon, 1998; Visessanguan et al., 2006). Absence of proteinases (trypsin and chymotrypsin) and the presence of strong peptidase (leucine-, valine-, and cystine-arylamidase) activities produced by the predominant LAB strains possibly impart flavor during sausage production as the composition and concentration of these compounds contribute to the overall flavor in cured meat products such as dry sausages (Henriksen & Stahnke, 1997) and ham (Toldrá, Flores, & Sanz, 1997). *Lb. sake*, which was also recovered in the Himalayan sausage, is responsible for the generation of free amino acids and contributes to flavor improvement of dry fermented sausage (Demeyer et al., 2000).

Almost all of the strains of LAB isolated showed some antimicrobial activity against *K. pneumoniae* subsp. *pneumoniae* BFE 147 and *E. agglomerans* BFE 154 (Table 7). However, the cell-free supernatant fluid extracts of LAB strains isolated could not produce bacteriocin under the applied conditions. None of the strains were found to produce biogenic amines. Biogenic amines are present in dry sausages, fishery products, cheese, wine, beer, and other fermented foods (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994). Samples with moderate or high levels of biogenic amines could be considered of poorer quality and their consumption could be a problem for sensitive individuals (Latorre-Moratalla et al., 2007). The inability of the strains of LAB to produce biogenic amines is a good indication of their acceptability and potential for their development as a starter culture. Bacterial adherence to hydrocarbons such as hexadecane, is a simple and rapid method to determine cell surface hydrophobicity (Vinderola, Medici, & Perdigón, 2004). Only two strains, *Lb. brevis* and *Lb. plantarum* isolated from *faak kargyong* and *yak kargyong*, respectively had more than 70% hydrophobicity (Table 8). Percent of hydrophobicity greater than 70% was arbitrarily classified as hydrophobic (Nostro et al., 2004). The ability to adhere to the intestinal mucosa is considered one of the main criteria in the selection of potential probiotic cultures (Holzapfel & Schillinger, 2002).

4. Conclusion

Drying, smoking or fermentation of meat is an important step in traditional meat processing in the Himalayas. A diversity of microorganisms from species of lactic acid bacteria, bacilli, micrococci and a few yeasts was observed in the Himalayan ethnic meat products. Strains of LAB from the products exhibited some functionality such as antimicrobial, a range of enzymatic activities,

non-production of biogenic amines and a few are hydrophobic, essential for probiotics.

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