Microbiology of Traditional Meat Products of Sikkim and Kumaun Himalaya

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Microorganisms transform the chemical constituents of raw substrates during fermentation into acceptable food products with improved flavour, aroma and texture, enhancing nutritional value and other health benefits (Steinkraus, 1996; Stiles and Holzapfel, 1997; Tamang, 2007). Microorganisms are mostly present in or on the raw materials, ingredients, utensils, environment, and are selected through adaptation to the substrate for fermentation (Hesseltine, 1983; Tamang, 1998). Any animal can be used as a source of meat, ranging from domesticated cow, pig and chicken, to deer and camels, and it is a good source of easily digestible protein and contains essential amino acids which are vital for growth and maintenance of the body (Fellows and Hampton, 1992). Raw meat gets spoiled at high ambient temperatures within a few hours due to its high moisture and protein contents (Dzudie *et al*., 2003). Fermentation or drying or smoking prolongs the shelf-life of perishable raw meat (Rantsiou and Cocolin, 2006). Traditional cured meat products, made from whole meat pieces of pork or beef, are commonly produced and consumed in different countries throughout the world (Vilar *et al.,* 2000). In developed countries, a wet-curing process for meat has been evolved which involves use of a solution of salt, sodium nitrate/nitrite, whereas in under-developed and developing countries, preserving meat is done by curing with salt followed by drying or smoking or fermentation (Zapata *et al*., 1990; Romano *et al.,* 2006).

Fermented meat products are divided into two categories: those made from whole meat pieces or slices, such as dried meat and jerky, and those made by chopping or comminuting the meat, usually called sausages (Campbell-Platt, 1987). Meat processing is the combination of chemical curing, microbial fermentation and drying which together give stable, safe, ready-to-eat products (Bacus, 1984). Pederson (1980) gave an account of the history of meat preservation through fermentation. Southern and Central Europe, dating back to Roman times, is the original home of many of these cured and fermented meat products, most made from pork and beef. The name salami is believed to have originated from the city of Salamis in Cyprus, which was destroyed over 2000 years ago (Lücke, 1985). Emigrants carried knowledge of these processes and practices to North America and Australia. These regions now share a range of whole-meat *bacon*, to be cooked before eating, ready-to-eat country *ham*, and chopped semi-dry *cerevalat*, dry German *salami* and *pepperoni*, which are smoked, and dry Italian *salame* and *chorizo*. Cooked fermented meat products such as *mortadello*, *kochsalami* and *thüringer* are less common (Campbell-Platt, 1987). *Salsiccia* and *soppressata* are traditional dry fermented sausages produced in Basilicata in Southern Italy (Parente *et al*., 2001a). In North America, the Inuits produce milkiyuk from whale and *iqunaq* from duck (Bacus, 1984). The hotter regions of Africa and Asia are the home of relatively few fermented meat products,

although whole-meat dry uncooked jerky is produced in Africa as well as America (Klettner and Baumgartner, 1980; Campbell-Platt, 1987). Some of the common fermented meat products of Thailand are *nham* (fermented beef or pork sausage), *naang* (fermented pork or beef) *nang-khem* (fermented buffalo skin) and *sai-krork-prieo* (fermented sausage) (Phithakpol *et al.*, 1995). Many traditional meat products of different countries have been well documented and studied such as fermented sausages, *salami* of Europe (Campbell-Platt and Cook, 1995), *ham* (Simoncini *et al*., 2007), *alheira* of Portugal (Ferreira *et al*., 2006), *androlla* of Spain (Garcia Fontán *et al*., 2007), *salsiccia* and *soppressata* of Italy (Parente *et al*., 2001a), *jerky* of America (Baruzzi *et al.,* 2006) and Africa (Klettner and Baumgartner, 1980), *nham* of Thailand (Visessanguan *et al*., 2006), *lup cheong* of China (Leistner, 1995).

Lactic acid bacteria (LAB) exert an important effect on the production and quality of the various fermented meat products (Schillinger and Lücke, 1987; Hammes and Hertel, 1998). *Pediococcus* and *Lactobacillus* are active in producing lactic acid and thus help in lowering of pH, which helps preserve meat (Bacus, 1986). The microflora of fresh raw meat, stored aerobically under refrigeration, largely consists of Gram-positive, and oxidase positive rods, particularly psychrotrophic pseudomonads (McMeekin, 1982), enterobacteriaceae (Gill, 1982) and low numbers (10^2-10^3 cfu/g) of LAB (Hammes and Knauf, 1994). During meat

fermentation, water activity is reduced and the oxygen present is rapidly consumed, thus pseudomonads, which require oxygen and are sensitive to salt and nitrite are inactivated (Hechelmann *et al*., 1977). Similarly, enterobacteriaceae is also reduced at low oxygen tension, low pH and in presence of salt (Grau, 1981), which results the rapid growth of LAB (Hammes and Knauf, 1994) and also micrococci (Čavlek *et al*., 1971). In fermented meat products, the dominant microorganisms are mostly species of LAB- *Lactobacillus*, e.g., *Lactobacillus sakei, Lactobacillus curvatus* and *Lactobacillus plantarum*; entorococci mainly *Enterococcus faecium*; *Pediococcus pentosaceus*, *Leuconostoc carnosum, Leuc. gelidium, Leuc. pseudomesenteroides*, *Weissella*, etc. (Collins *et al*., 1993; Parente *et al*., 2001a,b); and also coagulase-negative staphylococci (Hugas *et al*., 2003). Species of *Micrococcus* and *Staphylococcus* help reduce nitrate if added, to nitrite in fermented sausages (Lücke, 1985). *Staphylococcus aureus* is regularly found in meat and fermented sausages (Barber and Deibel, 1972). Raw sausage mixtures may contain considerable numbers of *Bacillus* spores, of which spices are a major source (Neumayr *et al*., 1983). Some yeasts and moulds may develop on the surface of dry fermented sausages during ripening (Lücke, 1988; Tamang and Fleet, 2008). Species of yeasts *Debaryomyces, Candida, Cryptococcus* and *Trichosporon* have been reported in traditional Greek dry *salami* (Metaxopoulos *et al*., 1996). Species of *Penicillium* constituted the surface mycoflora of *chorizo de*

Cantimpalos, a Spanish variety of fermented sausage (López-Díaz *et al*., 2001).

Ability of LAB to inhibit putrefactive, pathogenic and toxinogenic bacteria has been reviewed periodically (Holzapfel *et al.,* 1995; Ouwehand, 1998; Nout, 2001). Bacteriocinogenic enterococci can be used to enhance preservation in meat products (Hugas *et al*., 2003). During meat fermentation, the microbial growth, the acidification and the proteolysis offer favourable conditions for the production of biogenic amines (Hugas *et al*., 2003). The production of biogenic amines in fermented meat has been attributed to the action of several microorganisms such as pseudomonads, enterobacteriaceae, enterococci and lactobacilli (Stratton *et al*., 1991; Halász *et al*., 1994; Silla-Santos, 1996; Bover-Cid *et al*., 2001). Dry fermented sausages are worldwide diffused fermented meat products that can be a source of biogenic amines (Suzzi and Gardini, 2003). Excessive consumption of biogenic amines can be a health concern that may lead to a variety of cutaneous, gastrointestinal and neurological symptoms (Taylor, 1986; Suzzi and Gardini, 2003).

Sikkim (Fig. A) is the Himalayan state of India with an area of 7096 sq. km and the altitude ranges from 300 m to 8500 m. The state comprises of four districts: North, East, South and West. A total population of Sikkim is 540,493 (Census, 2001) and is populated by three major ethnic groups of people, the Nepali, the Bhutia and the Lepcha. The domestic

livestock of Sikkim includes cattle, sheep, goats, pigs, yaks, poultry, etc. which is mainly used for meat, milk and milk products (Balaraman and Golay, 1991). Yaks (*Bos grunniens*) are reared mostly on extensive alpine and sub alpine scrub lands between 2100 m to 4500 m altitude for milk products and meat (Sharma *et al.*, 2006). Traditional food has always been rich ingredients to the people of the Eastern Himalayas including North East India (Tamang, 2001). About 12.6 % of the total daily food consumed in local diet represents fermented foods (Tamang *et al*., 2007). The food survey shows that 11.7 % of people in Sikkim are vegetarian and 88.3% are non-vegetarians (Tamang *et al*., 2007). The Bhutia and the Lepchas usually prefer beef, yak and pork. Beef is taboo to majority of Nepalis except Tamang and Sherpa castes. The Newar caste of Nepalis prefers to eat buffalo meat. The Bhutias and the Lepchas in North Sikkim, slaughter yaks occasionally, consume the fresh meat and the rest is preserved by smoking or drying or fermenting. Varieties of ethnic fermented foods and beverages are indispensable cultural foods of the people of Sikkim Himalayas including different types of meat based products (Tamang, 2005a).

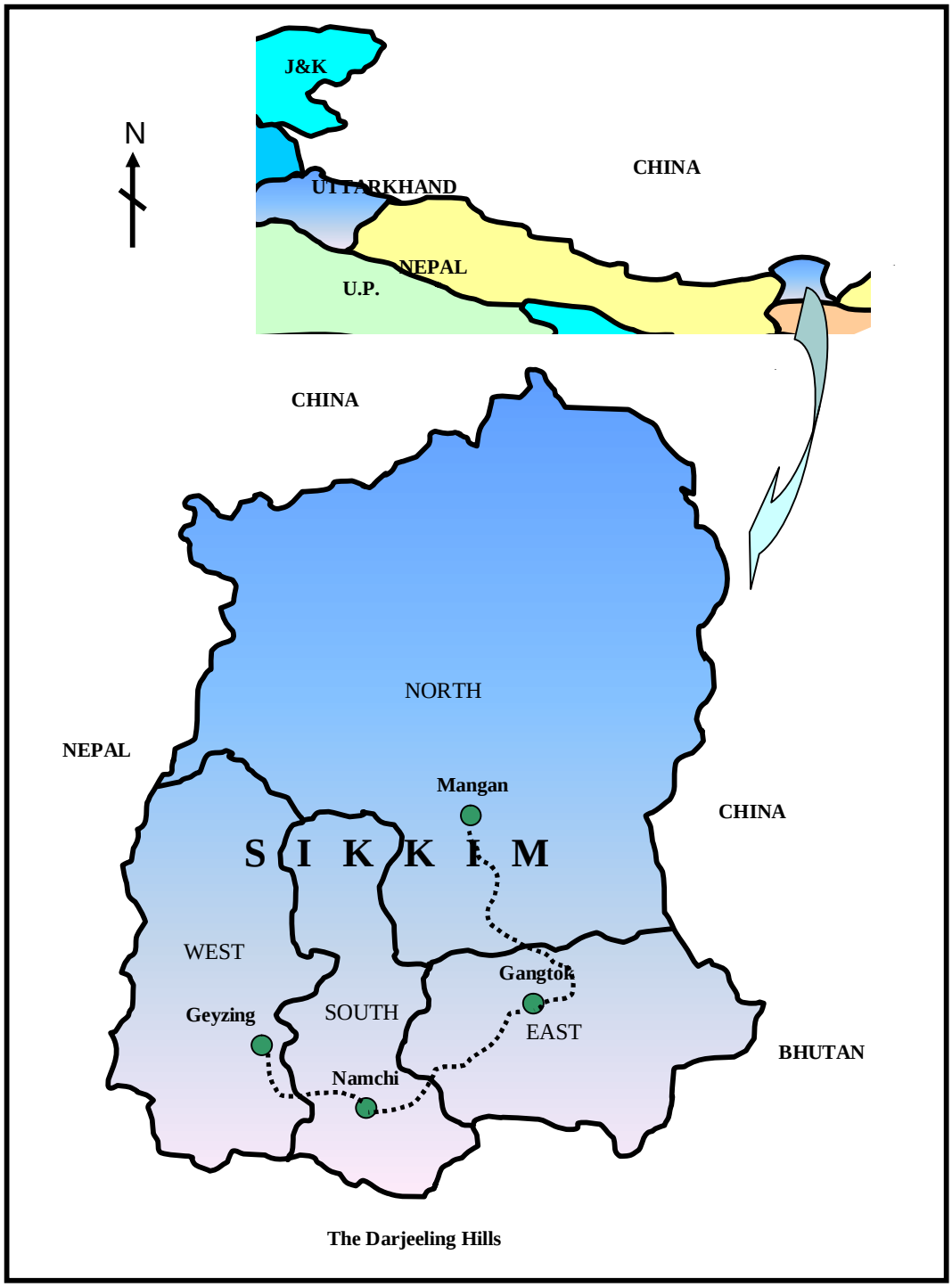


Fig A. Map showing the Sikkim Himalaya

Kumaun (Fig B), hills of Uttarakhand is situated in the Central Himalaya at the tri-junction of Nepal, Tibet (China) and India, and the region extends from 28°44' & 30°49' N latitudes to 78°45' & 81°5'E longitudes with an area of 21,033 km^2 (Nandy *et al*., 2006). A total population of Uttarakhand is 8479,562 (Census, 2001). Five major tribes, i.e., the Tharus, the Jaunsaries, the Buxas, the Bhutias and the Rajis numbering 179,002 inhabit the Central Himalayan region of Uttarakhand. The Tharus, the Buxas and the Jaunsaries are agriculturists while the Bhutias are pastoralists and the Rajis are mostly hunters and gatherers. The Bhutias, a transhumant community of mongoloid origin, inhabit the high altitude regions of Uttarakhand at Indo-Tibetan & Indo-Nepal borders, a zone of ethnic intermixing and cultural assimilation. They show close racial and cultural affinity to the Tibetans. The eight major Bhutia groups are the Johari, Jeethora, Darmi, Chaudansi, Byabsi, Marchha, Tolcha and Jad, and are scattered over eight main river valleys known as Johar, Darma, Byans, Chaudans (Pithoragarh district in Kumaun), Mana, Niti (Chamoli district in Garhwal), Nilang and Judun (Uttarakashi district in Garhwal) (Nandy *et al.*, 2006). The ethnic people of the Kumaun Himalaya also prepare and consume indigenous meat products.

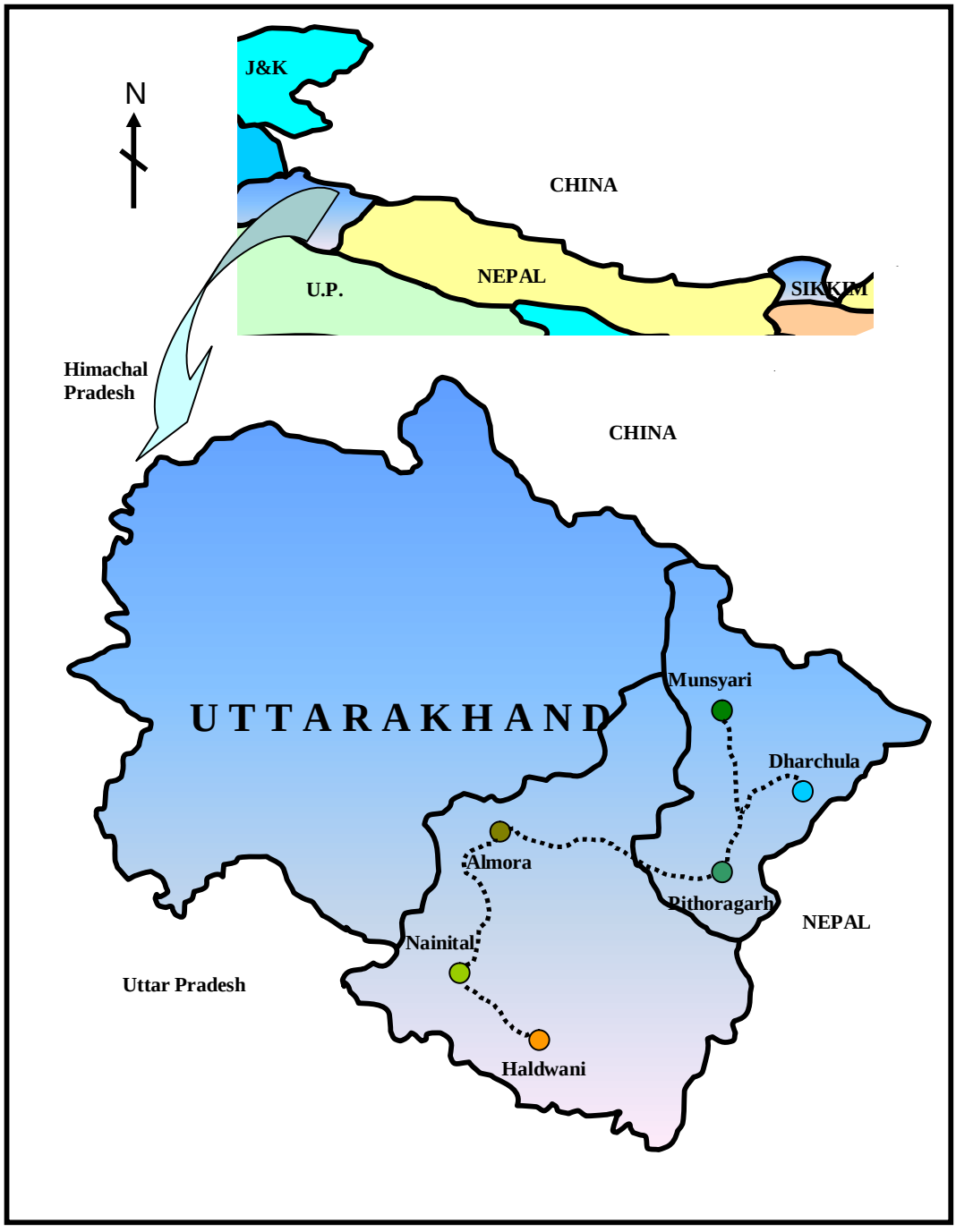


Fig B. Map showing the Kumaun Himalaya of Uttarakhand

Meat is a part of the daily diet for many ethnic people dwelling in the Himalayan regions of India, Nepal, Bhutan and China (Tamang, 2005b). However, documentation of meat products, their traditional processing methods, microbiological profiles, food safety and nutritional aspects have not been studied in details. This would be the first major research work on traditional processing of meat products of the Central (Kumaun) and Sikkim Himalaya concerning microbiology and biochemical aspects of traditionally processed meat products.

Objectives of the Thesis were to:

- to document the different types of traditional meat products (fermented, smoked and dried) of the Central (Kumaun) and the Sikkim Himalaya, and their indigenous knowledge of preparation of these products;
- to isolate, to purify, to characterize and to identify the predominant microorganisms associated with the traditional meat products;.
- to study the population dynamics of major microbial groups;
- to study the enumeration of pathogenic contaminants in the finish products;
- to characterize the predominant lactic acid bacteria in respect of antagonism activities and bacteriocin production, enzymatic profiles, screening for biogenic amine production, and determination of hydrophobicity for presumptive 'probiotic' properties;
- to analyze the proximate composition of the products.

An extensive and updated review on meat products of the world was prepared in this chapter referring journals, reprints, books, monographs, reports, dissertation, etc. Table A shows a compilation of the major meat products of the world.

Table A. Major meat products of the world

Product	Raw material	Nature	Region	Microorganisms	References
(I) Sausages					
Pastirma	Chopped beef lean meat with lamb fat, not smoked, heavily seasoned	Dry / semi-dry	Turkey, Iraq	*Lb. plantarum, Lb. sake, Pediococcus, Micrococcus, S. xylosus, S. carnosus*	Aksu *et al.* (2005)
Soppressata	Chopped lean pork meat, NaCl and spices	Dry / semi-dry	Italy	LAB, Yeast, staphylococci, micrococci, enterobacteriacea e	Parente *et al.* (2001a)
Salsiccia	Chopped pork meat, spices, NaCl	Dry / semi-dry	Italy	LAB, Yeast, enterobacteriacea e staphylococci, micrococci	Parente *et al.* (2001a,b)
Sucuk	Chopped meat, pork or beef, curing salts and various spices	Dry	Turkey	LAB, micrococci, staphylococci, enterobacteriacea e	Genccelep *et al.* (2008)
Alheira	Pieces of meat, pork or beef, bread	Dry / semi-dry	North of Portugal	LAB, staphylococci, micrococci, yeast	Ferreira *et al.* (2006)

	chopped fat and spices, salt				
Chorizo	Pork, coarse chopped, spices, NaCl	Dry	Spain	*Lb. sake, Lb. curvatus, Lb. plantarum*	Garcia-Verona *et al.* (2000)

Continued (Table A)

Salchichon	Pork or beef meat, fat, NaCl, spices	Dry	Spain	LAB, Yeast, micrococcaceae, enterobacteriaceae, moulds	Fernandez-Lopez *et al.* (2008)
Androlla	Ground lean pork, jowl, back fat, ribs with their fleshy parts, salt, sweet paprika, spicy paprika, garlic, marjoram sugars	Dry	Spain	*Lb. sake, Lb. curvatus, Lb. alimentarius, , Lb. plantarum, S. xylosus, S.epidermis, S.equorum, S. capitis, S. saprophyticus,* micrococci	Garcia-Fontan *et al.* (2007)
Nham	Pork meat (trimmed), minced, salt, rice and seasoning, traditionally wrapped in banana packets.	Dry / semi-dry	Thailand	*Lb. plantarum, Lb. brevis,* micrococcaceae, yeast	Yanasugondha (1977)
(II) Ham	Cured pork, salted and dried, usually uncooked, smoked	Semi-dry	Spain, Italy	LAB, yeasts, micrococcaceae	Simoncini *et al.* (2007)
(III) Bacon	Slices of cured pig,	Dry / semi-	Germany ,	LAB, yeast,	Tanaka *et*

	beef	dry	Belgium, Spain	micrococcaceae	*al.* (1980)
(IV) Jerky	Beef meat that has been cut into strips trimmed of fat, salt, sweet liquid	Dry / semi-dry	South America	LAB, yeast, moulds, micrococcaceae	Delong (1992)
(V) Dried meat	Lean meat of beef, Buffalo, deer etc	Dry	North America	LAB, micrococcaceae, yeast, moulds	Sinclair *et al.* (2001)

(I) Sausage

The sausage is usually made from comminuted meat and fat, mixed with salts, curing agents, sugar and spices and filled into the casings, and are referred to as *fermented sausage* (Lücke, 1985). Traditional sausages are considered by interviewed consumers as an important part of their habit (Conter *et al.,* 2008). Fermented sausages are produced from comminuted lean and fatty tissue, mixed with salt, spices, sugar, and in most cases curing agents (nitrite, nitrate, ascorbate) and the mixture is stuffed into the casings which are then subjected to microbial fermentation (Lücke, 2003). Fresh raw sausage mixture contains 50-70 % lean meat, which is mammalian skeletal muscle tissue (Lücke, 1985). The shelf-life of meat can be extended by addition of fermentable carbohydrate (Shelef, 1977).

However, it is much more effective to remove oxygen (e.g. by vacuum-packaging) because this favoured the development of lactic acid

bacteria (LAB) and inhibits the degradation of amino acid, if enough fermentable carbohydrates are present, LAB lower the pH value sufficiently to suppress other bacteria, at least temporarily (Lücke, 1985). The pH of the raw materials during the manufacture of the sausages should be 5.8 or lower to prevent the growth of undesired bacteria (Lücke, 2003). Usually 2.4-3.0 % NaCl is added to the raw sausage mixtures so that the initial water activity is 0.97-0.96 % which inhibits the growth of many undesired microorganisms and favours the development of lactobacilli and micrococci (Lücke, 1985). The main microbial groups involved are LAB and coagulase-negative cocci which in addition, and depending on the product, other groups may play a role, such as yeasts and enterococci (Rantsiou, 2006). Vilar *et al.* (2000) suggested that micrococcaceae, LAB, yeasts are the typical microflora in meat products with the involvement of micrococcaceae being the most important. Sodium chloride interacts with the myofibrillar structure and solubelises proteins which form a sticky film around meat particles (Lücke, 1985). The lipolytic and proteolytic activities for the manufacture of traditional fermented sausages of Southern Italy where the three starter formulation including *Lactobacillus curvatus* and *Staphylococcus xylosus* strains selected *in vitro* (Casaburi *et al.,* 2008). The sausage inoculation of yeast strains resulted at the end of ripening, in more pronounced proteolysis and lipolysis (Patrignani *et al.,* 2007). Proteolysis and lypolysis were observed

in sausages inoculated with proteolytic and lypolytic *S. xylosus* coupled with *Lb. curvatus* while sausage started with only *S. xylosus* without lactobacilli was identical to the non-inoculated control indication that the proteolysis could be due to both microbial activity and endogenous processes activated by the decrease in pH (Casaburi *et al.,* 2008).

The starter culture initiate the rapid acidification of the raw meat batter and that laid to a desirable sensory quality of the end product, used for the production of fermented sausage (Leroy *et al.*, 2006). The use of LAB is important for the production of safe fermented sausages (Lücke, 2003). The 'atypical lactobacilli' (*Lb. sake, Lb. curvatus*) predominate in naturally fermented dry sausage (Reuter, 1967; Kagermeier, 1981), but attempts to improve sausage manufacture and quality by inoculating selected strains into sausage mixture were of limited success (Reuter, 1972). Wirth (1973) stated that fermented sausages made without nitrite or nitrate have a grayish colour and poor flavour and spoil rapidly due to oxidative rancidity. The selection of functional starter culture could protect consumers from harmful bacteria either by rapid acidification or by the production of antimicrobials (Ammor and Mayo, 2007). Dry sausages made with nitrate or low amounts of nitrite frequently taste better than those made with the usual amount of nitrite which indicates that high concentrations of nitrite present at the beginning of the fermentation may suppress microorganisms active in synthesizing

flavour components or their precursors (Wirth, 1983). Ground pepper is usually present in all types of fermented sausages at the 0.2-0.3 % and other spices commonly used include paprika, garlic, mace, pimento and cardamom (Vandendriessche *et al*., 1980). Some spices (e.g. red pepper, mustard, mace) have been found to stimulate the rate of lactic acid formation (Nes and Skjelkvale, 1982). This may be due to manganese present in the spices which is required by the LAB for various enzyme activities including the key enzyme of glycolysis, fructose 1, 6-diphosphate aldolase (Kandler, 1982). Some spices (garlic, rosemary, sage) contain powerful antioxidants and may thus extend the shelf-life of dry sausages (Hammer, 1977). The products of carbohydrate fermentation (lactic acid with small amounts of acetic acid) give the sausage their typical 'tangy taste' and this flavour predominates in non-dried and semi-dry products which are sold after only a few days ripening (Langner, 1969). The pH increases and may be high as 7 in some thin, mould ripened sausages which taste quite different to the smoked 'tangy' sausages (Lücke, 1985). The longer the ripening time and the higher the activity of microorganisms other than lactic acid bacteria, the more flavouring compounds are found (Langner, 1969, 1972). In the presence of oxygen, moulds and yeasts not only form flavouring compounds but also oxidise lactic acid and these strains are available as starter cultures for air-dried (not or only lightly, smoked) sausages where these organisms

readily colonise the surface (Mintzlaff and Leistner, 1972). In order to avoid the colonization of wrong mould bearing unsatisfactory product, the selection of strain, *Penicillium nalgiovense* as a starter culture gave the best result as it does not form mycotoxins (Mintzlaff and Leistner, 1972). Yeast mostly species of *Debaryomyces* predominate on dry sausages (Leistner and Bem, 1970; Comi and Cantoni, 1980). Coretti (1977) reported that a combination of *Debaryomyces hansenii*, lactobacilli and micrococci gave best result.

The use of an appropriate starter culture can influence the aroma characteristics and the microbiological quality (Luongo *et al*., 2001). The advantages of the use of starter culture for sausage ripening have been reviewed by Coretti (1977). The presence of 10^6 - 10^7 rather than 10^3 active LAB per gram in the fresh sausage mixture obviously leads to more predictable and more rapid pH decrease and to earlier development of firmness (Bacus and Brown (1981). The suppression of pathogenic organisms like salmonellae (Sirviö *et al*., 1977) and *Staphylococcus aureus* is a major argument at high fermentation temperatures (Niskanen and Nurmi, 1976). During drying, enterobacteriaceae including salmonellae are slowly inactivated (Scharner, 1968; Smith *et al.,* 1975; Stecchini *et al*., 1982). Meat consumption LAB are often utilized to control food borne pathogens (Kostrzynska, 2006).

The prevalence of *D. hansenii* in all batches of Greek dry salami suggested that its potential use as a starter culture in Greek dry salami (Metaxopoulos *et al*., 1996). The use of selected lactic acid bacteria (LAB) starter culture of dairy origin in the production of low-acid fermented sausages (*salame nostrano*) greatly reduced the rate of isolation of pathogens and increased the acceptability of full-ripened salami (Cenci-Goga *et al.,* 2008).

Raw sausage mixture may contain considerable numbers of *Bacillus* spores, of which spices are the major source (Neumayr *et al*., 1983). The results for the technological properties of *Bacillus* strains isolated from southern Italian sausage made without a selected starter, suggest that *Bacillus* strains, always present in meat curing could play a role in the development of texture and organoleptic characteristics of the sausages (Baruzzi *et al*., 2006). Prolonged drying of sausages at low relative humidity markedly reduces the number of vegetative bacteria (Neumayr *et al*., 1983). The latter authors concluded that *Bacillus* sp. is controlled in fermented sausages by a combination of low water activity, low pH and absence of oxygen (Lücke, 1985).

Parente *et al*. (2001b) reported the occurrence and evolution of biogenic amines during ripening of fermented sausages and their relationship with physico-chemical and microbiological properties of the product and found no correlation between individual biogenic amine

content, microbial counts or physiochemical parameters. Bover-Cid *et al*. (2001) reported the presence of biogenic amines in a decarboxylase synthetic broth was determined by ion-pair high performance liquid chromatography, among which LAB particularly *Lb. curvatus* and all enterococci tested were amine producers. The results obtained for biogenic amine production by bacteria in a synthetic medium suggest that amino-acid-decarboxylase activity is strain dependent rather than being related to specific species (Bover-Cid *et al*., 2001). Samples with moderate, high or very high levels of biogenic amines could be considered as products of less quality and their consumption could be unhealthy for sensitive individuals (Latorre-Moratalla *et al.,* 2008). Starter cultures did not necessarily prevent the production of biogenic amines (Parente *et al*., 2001b). However, Kueper and Trelease (1974) did not detect any nitrosamines at all in summer sausage.

The development of starters is very promising because it enables sausages to be produced with both high sanitary and sensory qualities (Talon *et al.,* 2007). Recently bioprotective LAB, which in addition to the production of antimicrobial lactic acid have been found to contribute to the safety of the dry sausages by producing antimicrobial peptide, i.e. bacteriocins and other low-molecular-mass compounds (Tyopponen, 2002). As one possible mode of action for probiotics is the production of antimicrobial compounds, LAB may act as both probiotic and

bioprotective culture as well as fermenting agent in meat product, such as dry sausages (Tyopponen, 2002). Enterocins could be considered as an extra biopreservation hurdles for listeria prevention in dry fermented sausage (Aymerich *et al*., 2000). A bacteriocin produced by *Pediococcus acidilactici* had an inhibitory and bactericidal effect on *Listeria monocytogenes* associated with fresh meat (Nielsen *et al*., 1990). The compound excreted by *Lb. sake* Lb 706 was active against various LAB and *Listeria monocytogenes* (Schillinger and Lücke, 1989). The survival and toxinogenic properties of a strain of *E. coli* O157: H7 were evaluated in a typical fermented sausage during ripening and was found that *E. coli* O157:H7 declined progressively over time even though it was still detected at the end of the ripening process (Normanno *et al*., 2002).

Traditional processes for making salami lead to a microbiologically safe product when applied under industrial manufacturing conditions but should be closely monitored to give a less acidified and delicately flavoured final product (Metaxopoulos *et al*., 2001).

An excessive intake of meat products, particularly dry fermented sausages, is not recommended from a health point of view, at least for some population groups, due to their high level of sodium and animal fat (Muguerza *et al*., 2004). Lebert *et al.* (2007) studied the diversity of microorganisms in the environment and fermented sausage of processing unit where in two of the processing units, the final sausages were

contaminated with *S. aureus* and *L. monocytogenes* respectively, at the levels exceeding the maximum tolerable limit indicating insufficient cleaning and disinfection procedures.

Different types of sausages

Pastirma

Pastirma is a dry-cured meat product, categorized as an intermediate moisture food and it is consumed sliced without cooking (Aksu *et al.,* 2005). It is dry or semi-dry sausages of Turkey and Iraq, prepared from chopped beef lean meat with lamb fat, not smoked, and is heavily seasoned Aksu *et al.,* 2005). It is the Turkish meat product which is pasted with cemen (fenugreek) and this paste is prepared from ground fenugreek, garlic and red hot pepper (Yetim *et al.,* 2006). *Lactobacillus sake, Pediococcus, Micrococcus, Staphylococcus xylosus, S. carnosus* were reported from *pastirma* (Aksu *et al.,* 2005). Akta *et al.* (2005) reported the effects of different commercial starter cultures (*Staphylococcus carnosus*, *S. carnosus* + *Lactobacillus pentosus* and *Staphylococcus xylosus* + *Lactobacillus sakei*) on myofibrillar proteins were investigated using differential scanning calorimetry during the processing of *pastirma* and found that the stage of *pastirma* production significantly decreased the thermal stabilities of myosin and actin.

Soppressata

Soppressata is the most appreciated traditional dry fermented sausage in Basilicata, Southern Italy (Amato *et al.,* 1997). It is produced by casing a mix of lean pork meat lard, NaCl and spices in 45-60 mm natural

casings (Parente *et al.,* 2001a). Coppola *et al.* (1998) isolated and characterized 183 LAB from *soppressata* at different stages of ripening using phenotypical test. LAB, mostly *lactobacillus sake* is dominant while *Lb. curvatus,* heterofermentative lactobacilli and *Leuconostoc* sp. occurred in lower numbers (Parente *et al.,* 1994). In addition staphylococci, micrococci, enterobacteriaceae were also reported from *soppressata* (Parente *et al.,* 2001a). A traditional dry fermented sausage from southern Italy *soppressata* of Vallo di Diano was characterized by high microbial loads of both staphylococci and lactobacilli (Villani *et al.,* 2007).

Salsiccia

Salsiccia is the traditional dry fermented sausage in Basilicata of Italy (Amato *et al.,* 1997). It is prepared by mixing of pork meat, sodium chloride and spices in 20-25 mm natural casings, followed by drying and ripening. LAB, staphylococci, micrococci, enterobacteriaceae were reported from *salsiccia* (Parente *et al.,* 2001a).

Sucuk

Sucuk is a popular fermented sausage in Turkey (Colak *et al.,* 2007). It is a dry fermented sausage prepared by mixing ground lean or semi-lean beef and/or sheep meat, fallow fat, garlic, salt, nitrate, ascorbic acid, sucrose and spices (Tamelli *et al.,* 2005). LAB, *Micrococcus, Staphylococcus*

and enterobacteriaceae have been reported from *sucuk* (Genccelep *et al.,* 2008). Colak *et al.* (2007) also reported the presence of *Listeria* sp. and *Listeria monocytogenes* in *sucuk* at the level of 21.0 % and 11.6 %, respectively. In addition to microbial hazards, the presence of biogenic amines in *sucuk* has also been reported (Bozkurt and Erkmen, 2004). However, high quality raw materials and suitable starter culture should be used in the production of *sucuk* (Erkmen and Bozkurt, 2004). Genccelep *et al.* (2008) studied the biogenic amines content of *sucuk* and found the tyramine followed by putrescine.

Alheira

Alheira is a traditional smoked naturally fermented meat sausage produced in north part of Portugal (Ferreira *et al.,* 2006). It is prepared by boiling meats in lightly salted and spiced water, soaking the thinly sliced bread in some of the broth formed during the boiling of the meats until it is soft enough, then spices and olive oil are added to the bread/broth mixture. Finally the meat paste is stuffed into the cattle intestinal or cellulose casings (Ferreira *et al.,* 2006). In addition to LAB, micrococcaceae, yeast and moulds also play the important role in the development of the organoleptic characteristics of *alheira* (Mauriello *et al.,* 2004). *Pediococcus pentosaceous* isolated from *alheira* have been found to have antibacterial activities against *Listeria innocua* and *L. monocytogenes* (Albano *et al.,* 2007).

Chorizo

Chorizo is the Spanish dry fermented cured sausage made from pork or beef, not usually smoked or cooked, heavily seasoned and coloured with pimento and other seasonings (González and Díez, 2002). *Lb. sake*, *Lb. curvatus* and *Pediococcus* sp. have been reported (Santos *et al.,* 1998). García-Varona *et al.* (2000) reported the predominance of *Staphylococcus xylosus* among micrococcaceae in *chorizo.* Latorre-Moratalla *et al.* (2007) evaluated the application of high hydrostatic pressure (200 MPa) to meat batter just before sausage fermentation also studied and the inoculation of starter culture to improve the safety and quality of *chorizo*, traditional Spanish fermented sausages.

Salchichon

Salchichon is the Spanish dry-fermented sausage (Fernandez-Lopez *et al.,* 2008). It is prepared from pork or beef meat, fat, salt and spices and other additives (Bover-Cid *et al.* (2000 a,b) LAB, enterobacteriaceae, micrococcaceae, moulds and yeasts were reported from *salchichon* (Fernandez-Lopez *et al.,* 2008). Bover-Cid *et al.* (2000b) reported the higher concentration of tyramine in this product.

Androlla

Androlla is a traditional raw-cured sausage of Spain that has a good appreciation among consumers and a great installation in the local markets (Garcia Fontan *et al.,* 2007). The studies carried out in this product only refer to the biochemical characteristics of the final product (Lorenzo *et al.,* 2000) and to the biochemical changes during the manufacture (Franco *et al.,* 2002). Garcia Fontan *et al.* (2007) reported the presence of micrococcaceae, enterobacteriaceae and LAB such as *Lb. sake* followed by *Lb. curvatus, Lb. alimentarius* and *Lb. plantarum*.

Nham

Nham is the Thai semi-dry, uncooked, fermented pork or beef sausage, with rice and seasoning traditionally wrapped in small banana-leaf packets (Yanasugondha, 1977). It is one of the popular meat products of Thailand prepared from ground pork, shredded cooked pork rind, sucrose, garlic, salt, cooked rice, sodium erythrobate, trisodium polyphosphate, monosodium glutamate, whole bird chili and potassium nitrite were thoroughly mixed, stuffed into a plastic casing and sealed tightly (Visessanguan *et al.,* 2004). LAB, notably lactobacilli and staphylococci / micrococci were dominant with the lower counts of yeasts and moulds (Visessanguan *et al.,* 2006). The fermentation of *nham* takes 3-5 days and relies mainly on adventitious microorganisms, which are

normally found in raw materials (Khieokhachee *et al.,* 1997). The occurrence of pathogenic bacteria viz. *Salmonella* sp*., Staphylococcus aureus* and *Listeria monocytogenes* were also reported in *nham* with pH higher than 4.6 (Paukatong and Kunawasen, 2001). Thus, the application of *Lb. curvatus* as a starter culture in *nham* provide a tool for enhancing the competitiveness of the starter organisms in favour of the fortuitous flora as well as preventing the outgrowth of pathogenic bacteria (Visessanguan *et al.,* 2006). Based on the physiological properties and consumer acceptability, *Lb. curvatus* is a potential starter for *nham* fermentation. (Visessanguan *et al*., 2006).

(II) Bacon

Bacon is slice of cured pig meat, occasionally beef, eaten fried or grilled, usually eaten as breakfast and the product is consumed in Ireland, Britain, Netherlands, Denmark, USA, Canada, Australia, New Zealand, West Africa, East Africa and South Africa, and to some extent, India, Central and South America (Campbell-Platt, 1987). These products are made from pork sides, 22-28 kg, with back fat generally 30-40 mm thick, cured, curing, normally by pumping in curing salts, containing some or all of: sodium chloride, potassium nitrate, sodium nitrite, sugars and ascorbic acid (Kramlich *et al.,* 1973; Lawrie, 1979). *Micrococcus* and *Staphylococcus* reduce the nitrate to nitrite, which is the active form in

producing stable pink nitroso compounds and *Lactobacillus* are active in aiding fermentation or curing and maturing, particularly in larger process (Campbell-Platt, 1987). The experiments on bacon inoculated with *Clostridium botulinum* indicate that a microbial formation of lactic acid in ungrounded meat products also reduces the microbial hazards (Tanaka *et al*., 1980).

(III) Ham

Ham is semi-dry cured pork, salted and dried meat product, usually eaten as uncooked product; may be smoked and eaten cold and these product are consumed in Germany, Belgium, Scandinavia, France, Italy, Spain, Britain, Ireland, USA, Thailand, China (Campbell-Platt, 1987). Nitrate reduced to nitrite in curing process by the activity of *Micrococcus* and *Staphylococcus* (Bredholt *et al*., 2001). Hequet *et al*. (2007) studied the sliced cooked ham where the bacteriocinogenic bacteria, *Lb. sake* 2512, *Lb. curvatus* 2711 found to inhibit the growth of *Listeria*. Some LAB activity, principally *Lb. casei* and *Lb. plantarum* cause some lactic acid production. Some moulds usually *Penicillium nalgiovense* or *Aspergillus* spp. may appear on the surface of the dried hams, which are important in flavour development (Campbell-Platt, 1987).

The manufacture of raw hams may be accelerated if their pH is lowered to about 5.3 by injection of lab and fermentative carbohydrates

(Bartholomew and Blumer, 1980; Boshkovam *et al*., 1983). A strain of *Lb. sake* was isolated from cooked ham and inhibited growth of *Listeria monocytogenes* and *E.coli* O 157:H7 in this product. The findings presented confirm that *Lb. sake* strain is suitable for use as a protective culture and may technically easily be implemented in the commercial production of cooked meat products (Bredholt *et al*., 2001). Saldanha-da-Gama *et al*., (1997) reported that predominant species were *Debaryomyces hansenii, D. polymorphus, Cryptococcus laurentii, C. humicolus, and Pichia guillermondii*. The fatty acid profiles were roughly similar for all yeasts analysed suggesting that in these types of pork-based products the yeast lipid composition may have an ecological significance (Saldanha-da-Gama *et al*., 1997). During ripening of dry- cured ham, the moulds and yeasts that proliferate on the surface may contribute to flavour development and one strain each of *P. chrysogenum* and *D. hansenii*, selected from dry- cured ham by their proteolytic activity, were tested to determine their effect on the volatile compounds during ripening (Martin *et al*., 2003). Inoculation of pork loins with *P. chrysogenum* lead to a decrease in compounds attributed to lipid oxidation and to an increase of compounds derived from free amino acids and inoculation with *D. hansenii* seems to favour the formation of complex alcohol (Martin *et al*., 2003). The relationship between the superficial yeast population and the ripening condition of Italian dry cured hams has been studied and found that *Candida*

zeylanoides was the dominating yeast in early stages, whereas more than 99% of isolates were identified as *D. hansenii* (Nunez *et al*., 1996). The consumption of dry-cured ham is restricted by hypertensive consumers due to its high sodium content (Blesa *et al.,* 2008). Lorenzo *et al.* (2007) studied the biogenic amine content during the manufacture of dry cured bacon, where the tryptamine and spermine were the main in fresh meat while the tryptamine and cadaverine were the most abundant at the end of manufacturing process. However, the total biogenic amines content at the end of the manufacturing process was low as expected Lorernzo *et al.* (2007). The use of selected fungi such as *P. chrysogenum* and *D. hansenii* as starter cultures may be useful to obtain high-quality and safe dry cured-ham (Martin *et al*., 2006). Non-enteric Gram-negative bacteria were the dominant microbial group, sharing a positive correlation with the moisture of spoiled hams (Martin *et al*., 2008). The catalase positive cocci growth was favoured by high NaCl concentrations in the spoiled hams, whereas the counts of enterobacteriaceae were negatively affected by high NaCl concentration (Martin *et al*., 2008). The combination of low storage temperature (1º C, high pressure processing and addition of lactate-diacetate reduced the level of *Listeria monocytogenes* in sliced cooked ham (Marcos *et al.,* 2008).

(IV) Dried meat

Dried meat are those which has been cured, during which mild fermentation takes place, then often smoked and dried (Campbell-Platt, 1987). The use of LAB as protective cultures in vacuum-packed chill-stored meat has potential application for assuring and improving food quality (Jones *et al.,* 2008). Dry-cured meat products are well known for their unique sensory characteristics (Arnau, 2007). The lactic acid has been used as an efficient decontaminant in meats aimed for direct consumption and the meat preservation by lactic fermentation with selected strains can be an alternative when keeping meat protein functional properties unaltered (Signorini *et al.,* 2007). Usually eaten cold, thinly sliced, consumed in Europe particularly Switzerland, Middle East, North America, South America, Indonesia, and Thailand (Campbell-Platt, 1987). Meat, often beef, cut into 2-8 kg pieces and heavily salted with dry curing salts, of NaCl, potassium or sodium nitrate or sodium nitrite, with small amount of sugars, spices and seasonings added (Campbell-Platt, 1987). The shelf-life of aerobically stored ground beef can be extended by the addition of lactic acid bacteria and fermentable carbohydrate (Reddy *et al*., 1970). It is also possible to extend the shelf-life of pack pasteurized meat product by lowering the pH prior to heat treatment (Mol and Timmers, 1970). The potential bioactive packaging technologies to prevent the development and spread of spoilage and pathogenic

microorganism via foodstuffs, antimicrobial packaging materials could be the potential alternative solutions for extended self-life of meat based products (Coma, 2008). The combination of several non-thermal and thermal preservation technologies under the so-called hurdle concept has also been investigated in order to increase their efficiency (Aymerich *et al.,* 2008). Quick thermal technologies such as microwave and radiofrequency funnels of steamed pasteurization bring new possibilities to the pasteurization of meat products especially in ready to eat meals and their application after final packaging will prevent further cross-contamination during post-processing handling (Aymerich *et al.,* 2008).

(V) Jerky

Jerky is meat that has been cut into strips trimmed of fat, marinated in a spicy, salty or sweet liquid, and then dried with low heat (usually under 70°C/160°F) or occasionally salted and dried in sun (Lücke, 2003). The result is a salty, stripped, semi-sweet snack that can be stored without refrigeration and it is an early application of food preservation techniques (Delong, 1992). When the growth of aerobic spoilage bacteria is inhibited, lactic acid bacteria may become the dominant component of the microbial flora of meats. This occurs with cured meats and with meats packaged in films of low gas permeability (Egan, 1983). Jerky is the lean meat, salted and sun dried or hot-air dried

in stripes or thin sheets in hot climates to dry product with good keeping quality at high temperatures and consumed in North, Central and South America and West Indies; Africa; eaten as snack, or crumbled into soups and stews (Campbell-Platt, 1987). Meat pieces salted with often impure sodium chloride, which may also contain some nitrate, cut into stripes, further salted and sun or air-dried for several days to give very dry product which keeps well without refrigeration (Egan, 1983). *Micrococcus* and *Staphylococcus* active in reducing nitrate to nitrite, and some involvement of LAB in flavour development. Some protein hydrolysis takes place during maturation (Campbell-Platt, 1987).

MEDIA USED

(1). Arginine Hydrolysis Medium (Thornley, 1960)

Peptone	10.0 g
Yeast extract	5.0 g
D (+) glucose	0.5 g
$K_2HPO_4.3H_2O$	2.0 g
Magnesium sulphate	0.1 g
Manganese sulphate	0.05 g
Sodium acetate	5.0 g
Tri-sodium citrate	20.0 g
Tween 80	1.0 ml
Arginine	0.3 %
Phenol red	0.01 g
Distilled water	1000 ml
pH	5.0

(2). Ascospore Agar (M804, HiMedia)

(3). *Bacillus cereus* Agar Base (M833, HiMedia)

(4). Bacteriocin Screening Medium (Tichaczek *et al*., 1992)

Peptone	10 g
Beef extract	5.0 g
Yeast extract	5.0 g
Glucose	2.0 g
K_2HPO_4	2.0 g
Tween 80	1.0 g
Diammonium citrate	2.0 g
Sodium acetate	5.0 g
$MgSO_4$	0.1 g
$MnSO_4$	0.05 g
Distilled water	1000 ml
pH	6.5
Agar	12 g

(5). Baird Parker Agar Base (M043, HiMedia)

(6). Bile salt (MG3 M531040, Merck)

(7). Biogenic amine Culture Medium (Bover-Cid and Holzapfel, 1999)

MRS Broth (M369, HiMedia, Mumbai)	52.2 g
D-Tyrosine (RM 1520, HiMedia, Mumbai)	1.0 g
L-Histidine monohydrochloride (Merck)	1.0 g
L-Lysine monohydrochloride (Merck)	1.0 g
L-Ornithine monohydrochloride (Merck)	1.0 g
Pyridoxal-5-Phosphate (RM 1554, HiMedia)	0.001 g
Distilled water	1000 ml
pH	6.00

(8). Biogenic Amine Screening Medium (Joosten and Northold, 1989)

Tryptone	5.0 g
Yeast extract	5.0 g
Meat extract	5.0 g
Sodium chloride	2.5 g
Glucose	0.5 g
Tween 80	1.0 g
K_2HPO_4	2.0 g
Ammonium citrate	2.0 g
Calcium carbonate	0.1 g
$MgSO_4.7H_2O$	0.2 g
$MnSO_4.4H_2O$	0.05 g
$FeSO_4.7H_2O$	0.04 g
Thiamine	0.001 g
Pyridoxal-5-phosphate	0.005 g
Bromocresol purple	0.05 g
Agar	22.0 g
Amino acid	5.0 g
Distilled water	1000 ml

Amino acids used were D-Tyrosine (pH 5.3) (RM 1520, HiMedia); L-Histidine monohydrochloride (pH 5.0) (Merck, Germany); L-Lysine monohydrochloride (pH 5.15) (Merck, Germany); L-Ornithine monohydrochloride (pH 5.0) (Merck, Germany).

(9). Egg Yolk Emulsion (FD045, HiMedia)

(10). Egg Yolk Tellurite Emulsion (FD046, HiMedia)

(11). Fermentation Basal Medium for yeasts (Wickerham, 1951)

Yeast extract	4.5 g
Peptone	7.5 g
Distilled water	1000 ml
Bromothymol blue (Till sufficiently dense green colour appears)	

(12). Furazolidone (FTO) Agar

Peptone	10 g
Yeast Extract	5 g
NaCl	5 g
Glucose	1 g
Agar	12 g
Distilled water	1000 ml
pH	7.0

Furazolidone (F9505, Sigma) – 100 ml of a 0.02 % acetone solution of furazolidone are mixed under slow stirring with the basal medium. Before pouring the plates the flask are left open in a water bath for 3-5 minutes to allow the evaporation of acetone (von Rheinbaben and Hadlok, 1981)

(13). Lactate Configuration Medium (Tamang *et al*., 2005)

Peptone from casein	10.0 g
Yeast extract	4.0 g
Glucose	20.0 g
Di-potassium hydrogen phosphate	2.0 g
Tween 80	1.0 g
Di-ammonium hydrogen phosphate	2.0 g
Magnesium sulphate	0.2 g
Manganese sulphate	0.04 g
Distilled water	1000 ml

(14). *Listeria* Identification Agar Base (M 1064, HiMedia)

(15). *Listeria* Selective Supplement (FD 061, HiMedia)

(16). Luria Burtani Broth (Duc *et al.,* 2004)

Tryptone	10 g
Yeast extract	5.0 g
NaCl	10 g
Distilled water	1000 ml
pH	7.2 – 7.4
Bile salt	2.0 %

(17). Malt Extract Agar (M137, HiMedia)

(18). Milk Agar (Gordon *et al*., 1973)

Skim milk powder (RM1254, HiMedia)	5.0 g in 50 ml distilled water
Agar	1.0 g in 50 ml distilled water

(19). Mannitol-salt Phenol-red Agar (Merck)

(20). MRS Agar (M641, HiMedia)

(21). MRS Broth (M369, HiMedia)

(22). Nitrate Broth (Gordon *et al*., 1973)

Peptone	5.0 g
Beef extract	3.0 g
Potassium nitrate	1.0 g
Distilled water	1000 ml
pH	7.0

Autoclaved separately at 121^{o} C for 20 min, cooled to 45^{o} C, mixed together and poured into petridishes. The plates were allowed to stand at 37^{o} C for 24 h to dry the surface of the agar.

(23). Nutrient Agar (MM012, HiMedia)

(24). Nutrient Broth (M002, HiMedia)

(25). Plate Count Agar (M091, HiMedia)

(26). Potato Dextrose Agar (M096, HiMedia)

(27). *Salmonella–Shigella* Agar (M108, HiMedia)

(28). Simmons Citrate Agar (M099, HiMedia)

(29). Skim Milk Powder (RM1254, HiMedia)

(30). Starch Agar (Gordon *et al*., 1973)

Starch (HiMedia RM089)	1.0 g in cold distilled water
Tryptone	5.0 g
Yeast extract	15.0 g
Potassium dihydrogen phosphate	3.0 g
Agar	20.0 g
Distilled water	1000 ml

(31). Sucrose Broth (Garvie, 1960)

Tryptone	10 g
Yeast extract	5.0 g
K_2HPO_4	5.0 g
Trisodium citrate	5.0 g
Sucrose	50 g
Distilled water	1000 ml

(32). Tryptone Soya Agar (M290, HiMedia)

(33). Voges-Proskauer (VP) Broth (Gordon *et al*., 1973)

Peptone	7.0 g
Glucose	5.0 g
Sodium chloride	5.0 g
Distilled water	1000 ml
pH	6.5

(34). Violet Red Bile Glucose Agar w/o Lactose (M581, HiMedia)

(35). Yeast-Malt Extract (YM) Agar (M424, HiMedia)

(36). Yeast Malt Extract (YM) Broth (M425, HiMedia)

(37). Yeast Morphology Agar (M138, HiMedia)

(38). Yeast Nitrogen Base (M139, HiMedia)

REAGENTS

(1). Burke's Iodine Solution (Bartholomew, 1962)

Iodine	1.0 g
Potassium iodide	2.0 g
Distilled water	100 ml

(2). Gram's Crystal Violet (S012, HiMedia)

(3). Malachite Green (S020, HiMedia)

(4). Nessler's Reagent

Potassium iodide	50.0 g
Mercuric chloride (saturated)	35.0 ml
Distilled water (ammonia free)	25.0 ml
Potassium hydroxide (50 %)	400.0 ml

Potassium iodide was dissolved in 35 ml of distilled water followed by addition of saturated aqueous solution of mercuric chloride till the appearance of precipitate. Then, 400 ml of potassium hydroxide was added and made the final volume to 1000 ml by adding distilled water. The solution was left for a week; the supernatant was decanted and stored in capped amber bottle at 4^{o} C.

6). Nitrate Reduction Test Reagent

Solution A

Sulphanilic acid	0.8 g

5 N acetic acid		100 ml
(Glacial acetic acid: water, 1: 2.5)		
Solution B		
∝-Napthylamine	0.5 g	
5 N acetic acid		100 ml

The solutions A and B were mixed in equal quantities just before use.

(5). Phenolphthalein (I009, HiMedia)

(8). Ringer solution (Merck, Germany)

(7). Safranin (S027, HiMedia)
Reference Strains: used as indicator strains for antimicrobial activity

Reference Strains	Origin
Bacillus cereus CCM 2010	CCM
Enterobacter agglomerans BFE 154	BFE
Klebsiella pneumoniae subsp. *pneumoniae* BFE 147	BFE
Listeria innocua DSM 20649	DSM
Listeria monocytogenes DSM 20600	DSM
Pseudomonas aeruginosa BFE 162	BFE
Staphylococcus aureus S1	FMR

Originally, these reference strains were obtained from DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany), CCM (Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia), BFE (Institute of Hygiene and Toxicology, Karlsruhe, Germany), and FMR (Food Microbiology Laboratory, Sikkim Government College, Gangtok, India).

Listeria monocytogenes DSM 20600, *Staphylococcus aureus* S1, *Bacillus cereus* CCM 2010, and *Klebsiella pneumoniae* subsp. *pneumoniae* BFE 147, *Pseudomonas aeruginosa* BFE 162, *Listeria innocua* DSM 20649, *Enterobacter agglomerans* BFE 154, were propagated in standard nutrient agar. The cultures were maintained as frozen stocks at –20° C in 15 % glycerol.

METHODOLOGY

Survey

A field survey was conducted in different places of the Sikkim Himalaya and the Kumaun Himalaya to document the indigenous knowledge of ethnic people on meat processing. The information gathered from ethnic communities representing Bhutias, Lepchas, Nepalis and Tibetans. Indigenous knowledge on the types of traditional meat products, their methods of preparation, culinary and their mode of consumption, socio-economy and ethnical importance of the product was also documented based on the information sought from the local people of the respective places.

Collection of samples

A total of 52 samples of traditionally prepared meat products were collected directly from different villages located in the Sikkim Himalayas. Out of 52 samples collected, 17 samples were *lang kargyong*, 7 samples

were *yak kargyong*, 8 samples were *faak kargyong*, 8 samples were *lang satchu*, 6 samples were *yak satchu* and 6 samples were *suka ko masu/sheakua*. Similarly, 16 samples of traditional meat products were also collected from different places of Kumaun Himalaya in Pithoregarh district of Uttarakhand. Samples were collected aseptically in pre-sterile poly-bags as well as sterile bottles and were, sealed and labeled. Samples were stored at –20° C for further microbial and biochemical analyses.

Microbiological analysis

Ten g of sample were homogenised with 90 ml of 0.85 % (w/v) sterile physiological saline in a stomacher lab-blender (400, Seward, UK) for 1 min. A serial dilution (10^{-1} to 10^{-8}) in the same diluent was made.

LAB: Lactic acid bacteria commonly called LAB were isolated on MRS agar (M641, HiMedia) plates supplemented with 1 % $CaCO_3$, and were incubated at 30° C under anaerobic condition kept in an Anaerobic Gas-Pack container (LE002, HiMedia) for 48-72 h.

Micrococcaceae: Mannitol-salt Phenol-red Agar (Merck) media were used for the detection of micrococcaceae in the samples following the method of Papamanoli (2002). Inoculated plates were incubated at 30° C for 48 h. Identity of micrococci was further confirmed by growing in FTO (furazolidone) agar (von Rheinbaben and Hadlok, 1981). Spread plates of Baird Parker agar base (M043, HiMedia) with appropriate additions of

Egg yolk tellurite emulsion (FD046, HiMedia) was used for selective enumeration of *Staphylococcus aureus*. After serial dilution plates were overlaid with the medium and incubated at 30° C for 48 h. Black colonies surrounded by a clear zone extending 2-5 mm into the opaque medium appeared were regarded as presumptive *Staphylococcus aureus*.

Bacilli: Spore-forming bacilli were isolated on nutrient agar (MM012, HiMedia), after inactivation of vegetable cells by heating at 100° C for 2 min (Tamang and Nikkuni, 1996) and then incubated at 37° C for 24 h.

Fungi : Moulds and yeasts were isolated on potato dextrose agar (M096, HiMedia) and yeast-malt extract (YM) agar (M424, HiMedia), supplemented with 10 IU/ml benzylpenicillin and 12 μg/ml streptomycin sulphate, respectively; and plates were incubated aerobically at 28° C for 72 h.

TVC: Total viable count (TVC) was determined in the plate count agar (M091A, HiMedia) plates which were incubated at 30° C for 48-72 h.

Colonies of all microorganisms were selected randomly or all sampled if the plate contained less than 10 colonies according to Leistner *et al*. (1997). 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. Microbiological data obtained were transformed into logarithms of the numbers of colony

forming unit (cfu) per g of sample. Identified strains of microorganisms were preserved in respective media using 15 % (v / v) glycerol at –20° C.

Characterisation of Bacterial Isolates

Cell morphology

The smear of a 24 h-old bacterial culture was made in a grease free slide, air-dried (not heated-fixed), stained for 30 sec with safranin (S027, HiMedia), washed in water, air-dried (Harrigan, 1998) and observed under oil-immersion objective. Cell dimensions were measured with a standardized ocular micrometer.

Gram staining

Bacterial isolates were Gram-stained following the method of Bartholomew (1962). A suspension of a 24 h-old bacterial culture on slant was prepared, and smear was made in a grease-free slide. Then heated-fixed, flooded by crystal violet stain for 1 min, and washed for 5 sec with water. The smear was flooded with Burke's iodine solution, allowed to react for 1 min, and washed again for 5 sec with water. Holding the slide against a white surface, 95 % ethanol was poured drop-wise from the top

edge of the slide until no more colour came out from the lower edge of the slide. After washing with water, the smear was stained with safranin for 1 min and washed again with water. The slide was air-dried and observed under oil-immersion objective.

Motility

A hanging drop of a 24 h-old culture in MRS broth was prepared in a cavity slide following the method of Harrigan (1998). The prepared culture was observed in a phase contrast microscope (Olympus CH3-BH-PC, Japan) for motility of the strains

Production of catalase

The production of gas bubbles by the isolates were observed by adding 0.5 ml of 10 % hydrogen peroxide solution (Merck) to the cultures indicating the presence of catalase (Schillinger and Lücke, 1987).

Hydrolysis of arginine

Tubes of 5 ml arginine hydrolysis medium (Thornley, 1960) were inoculated with 24 h-old culture. The tubes were incubated at 30º C for 3 days and formation of ammonia from arginine was detected by spotting 100 µl cultures onto a white porcelain tile and adding equal volume of Nessler's reagent. Appearance of dark orange colour indicated presence of ammonia (Schillinger and Lücke, 1987).

Production of gas

Tubes of 10 ml MRS broth without citrate and containing inverted Durham tubes was inoculated with 24 h-old cultures and incubated at 30º C (Schillinger and Lücke, 1987). Accumulation of gas in the inverts indicated the positive result.

Production of dextran from sucrose

Production of dextran in sucrose broth (Garvie, 1960) was tested exclusively for leuconostoc isolates following the method described by Kelly *et al.* (1995).

Growth at different pH

The pH of MRS broth was adjusted to 3.9 and 9.6 using 1 N HCl or 10 % w/v NaOH. The medium was distributed into tubes containing 5 ml in each. They were autoclaved, cooled to room temperature and inoculated with 24 h-old MRS broth culture. The tubes were incubated at 30º C for 24-72 h and observed for growth (Dykes *et al*., 1994).

Growth at different temperatures

The 24 h-old cultures were inoculated in MRS broth and incubated at 8º C, 10º C and 15º C for 7 days, and 45º C for 3 days, respectively and observed for growth (Dykes *et al*., 1994).

Growth in different NaCl concentrations

Salt tolerance was tested by inoculating a loop-full of culture in MRS broth supplemented with 6.5 %, 10 % and 18 % NaCl, respectively, and incubated for 3 days at 30º C in a slanting position to improve aeration (Schillinger and Lücke, 1987). Cultures were observed for growth after incubation.

Acid from carbohydrates

The method was based on Schillinger and Lücke (1987). Tubes of 5 ml MRS broth without beef extract, containing 0.5 % w/v of different carbohydrates instead of glucose and 0.004 % phenol red indicator were inoculated and incubated at 30º C for 2-5 days. Colour change from red to yellow indicated acid production.

Lactic acid configuration

The configuration of lactic acid produced was determined enzymatically using D-lactate and L-lactate dehydrogenase kits (Boehringer-Mannheim GmbH, Cat. No.1112821, Germany). The LAB strains were grown in lactate configuration medium (Tamang *et al*., 2005) at 30º C overnight. One ml culture was centrifuged in a microcentrifuge (Heraeus, Germany) at 8,000 *g* for 5 min. The 20 µl of the supernatant was mixed with 980 µl of redistilled water to obtain 1:50 sample dilution. The 1 ml of Solution (1), 0.2 ml of Solution (2), 0.02 ml of Suspension (3), 0.1 ml sample solution and 0.9 ml of redistilled water was pipetted into a cuvette, followed by gentle swirling to mix the contents of the cuvette after closing it with parafilm. Similarly, a blank was prepared by adding all the reagents except the sample solution being replaced with 1.0 ml of redistilled water. After 5 minutes the absorbance of the solutions (A_1) was measured in UV-VIS Spectrophotometer (Analytik Jena, Germany) at 340 nm. The absorbance differences (A_2-A_1) for both, blank and sample was determined and the difference of the absorbance difference of the blank from that of the sample ($\Delta A_{D\text{-lactic acid}}$) was calculated. The reaction was started by adding 0.02 ml of Solution (4) to the sample as well as to the blank. The cuvettes were swirled gently to mix the contents by closing it with parafilm. After 30 minutes the absorbance (A_2) of the sample and the blank were measured immediately one after another at 340 nm. The 0.02

ml of Solution (5) was added to both the sample and the blank followed by mixing. These were allowed to stand for 30 minutes. The absorbance (A_3) was measured immediately one after another for the sample as well as for the blank at 340 nm. The absorbance differences (A_3-A_2) for both, blank and sample was determined and the difference of the absorbance difference of the blank from that of the sample ($\Delta A_{L\text{-lactic acid}}$) was calculated. The lactic acid isomer concentration was calculated as: $c = (V \times MW \times \Delta A)/(\varepsilon \times d \times v \times 1000)$ (g/l), where, V = final volume (ml), v = sample volume (ml), MW = molecular weight of lactic acid = 90.1 (g/mol), d = light path = 1 cm, ε = extinction coefficient of NADH at 340 nm = 6.3 (l/m mol $\times$ cm). The result was multiplied by the dilution factor.

Diaminopimelic acid (DAP)

The presence of *meso*-diaminopimelic acid in the cell walls of LAB was determined using thin-chromatography on cellulose plate (Tamang *et al*., 2000). Cells were harvested from 48 h old MRS broth culture by centrifuging 5 ml culture at 8,000 *g* for 5 min, and washed with 3 ml of distilled water. The sediment was resuspended in 1 ml of 6 N HCl and transferred to screw-capped tubes. The cells were hydrolysed overnight at 100° C in a water-bath. The contents of the tubes were blow-dried while immersed in boiling water. The sediment was resuspended in 1 ml of distilled water and blow dried again and oven dried for 1 hour. Finally,

the sediment was suspended in 0.1 ml of distilled water and each sample (5 µl) was spotted on thin-layer chromatography plates on cellulose plates (Merck, Germany). Descending one-dimensional chromatography was done by keeping the plates in a TLC chamber in a solvent solution containing methanol: pyridine: 10 N HCl: water (32:4:1:7). The solvent solution was prepared 1 h before use. After keeping for 4-5 h the plates were dried with a hair drier and the chromatograms were developed by spraying acidic ninhydrin and when almost dried, placed for 5 min in 100° C oven. Spots representing *meso*-diaminopimelic acid appeared dark green to grey and turned yellow within 24 hour. *Lactobacillus plantarum* DSM 20174 was used as standard (*meso*-DAP positive).

API (Analytical Profile Index) system

Carbohydrate fermentation patterns of LAB were determined using API 50 CHL and API 20 STREP commercial test strips (bioMérieux, France) according to manufacturer's instructions as well as the method described by Tamang and Holzapfel (1999). Cultures were grown on MRS agar at 30º C for 48 h. The growth was harvested in 2 ml sterile water which was used to prepare suspensions, corresponding to 10^7 cells/ml. Incubation box was prepared by distributing about 10 ml of sterile water into the honeycombed base of the 50 CHL trays. Strips were unpacked, placed them in the trays and the tubes were filled with the bacterial

suspensions. Inoculated strips were kept slightly tilted and incubated at 30º C for 48 h. The results were read by referring to the manufacturer's interpretation chart at 24 h and 48 h, respectively. All spontaneous reactions were recorded. The APILAB PLUS database identification software (bioMérieux, France) was used to interpret the results.

Phenotypic identification

Bacterial species were identified following the taxonomic keys of Bergey's Manual (Sneath *et al*., 1986), Simpson and Taguchi (1995), Wood and Holzapfel (1995), and by APILAB PLUS database software (bioMérieux, France).

Characterisation of yeast Isolates

Cell morphology

Cell morphology and mode of vegetative reproduction of yeast was observed following the method of Yarrow (1998). Sterile yeast morphology agar (M138, HiMedia) slants were inoculated with an actively growing (24 h-old) yeast culture and incubated at 28º C for 3 days. Dimensions of cells were measured with a standardized ocular micrometer.

Pseudo- and True-mycelium

For observation of pseudo-mycelium and true-mycelium of yeast isolates, the slide culture method described by Kreger-van Rij (1984) was followed. A petri-dish, containing U-shaped glass rod supporting two glass slides, was autoclaved at 121º C for 20 min. The glass slides were quickly removed from the glass rod with a flame sterilised pair of tweezers, and were dipped into the molten potato dextrose agar (M096, HiMedia) after which they were replaced on the glass rod support. The solidified agar on the slides was inoculated very lightly with yeast isolates in two lines along each slide. Four sterile coverslips were placed over part of the lines. Some sterile water was poured into the petri-dish to prevent the agar from drying out. The culture was then incubated at 28º C for 4 days. The slides were taken out of the petri-dish and the agar was wiped off from the back of the slide. The edges of the streak under and around the coverslips were examined microscopically for the formation of pseudo-mycelium or true-mycelium.

Characteristics of asci and ascospore

Sterile ascospore agar (M804, HiMedia) slants were streaked with actively grown yeast cultures, incubated at 28º C for 3 days and examined at weekly intervals up to 4 weeks for observation of asci and ascospores. A heat fixed smear was flooded with 5 % w/v aqueous malachite green (S020, HiMedia) for 30 to 60 sec, heated to steaming 3 to 4 times over the

flame of a spirit lamp and counterstained with safranin (S027, HiMedia) for 30 sec and observed under the microscope (Yarrow, 1998).

Reduction of nitrate

Yeast cultures were grown in 5 ml nitrate broth incubated at 28º C. After 3, 7 and 14 days, 1 ml of the culture was mixed with 3 drops of the reagent for nitrate reduction test and observed for the development of a red or yellow colour, indicating the presence of nitrate. A small amount of zinc dust was added to the tube that was negative even after 14 days and observed for the development of red colour, indicating the presence of nitrate, i.e. absence of reduction (Yarrow, 1998).

Growth at 37º C

Slants of malt-extract agar (M137, HiMedia) were inoculated with actively grown yeast isolates and incubated at 37º C for 4 days and observed for growth (Yarrow, 1998).

Sugar fermentation

Yeasts isolates were grown at 28º C on yeast-malt extract (YM) agar (M242, HiMedia) slants for 3 days. Tubes of 10 ml of fermentation basal

medium (Wickerham, 1951) supplemented with 2 % w/v sterile sugars containing inverted Durham tubes, were inoculated with the above yeast culture and incubated at 28º C and were shaken regularly to observe gas accumulation in the inverts (Yarrow,1998).

Sugar assimilation

Yeast isolates were grown at 28º C on yeast-malt extract (YM) agar (M242, HiMedia) slants for 3 days. Tubes containing 5 ml mixture of yeast nitrogen base (M139, HiMedia) and carbon source were inoculated with cultures and incubated at 28º C for 3 to 7 days. Control test tube was made by adding 0.5 ml of yeast nitrogen base in 4.5 ml of sterilised distilled water (devoid of any carbon source). Assimilation of carbon sources was observed by comparing with the control (Yarrow, 1998).

Identification of Yeast

Yeast isolates were identified to the genus level according to Kreger-van Rij (1984), Kurtzman and Fell (1998) and Yarrow (1998).

Pathogenic bacteria

Enumeration of pathogenic bacteria from the meat samples were done in selective media such as *Bacillus cereus* agar base (M833, HiMedia) for *Bacillus cereus*, Violet Red Bile Glucose agar w/o lactose (M581,

HiMedia) for enterobacteriaceae (Han *et al*., 2001). *Salmonella-Shigella* Agar (M108, HiMedia) was used for the detection of *Salmonella* and *Shigella* and *Listeria* identification agar base (M1064, HiMedia) with *Listeria* selective supplement (FD 061, HiMedia) for *Listeria* in the samples following the standard method of Metaxopolous *et al*. (2001). Ten g of sample were blended with 90 ml of peptone-physiological saline (0.1 % neutral peptone, 0.85 % NaCl) and homogenized in a stomacher lab-blender 400 (Seward, UK) for 1 min. Serial decimal dilution series was prepared in the same diluent in duplicate sets.

Bacillus cereus: Selective enumeration was carried out on spread plates of *Bacillus cereus* agar base (M833, HiMedia) with appropriate additions of Polymyxin B Selective Supplement (FD003, HiMedia) and Egg yolk emulsion (FD045, HiMedia). The inoculated plates were incubated at 30º C for 24-48 h. Characteristic turquoise to peacock blue colonies surrounded by a zone of precipitate of the same colour was regarded as presumptive *Bacillus cereus*.

Enterobacteriaceae: Sample dilutions in tryptone soya broth (M011, HiMedia) were allowed to resuscitate on thinly plated tryptone soya agar (M290, HiMedia) plates for 2 h at 27° C, followed by a thick overlay of selective Violet Red Bile Glucose agar (without lactose) (M581, HiMedia)

medium and incubated at 30° C for 20 h. Pink colonies appeared were regarded as presumptive enterobacteriaceae.

Listeria sp: *Listeria* identification agar base (M1064, HiMedia) with *Listeria* selective supplement (FD061, HiMedia) media were used for detection of *Listeria* in the samples following the method of Metaxopolous *et al*. (2001). Inoculated plates were incubated at 30º C for 48 h and observed in dark background for smooth glistening colonies indicating presence of *Listeria*

Salmonella and *Shigella*: *Salmonella-Shigella* (SS) agar (M108, HiMedia) was used for the detection of *Salmonella* and *Shigella* in samples following the method of Metaxopolous *et al*. (2001). Serial dilution plates were inoculated which was followed by an overlay of the SS agar, and incubated at 37º C for 48 h and observed in dark background for presumptive colonies. Dark-centred colonies were presumed as *Salmonella*, while colourless colonies presumptive *Shigella*.

Voges-Proskauer reaction

Tubes of 10 ml Voges-Proskauer broth were inoculated with the isolates and incubated at 37º C for 7 days. To the culture, 0.6 ml 5% w/v ethanolic α- naphthol and 0.2 ml 40% w/v aqueous potassium hydroxide were added and kept for 1 h at room temperature for the production of a

pink colour, indicating positive reaction. Initial and final pH of the broth was measured using pH meter (Gordon *et al*., 1973).

Anaerobic growth

Anaerobic agar (M228 HiMedia) was distributed into culture tubes in amount sufficient to give 7.5 cm depth of the medium and sterilized by autoclaving at 121º C for 20 min. The tubes were inoculated with a small loopful of 24 h-old nutrient broth culture by stabbing up to the bottom of the column. Cultures were incubated at 37º C for 3 and 7 days, and observed for growth along the length of the stab (anaerobic) and on the surface of the agar (aerobic) as described by (Claus and Berkeley, 1986).

Hydrolysis (decomposition) of casein

Milk agar plates were streaked with 24 h-old cultures and examined after incubating at 30º C for 7 days (lactic acid bacteria) and 37º C for 3 days (spore forming bacteria) for any clearing of casein around and underneath the growth (Gordon *et al*., 1973).

Hydrolysis of starch

Starch agar plates were streaked with 24 h-old cultures and incubated at 30º C for 3 days (lactic acid bacteria) and 37º C for 3 days (spore forming bacilli). After incubation the plates were flooded with iodine solution for 15-30 min and measured the clear zone underneath (after the growth was scrapped off).

Bile salt tolerance

The Luria Burtani broth supplemented with 0.2 % bile salts were inoculated with 24 h old culture of *Bacillus* isolates and incubated at 37º C for 7 days. Broths were observed for growth in 3rd and 7th days (Duc *et al.,* 2004).

Citrate utilization test

Simmon's citrate agar (M099, HiMedia) is a defined medium containing sodium citrate as a sole carbon source and the ammonium ion as the sole nitrogen source. The pH indicator, bromocresol blue, will turn from green at neutral pH (6.9) to blue when a pH higher than 7.6 is reached (basic or alkaline). If the citrate is utilized, the resulting growth will produce alkaline products (pH>7.6), changing the color of the medium from green to blue (Osbourne and Stokes, 1955).

Technological Properties of Isolates

Enzymatic profile by API-zym

The enzymatic profile of LAB and yeast isolates were assayed following the method of Arora *et al*. (1990) using API-zym (bioMérieux, France) galleries by testing for the activity of the following 19 enzymes: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine-, valine- and cystine-arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. LAB cultures were grown on MRS broth, and yeast cultures were grown on YM broth, respectively and cells were harvested in 2 ml sterile distilled water which was used to prepare suspension of 10^7 cells/ml. The strip was unpacked and 2 drops of cell suspensions was inoculated in each cupule of the strip containing ready-made enzyme substrates and incubated at 30º 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.

Antimicrobial and bacteriocin activity

Agar Spot Test: The LAB isolates were screened for antimicrobial activity against some of the pathogenic bacteria by agar spot method of Schillinger and Lücke (1989). Cultures were grown on the respective

broth media for 24 h. Sterilised petri-plates were plated with MRS agar (containing 0.2 % glucose) and allowed to dry. These were spotted with a drop of the broth culture of the producer strain and incubated at 30° C for 24 h. The indicator strains *Bacillus cereus* CCM 2010, *Enterobacter agglomerans* BFE 154, *Klebsiella pneumoniae* subsp. *pneumoniae* BFE 147, *Listeria innocua* DSM 20649, *Listeria monocytogenes* DSM 20600, *Pseudomonas aeruginosa* BFE 162 and *Staphylococcus aureus* S1 were propagated in standard nutrient agar (M002, HiMedia). The 0.1 ml of an overnight culture (~10^7 cells) of each indicator strain was inoculated into 7 ml of soft MRS agar (containing 0.7 % agar) and poured over the plate on which the producer was grown, respectively. These were incubated at 30° C for 24 h. After incubation, the plates were checked for inhibition zones (clearing of the medium) around the producer colony.

Bacteriocin Activity: Bacteriocin activity of the LAB isolates was estimated using an agar spot assay as described by Uhlman *et al*. (1992) and Schillinger *et al.* (1993). The antimicrobial-positive strains were grown in MRS broth at 30° C for 24 h and a cell-free extract was obtained by centrifuging the culture in a microcentrifuge (Heraeus, Germany). The supernatant was heated at 100° C for 5 min in block-thermostat (Staurt Scientific, UK). The cell-free supernatant was adjusted to pH 6.5 by addition of 1 N NaOH. Agar plates overlaid with 7 ml soft MRS agar

(containing 0.7 % agar) were inoculated with 0.1 ml of an overnight culture of the indicator strains (as mentioned above), respectively. After incubation at 25° C for 24 h, 0.01 ml of the culture supernatant was spotted onto the agar surface. The plates were incubated at 30° C for 24 h and subsequently examined for zones of inhibition.

Biogenic amine

The ability of LAB isolates to produce biogenic amines was determined qualitatively on an improved screening medium as described by Bover-Cid and Holzapfel (1999) using a 'cocktail' of four precursor amino acids (histidine, lysine, ornithine and tyrosine). Cultures previously grown and sub-cultured twice in biogenic amine sub-culturing medium were spotted onto the plates containing screening medium. 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).

Hydrophobicity assay

Bacterial adhesion to hydrocarbons was determined and results were expressed according to Rosenberg (1984) and Perez *et al*. (1998), with modification. Fresh cultures were grown in MRS broth at 30º C for 24 h and centrifuged at 8,000 *g* for 5 min. The pellet was washed three times

with 9 ml of Ringer solution (Merck, Germany), and thoroughly mixed in a vortex. The 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) in duplicates 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. 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 (Martin *et al*., 1989; Nostro *et al*., 2004).

Titratable acidity

Titratable acidity of sample was calculated by titrating the filtrates of a well blended 10 g sample in 90 ml carbon-dioxide free distilled water with 0.1 N sodium hydroxide to end point of phenolphthalein (0.1 % w / v in 95 % ethanol) (AOAC, 1990).

Moisture

Moisture content of the batters was calculated by drying 2.5–3.0 g of well-mixed sample at 135 ± 1º C for 2 h to constant weight (AOAC, 1990).

Ash

A sample (~2 g) was accurately weighed into a previously dried and weighed porcelain crucible and placed in a muffle furnace, preheated to 550° C for 3 h. The crucible was transferred directly to desiccators, allowed to cool to room temperature and weighed immediately (AOAC, 1990). Process of heating for 30 min, cooling and weighing was repeated until the difference between two successive weighing was ≤ 1 mg.

Fat

Fat content of the sample was determined by ether extraction using glass soxhlet (AOAC, 1990). Flat-bottomed flask was oven dried and kept in a desiccator for cooling. The weight (W_1) of the round-bottomed flask was taken. A cellulose thimble (dry and fat free) was taken and in which ~ 2 g of sample was placed and put in the soxhlet. Fat was extracted by using petroleum ether with boiling range 40-60º C, on a heating mantle at 60º C for 5 h. The flat bottomed flask was dried for 1 h at 100º C to

evaporate ether and moisture, cooled in desiccator and weighed (W_2). Fat was calculated: Fat (%) = $W_2 - W_1$ / Sample weight $\times$ 100.

Protein

Total nitrogen of the sample was determined following the method described in AOAC (1990). Approximately 1 g of sample was taken in a digestion flask, 0.7 g catalyst ($CuSO_4$: K_2SO_4, 1:9) and 25 ml of concentrated H_2SO_4 were added to it. The flask was heated gently until frothing ceased, boiled briskly until the solution became clear and then continued the boiling for about 1 h. The solution was transferred quantitatively to a round-bottomed flask, and mixed with approximately 100 ml of distilled water and 25 ml 4 % w/v aqueous Na_2S to precipitate mercury. A pinch of zinc granules to prevent bumping and a layer of 40 % w/v NaOH were added carefully. The flask was immediately connected to a distillation apparatus and the tip of the condenser was immersed in standard 0.1 N H_2SO_4 containing about 5 drops of methyl red indicator (I007, HiMedia). The flask was rotated to mix the contents thoroughly and heated until all the ammonia had distilled. The receiver was removed and the tip of the condenser was washed with distilled water. The remaining acid in the receiver was titrated with standard 0.1 N NaOH solutions. The blank determination on reagents was considered for correction.

Nitrogen was calculated in percentage. Total nitrogen (%) = [(ml of standard acid × N of standard acid) – (ml of standard NaOH – C.F.) × N of standard NaOH] ×1.4007 / weight of sample (g). Correction factor (C.F.) = (titre of standard NaOH against blank – ml of standard acid).

Protein content was determined by multiplying total nitrogen value with 6.25 (AOAC, 1990). Protein (%) = Total Nitrogen (%) × 6.38

Carbohydrate

The carbohydrate content of the samples was calculated by difference: 100 – (% protein + % fat + % ash) (Standal, 1963).

Food Value

Food value of each batter sample was determined by multiplying the protein, fat and carbohydrate contents by the factors 4, 9 and 4, respectively, and adding all the multiplication values to get kcal per 100 g (Indrayan *et al*., 2005).

Statistical analysis

The statistical data were analysed by determining the standard deviation (SD) as described by Snedecor and Cochran (1989).

A field survey was conducted in different regions of Sikkim, covering all districts viz. North, South, West and East (Fig A). The following villages and places of Sikkim were surveyed for collection of information and samples: North district (Mangan, Pangthang, Sankalan, Pakshyak, Lachen); East district (Lingtam, Rongli, Zuluk, Gnathang, Kupup, 4th mile, Ranka, Tadong); South district (Namchi); West district (Tashiding, Rinchenpong, Kewzing). Similarly, the following villages and places of Uttarakhand (Fig B) were surveyed for collection of information and samples: Pangu, Rumjum, Marchal and Sosa in Pithoregarh district of Uttarkhand. Based on personal observation and interviews with the producers, eleven types of indigenous meat products from the Sikkim Himalaya and three major types of meat products from Uttarkhand were documented (Tables 1 and 2).

Description of each meat product including a traditional method of preparation, preservation, culinary, mode of consumption and socio-economy were well documented in this thesis.

Table 1: Traditional meat products of the Sikkim Himalaya

Sl no.	Product	Meat	Raw materials	Nature / Use	Major consumer
1.	*Lang kargyong*	Beef	Intestine (fatty), chopped meat, fat, garlic, ginger, salt	Soft or hard, brownish; Sausage, curry	Bhutia, Lepcha, Sherpa
2.	*Yak kargyong*	Yak	Intestine (fatty), chopped meat, fat, garlic, ginger, salt	Soft or hard, brownish; Sausage, curry	Bhutia
3.	*Faak kargyong*	Pork	Intestine, boiled rice, blood, chopped meat, salt, garlic, ginger	Soft or hard, brownish; Sausage, curry	Bhutia, Lepcha, Sherpa
4.	*Lang satchu*	Beef	Long strands-like dried meat	Rough, hard, brownish; Dried meat, curry	Bhutia, Lepcha, Sherpa
5.	*Yak satchu*	Yak	Long strands-like dried meat	Rough, hard, brownish; Dried meat, curry	Bhutia, Lepcha, Sherpa
6.	*Suka ko masu (sheakua)*	Buff	Long strands-like dried meat	Rough, hard, brownish-chocolate; curry	Nepalis
7.	*Yak chilu*	Yak	Yak fat	Hard, used as substitute of an edible oil	Bhutia
8.	*Lang chilu*	Beef	Beef fat	Hard, used as an edible oil	Bhutia, Lepcha, Sherpa
9.	*Luk chilu*	Mutton	Sheep fat	Hard, used as an edible oil	Bhutia, Lepcha, Sherpa
10.	*Yak kheuri*	Yak	Chopped intestine (fatty), meat, fat, abdomen, salt	Curry	Bhutia
11.	*Lang kheuri*	Beef	Chopped intestine (fatty), meat, fat,	Curry	Bhutia, Lepcha,

			abdomen, salt		Sherpa

Table 2. Traditional meat products of the Kumaun Himalaya

Sl no.	Product	Meat	Raw materials	Nature / Use	Major consumer
1.	*Chartayshya*	Chevon	Chevon (small rough pieces), seasoned	Hard / Semi-soft; Curry	Bhutia
2.	*Jamma*	Chevon	Small intestine, finger millet powder, *Xanthoxylon* (yanna), garlic, blood, powdered chilli, salt	Soft; Curry	Bhutia
3.	*Arjia*	Chevon	Large fatty intestine, *Xanthoxylon* (yanna), garlic, blood, lungs, powdered chili, salt	Soft; Curry	Bhutia

LANG KARGYONG

Lang kargyong (Photo 1) is an indigenous sausage-like traditional meat product of the Sikkim Himalaya prepared from beef, and is mostly consumed by the Bhutia and Tibetan. Lepcha, Sherpa and Dhukpa also prefer this type of product. It is soft or hard and brownish in colour. Lepchas calls it 'tiklee'.

Traditional method of preparation

During traditional method of preparation of *lang kargyong*, the lean beef meat with its fat are chopped finely, and combined with crushed garlic, ginger, salt, and mixed with little amount of water. The mixture is stuffed into the segment of gastro-intestinal tract locally called *gyuma*, used as natural casings with 3-4 cm in diameter and 40-60 cm length. One end of the casing is tied up with rope, and other end is sealed after stuffing and boiled for 20-30 min (Photo 11). Cooked sausages are taken out and hung in the bamboo stripes above the kitchen oven for smoking and drying for 10-15 days or more to make *kargyong* (Fig 1). Due to use of natural casings, *kargyong* has a natural curve shape. The method of preparation of *lang kargyong* is same to all the ethnic groups but the ingredients varies from place to place, communities to communities.

Mode of consumption

Lang kargyong is eaten after boiling for 10-15 min, sliced and fried in edible oil by adding onion, tomato, powdered or ground chilies, and salt and is made into curry. It is also consumed as fried sausage with *raksi*, distilled liquor or *chyaang/kodo ko jaanr*, mild-alcoholic finger-millet-based beverage (Thapa and Tamang, 2004). *Lang kargyong* may also be eaten as cooked sausage before fermentation.

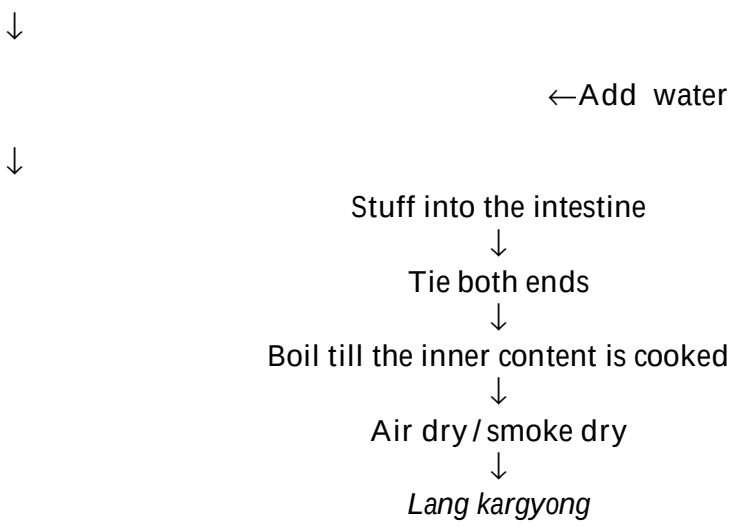


Fig 1. Flow sheet of traditional method of *Lang kargyong* preparation in Sikkim

Socio-economy

Lang kargyong is sold in the local restaurants and food stalls in Sikkim, the Darjeeling hills, Bhutan, etc. It is also prepared for home

consumption and also during marriages and festivals. *Lang kargyong* costs around Rs. 150-180 / kg.

YAK KARGYONG

Yak kargyong (Photo 2) is a traditional sausage-like meat product prepared from yak meat, and is mostly consumed by the Bhutia of Sikkim and Ladak in India, and Tibetan of China. Lepcha and Sherpa of Sikkim, Dukpa of Bhutan also prefer this type of product. It is usually prepared during November to December. It is soft or hard and brownish in colour.

Traditional method of preparation

Yak kargyong is a traditional sausage prepared from yak meat. During its preparation, yak meat and its fat are finely chopped and combined with crushed garlic, ginger, salt and mixed with water. The mixture is stuffed into the segment of gastro-intestinal tract of yak locally called *gyuma,* used as natural casings with 3-4 cm in diameter and 40-60 cm length. 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 taken out and hung in the bamboo stripes above the kitchen oven for smoking and drying for 10-15 days or more to make *kargyong* (Fig 2). These are dried or smoked as per their convenience.

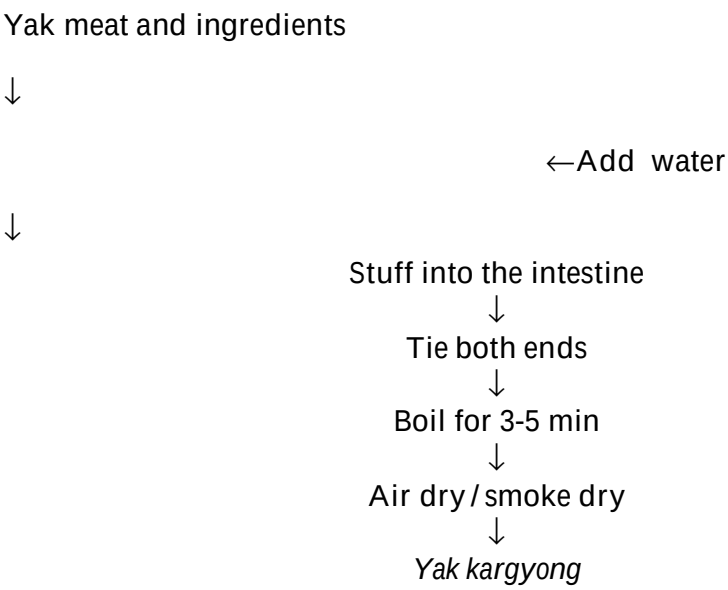


Fig 2. Flow sheet of traditional method of *Yak kargyong* preparation in Sikkim

Mode of consumption

Yak kargyong is consumed after boiling for 20 to 30 min or it is sliced and fried in edible oil by adding onion, tomato, powdered or ground chilies, salt and made into curry.

Socio-economy

Yak kargyong is not sold in the market; it is generally prepared for home consumption and on the special occasions as well.

FAAK KARGYONG

Faak kargyong (Photo 3) is a traditional sausage prepared from pork meat in the Sikkim Himalaya. It is commonly consumed by Bhutia, Lepcha, Sherpa and Tibetans.

Traditional method of preparation

During traditional method of preparation of *Faak kargyong*, the lean pork meats with its fat are chopped finely, combined with crushed garlic, ginger, soyabean sauce and required amount of salt and mixed with little amount of water. The mixture is stuffed into the segment of gastro-intestinal tract locally called *gyuma*, used as natural casings with 3-4 cm in diameter and 40-60 cm length. 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 taken out and hung in the bamboo stripes above the earthen-oven kitchen and smoke and dry for 10-15 days or more to make *kargyong* (Fig 3). Due to use of natural casings, *faak kargyong* has a natural curve shape.

Mixture of pork meat and ingredients

↓

←Add water

↓

Stuff into the intestine

↓

Tie both ends

↓

Air dry / smoke

↓

Faak kargyong

Fig 3. Flow sheet of traditional method of *Faak kargyong* preparation in Sikkim

Mode of consumption

Faak kargyong is consumed as curry frying in edible oil, mixed with chilli, garlic, salt and tomato. It is also sold in small hotels.

Socio-economy

Faak kargyong is sold in the local market. It costs Rs.170 per kg. It is also prepared during marriages and festivals and also for home consumption in the Sikkim Himalaya.

LANG SATCHU

Lang satchu (Photo 4) is a thread or strand like dried traditional beef meat product of the Sikkim Himalaya. This product is mostly consumed by Bhutia, Lepcha and Sherpa.

Traditional method of preparation

Red meat of beef is sliced into several strands of about 60-90 cm and is mixed thoroughly with turmeric powder, edible oil or butter and salt. The meat strands are hung in the bamboo stripes or wooden stick and are kept in an open air in corridor of the house or are smoked above the kitchen oven for 10-15 days as per the convenience of the consumers (Fig 4). *Lang satchu* can be kept at room temperature for several weeks. This is a natural type of preservation of perishable fresh raw meat in absence of refrigeration or cold storage.

Red meat of beef
↓
Cut into a thread / strands like
↓
Mixed with turmeric powder, mustard oil and salt
↓
Smoke or air dry
↓
Lang satchu

Fig 4. Flow sheets of traditional method of *Lang satchu* preparation in Sikkim

Mode of consumption

Lang satchu is made into curry by washing and soaking in water briefly, squeeze, and fry in cow butter with chopped garlic, ginger, chilli and salt. Thick gravy is made which is consumed with *thukpa* (noodles in soup) and boiled or baked potato by the Bhutias, Tibetans, Dhukpa, Lepchas and Sherpas. Deep fried *satchu* is popular side-dish of the ethnic people which is eaten with traditional alcoholic beverages in every house, or in special occasions. Sometimes, it can also be eaten as such in high altitudes of Sikkim.

Socio-economy

Lang satchu is sold in the local restaurants and food stalls in Sikkim, the Darjeeling Hills, Bhutan, etc. Per kilogram of *lang satch*u costs around Rs. 250-300/kg. Some ethnic people are economically dependent upon this product.

YAK SATCHU

Yak satchu is also a thread or strand like dried traditional yak meat product of the Sikkim Himalaya mostly consumed by Bhutias and Lepchas of high altitude in the Sikkim Himalaya.

Traditional method of preparation

Yak red meats are cut into several strands of about 60-90 cm and is mixed thoroughly with turmeric powder, edible oil or butter and salt. The meat strands are hung in the bamboo stripes or wooden stick and are kept in an open air in corridor of the house or are smoked above the kitchen oven for 10-15 days as per the convenience of the consumers (Fig 5). *Yak satchu* can be kept at room temperature for several weeks. This is a natural type of preservation of perishable fresh raw meat in absence of refrigeration.

Red yak meat
↓
Cut into a thread / strands
↓
Mixed with turmeric powder, mustard oil and salt
↓
Smoke or air dry
↓
Yak satchu

Fig 5. Flow sheets of traditional method of *Yak satchu* preparation in Sikkim

Mode of consumption

Yak satchu is made into curry by washing and soaking in water briefly, squeeze, and fry in yak butter with chopped garlic, ginger, chilli and salt. Thick gravy is made which is consumed with *thukpa* (noodles in soup) and boiled or baked potatoes by the Bhutias, Tibetans, Dhukpa, Lepchas and Sherpas. Deep fried *satchu* is popular side-dish of the ethnic

people which is eaten with traditional alcoholic beverages in every house, or in special occasions.

Socio-economy

Yak satchu is not sold in the market. The ethnic people generally prepare the product for home consumption.

SUKA KO MASU

Suka ko masu is a dried / smoked meat product like *satchu*. It is dried or smoked meat prepared from buffalo meat or chevon. It is commonly consumed by the Nepalis of the Darjeeling Hills and Sikkim in India and Nepal. The Newar community belonging to the Nepali calls it *sheakua*.

Traditional method of preparation

Suka ko masu is prepared by cutting the red meat of buffalo or chevon (goat meat) into a stripe up to 25-30 cm, and mixed with turmeric powder, mustard oil and salt. Mixed meat stripes are hung on bamboo and kept above the earthen kitchen oven and smoked for 7-10 days. After complete drying, the smoked meat product is called *suka ko masu* (Fig 6) which can be stored at room temperature for several weeks.

Red meat (Buffalo, Chevon)
↓
Cut into a thread / strands

↓

Mixed with turmeric powder, mustard oil and salt

↓

Smoke or air dry

↓

Suka ko masu

Fig 6. Flow sheets of traditional method of *Suka ko masu* preparation in Sikkim

Mode of consumption

Suka ko masu is washed and soaked in lukewarm water for 10 min, excess water is squeezed out and fried in heated mustard oil, with chopped onion, ginger, chilli power and salt. Coriander leaves are sprinkled over the curry and is eaten with boiled rice. *Suka ko masu* is usually grilled in charcoal and a popular side-dish in the region.

Socio-economy

Regular consumption of meat is expensive for a majority of the rural people. They slaughter domestic animals usually on special occasions, festivals and marriages. During *dasain* (a festival of Nepali), goats are ritually sacrificed to please the goddess Durga. After the ceremony, the meat is cooked and eaten. The remaining flesh of the meat is preserved by smoking to make *suka ko masu* for future consumption. It is sold in the local markets, costing Rs.350 / kg.

YAK CHILU

Yak chilu (Photo 5) is a stored fat product prepared in North Sikkim, Tibet in China and Bhutan. It is used in place of oil during the scarcity of the same. It is mostly consumed by the Bhutia and Lepcha.

Traditional method of preparation

Fatty portions of freshly slaughtered meat (yak) are separated, kneaded by hand and pressed into the cleaned and empty stomach of sheep (previously slaughtered) and then stitched. This stuffed meat is pressed with heavy stones for about 5-10 h, and are kept hanging in the corridor of the house in wooden plank for 10-15 days (Fig 7). *Chilu* can be used for a year or more.

It has been noted during survey that *chilu* production has declined in North Sikkim due to unavailability of sheep stomach.

Yak fat
↓
Put it into the sheep stomach
↓
Stitched
↓
Press with heavy load
↓
Hang for future use
↓
Yak chilu

Fig 7. Flow sheets of traditional method of *Yak chilu* preparation in Sikkim

Mode of consumption

Yak chilu is used in place of edible oil for cooking by the Bhutia, Lepcha, Tibetans, etc.

Socio-economy

Yak chilu is not sold in the market. The people of North Sikkim usually prepare the product for home consumption.

LANG CHILU

Lang chilu is a stored fat product prepared from beef fat in North Sikkim, Tibet in China and Bhutan. It is used in place of edible oil during the scarcity of the edible oil by the Bhutia and Lepcha in Sikkim.

Traditional method of preparation

Fatty portions of freshly slaughtered meat (beef) are separated, kneaded by hand and pressed into the cleaned and empty stomach of sheep (previously slaughtered) and then stitched. This stuffed meat is pressed with heavy stones for about 5-10 h, and are kept hanging in the corridor of the house in wooden plank for 10-15 days (Fig 8). *Lang chilu* can be used for a year or more.

It has been noted during survey that *chilu* production has declined due to unavailability of sheep stomach. *Lang chilu* is used as an oil.

Beef fat
↓
Put it into the sheep stomach and stitched
↓
Press with heavy load and hung for future use
↓
Lang chilu

Fig 8. Flow sheets of traditional method of *Lang chilu* preparation in Sikkim

LUK CHILU

Like othe *chilu, luk chilu* is a stored sheep fat product prepared in North Sikkim, Tibet in China and Bhutan. It is used in place of oil during the scarcity of the edible oil.

Traditional method of preparation

Fat of freshly slaughtered mutton meat (sheep) is separated, kneaded by hand and pressed into the cleaned and empty stomach of sheep (previously slaughtered) and then stitched. The stuffed meat is pressed with heavy stones for about 5-10 h, and is kept hanging in the corridor of the house in wooden plank for 10-15 days (Fig 9). *Luk chilu* can be preserved for a year or more. *Luk chilu* is used as an edible oil.

Sheep fat
↓
Put it into the sheep stomach and stitched
↓
Press with heavy load
↓
Hung for future use
↓
Luk chilu

Fig 9. Flow sheets of traditional method of *Luk chilu* preparation in Sikkim

YAK KHEURI

Yak kheuri (Photo 6) is a typical indigenous yak meat product of the Sikkim Himalaya and is consumed by Bhutias and Lepchas. It is prepared during the winter season or depending upon the availability of raw materials.

Traditional method of preparation

During the preparation of *yak kheuri*, yak meat, its intestine, and fat are chopped into pieces, mixed with required amount of salt. The meat mixture is filled into an empty stomach of sheep, locally called *khyabo* (previously cleaned and cleared sheep stomach), stitched the opening and is kept for 1 to 2 months in an open air outside the kitchen for fermentation (Fig 10). Now-a-days, the people of North Sikkim have stopped preparing *kheuri* because of unavailability of the sheep stomach due to ban on slaughtering high altitude sheep.

Pieces of yak intestine (fatty), meat, fat

↓

←Add water

↓

Stuff ingredients into the sheep stomach and stitched

↓

Keep for 1 to 2 months in open air / kitchen

↓

Yak kheuri

Fig 10. Flow sheet of traditional method of *Yak kheuri* preparation in Sikkim

Mode of consumption

Kheuri is prepared by frying in yak butter, locally called *maa*, mixed with chopped ginger, onion, garlic, powdered or ground chilies, salt and made into thick curry. It is also eaten simply by boiling for 10-15 min with salt. *Kheuri* dish is consumed with main meals by the Bhutias and the Lepchas as side-dish or curry with baked potatoes.

Socio-economy

Yak kheuri is not sold in the market. The ethnic people generally prepare the product for home consumption.

LANG KHEURI

Lang kheuri is a indigenous beef meat product of the Bhutia and Lepcha in the Sikkim HImalaya. It is prepared during the winter season or depending upon the availability of raw materials.

Traditional method of preparation

During the preparation of *lang kheuri*, beef meat, its intestine, and fat are chopped into pieces, mixed with required amount of salt. The meat mixture is filled into an empty stomach of sheep, locally called *khyabo* (previously cleaned and cleared sheep stomach), stitched the opening and are kept for 1 to 2 months in an open air outside the kitchen for fermentation (Fig 11). It is observed during the survey that the people of North Sikkim have stopped preparing *lang kheuri* because of unavailability of the sheep stomach due to ban on slaughtering high altitude sheep.

Pieces of beef intestine (fatty), meat, fat

↓

←Add water

↓

Stuff ingredients into the sheep stomach and stitched

↓

Keep for 1 to 2 months in air / kitchen

↓

Lang kheuri

Fig 11. Flow sheet of traditional method of *Lang kheuri* preparation in Sikkim

Mode of consumption

Lang kheuri dish is prepared by frying in yak butter, locally called *maa*, mixed with chopped ginger, onion, garlic, powdered or ground chilies, salt and made into thick curry. It is also eaten simply by boiling for 10-15 min with salt. *Kheuri* dish is consumed with main meals by the Bhutia and the Lepcha as side-dish or curry with baked potatoes. It is not sold in the local market.

CHARTAYSHYA

Chartayshya (Photo 7) is a traditional chevon (chevon means meat of goat) meat product of the Kumaun Himalayas, consumed by Bhutias of Dharchula and Munsiyari in the district of Pithoregarh of Uttarakhand. This product is preferred by the people of Darma, Chawdas and Byans valley of Dharchula. It is prepared mainly during the religious festival called 'Kolatch' (worshiping ancestral spirit).

Traditional method of preparation

Red goat meat (chevon) is cut into small pieces of 3-4 cm, mixed with salt, sewed in a long thread and is hung in the bamboo stripes or wooden stick and are kept in an open air in corridor of the house for 15-20 days (Fig 12). It can be kept at room temperature for several weeks for future consumption. In Western Nepal, a similar product called *sukha sikhar* is prepared from chevon.

Raw chevon meat
↓
Cut into small pieces; marinated with salt
↓
Smoke or dry for 15-20 days
↓
Chartayshya

Fig 12. Flow sheets of traditional method of *Chartayshya* preparation in Kumaun

Mode of consumption

Curry is made by frying in edible oil with tomato, ginger, garlic, onion and salt.

Socio-ethnical importance

The ethnic people of the Kumaun Himalaya prepare *chartayshya* curry especially during *kolatch* festival (worshiping the ancestral spirit) and offer to ancestors before eating. It is not sold in the Market.

JAMMA/ GEEMA

Jamma or *Geema* (Photo 8) is a traditional fermented sausage of the Kumaun Himalayas prepared from chevon meat. These products are also consumed by the Bhutias of Dharchula and Munsiari of Pithorgarh district.

Traditional method of preparation

Red goat meat is chopped into fine pieces; ground finger millet (*Eleusine coracana*), wild pepper locally called 'timbur' (*Zanthoxylum* sp.), chili powder and salt are added and mixed. A little amount of fresh animal blood is also added. Meat mixture is made semi-liquid by pouring water and stuffed into the small intestine of goat of about 2-3 cm in diameter and 100-120 cm length with the help of funnel, and tied the both ends of the long intestine (Photo 10). It is pricked randomly to prevent

bursting while boiling. After boiling for 15-20 min, stuffed intestine are smoked above the kitchen oven for 15-20 days (Fig 13.) or it can be eaten as such. The method of preparation of the product is similar to those of *kargyong* in the Sikkim Himalaya.

Goat meat and ingredients

↓

←Add blood and water

↓

Stuff into the small intestine; seal, prick

↓

Boil for 15-20 min

↓

Smoke / dry for 15- 20 days

↓

Jamma/ Geema

Fig 13. Flow sheet of traditional method of *Jamma* preparation in Kumaun

Mode of consumption

It is consumed as curry by mixing with onion, garlic, ginger, tomato and salt. It is also deep-fried and is eaten with local alcoholic

beverages. Sometimes, *Jamma* may be eaten as cooked sausage. It is not sold in the local market.

ARJIA

Arjia (Photo 9) is also a traditional sausage prepared from chevon meat. It is also an important food of the Kumaun.

Traditional method of preparation

Preparation method of *arjia* is similar to *jamma*, however, a mixture of chopped lungs of goat, salt, chilli powder, 'timbur' (*Zanthoxylum* sp.) and fresh animal blood are stuffed into the large intestine of goat, instead of small intestine, and boiled for 15-20 min. Pricking of stuffed large intestine is necessary to prevent bursting while boiling. It is dried / smoked for 15-20 days above the kitchen oven (Fig 14).

Goat meat, goat lungs, chilli, garlic

↓

←Add blood and water

↓

Stuff into the large intestine; boil for 15-20 min

↓

Dry / smoke for 15- 20 days

↓

Arjia

Fig 14. Flow sheet of traditional method of *Arjia* preparation in Kumaun

Mode of consumption

Arjia is consumed as curry or deep fried sausage along with main meal. It is not sold in the local market.

EXPERIMENTAL

Microbial population

A total of 68 samples (52 from the Sikkim Himalaya and 16 from the Kumaun Himalaya) of various types of meat products were collected. All the samples were analyzed for the microbiological population (Tables 3 and 4). In all traditionally prepared meat products, lactic acid bacteria (LAB) were found at the level of 10^5 - 10^8 cfu/g. Yeasts were also recovered in all samples representing 10^3 - 10^7 cfu/g. The count of bacilli was $<10^4$ cfu/g. Filamentous moulds were also detected in few samples of the meat products at the level of less than 10^4 cfu/g. The occurrence of micrococcaceae was found at the level of 10^5 - 10^7 cfu/g. The total viable count in all the samples of meat products collected from different places of the Sikkim and the Kumaun Himalaya was ranging in between 10^5 - 10^9 cfu/g (Table 3 and 4).

Table 3. Microbiological populations of meat products collected from different places of the Sikkim Himalaya

Product	Region	Place of collection	Log cfu / g sample					
			Bacteria			Yeast	Moulds	TVC
			LAB	Bacilli	Micrococcaceae			
Lang kargyong	North Sikkim	Mangan (n = 3)	7.4 ± 0.8	2.1 ± 0.4	6.3 ± 0.9	5.2 ± 0.5	<DL	8.1 ± 0.3
	North Sikkim	Pangthang (n = 3)	6.9 ± 0.5	1.3 ± 0.4	5.1 ± 0.4	5.6 ± 0.5	2.1 ± 0.8	8.4 ± 0.2
	North Sikkim	Sankalan (n = 2)	5.9 ± 0.6	2.4 ± 0.3	5.2 ± 0.2	5.5 ± 0.4	<DL	8.2 ± 0.2
	North Sikkim	Pakshyak (n = 2)	5.8 ± 0.4	1.4 ± 0.4	5.1 ± 0.3	4.1 ± 0.5	<DL	8.8± 0.4
	East Sikkim	Lingtam (n = 2)	6.4± 0.6	2.4 ± 0.4	5.4 ± 0.4	6.7 ± 0.1	2.6 ± 0.1	8.2 ± 0.3
	East Sikkim	Rongli (n = 2)	6.1 ± 0.5	1.2 ± 0.5	5.0 ± 0.1	5.1 ± 0.1	<DL	7.7 ± 0.1
	East Sikkim	Zuluk (n = 3)	7.2 ± 0.8	2.0± 0.6	6.8 ± 0.7	6.7 ± 0.3	3.8 ± 0.5	8.8± 0.4
Yak kargyong	East Sikkim	Gnathang (n = 2)	6.8 ± 0.5	1.3 ± 0.6	5.6 ± 0.1	4.9 ± 0.1	2.4 ± 0.1	8.4 ± 0.5
	East Sikkim	Kupup (n = 2)	6.1 ± 0.6	1.4 ± 0.8	5.4 ± 0.1	5.3 ± 0.5	1.4 ± 0.1	8.3 ± 0.6

	North Sikkim	Lachen (n = 3)	6.3 ± 0.7	2.3 ± 0.4	5.9 ± 0.5	4.4 ± 0.5	2.4 ± 0.8	7.3 ± 0.6

Continued (Table 3)

Product	Region	Place of collection	Log cfu / g sample					
			Bacteria			Yeast	Moulds	TVC
			LAB	Bacilli	Micrococcaceae			
Faak kargyong	East Sikkim	4th mile (n = 2)	8.0 ± 0.1	1.4 ± 0.4	6.4 ± 0.6	6.3 ± 0.5	<DL	8.5 ± 0.5
	East Sikkim	Ranka (n = 2)	8.1 ± 0.1	1.5 ± 0.3	6.6 ± 0.4	6.6 ± 0.1	<DL	8.8 ± 0.1
	West Sikkim	Tashiding (n = 2)	6.1 ± 0.4	2.2 ± 0.4	5.6 ± 0.1	4.3 ± 0.4	<DL	7.8 ± 0.1
	North Sikkim	Mangan (n = 2)	6.9 ± 0.3	1.0 ± 0.	5.5 ± 0.1	4.1 ± 0.3	<DL	7.8 ± 0.1
Lang satchu	East Sikkim	Ranka (n = 2)	6.6 ± 0.1	1.5 ± 0.5	6.2 ± 0.2	4.3 ± 0.1	<DL	7.8 ± 0.1
	East Sikkim	Tadong (n = 2)	7.4 ± 0.1	1.0 ± 0.7	7.0 ± 0.1	6.5 ± 0.1	<DL	8.1 ± 0.4

	West Sikkim	Rinchen-pong (n = 2)	7.5 ± 0.6	2.1 ± 0.5	6.6 ± 0.1	5.3 ± 0.6	1.0 ± 0.4	8.6 ± 0.6
	West Sikkim	Kewzing (n = 2)	6.7 ± 0.6	2.3 ± 0.4	6.2 ± 0.7	4.3 ± 0.1	<DL	7.8 ± 0.1

Continued (Table 3)

Product	Region	Place of collection	Log cfu / g sample					
			Bacteria			Yeast	Moulds	TVC
			LAB	Bacilli	Micrococcaceae			
Yak satchu	East Sikkim	Gnathang (n = 2)	7.1 ± 0.2	1.5 ± 0.4	6.4 ± 0.6	5.9 ± 0.1	<DL	8.3 ± 0.2
	East Sikkim	Kupup (n = 2)	6.2 ± 0.2	2.3 ± 0.5	5.6 ± 0.6	3.8 ± 0.5	0.6 ± 0.4	8.6 ± 0.7
	North Sikkim	Lachen (n = 2)	6.0 ± 0.5	1.3 ± 0.6	5.4 ± 0.5	4.1 ± 0.5	<DL	7.8 ± 0.1
Suka ko masu	South Sikkim	Namchi (n = 2)	5.5 ± 0.1	3.1 ± 0.1	5.1 ± 0.1	4.6 ± 0.1	<DL	5.5 ± 0.1
	East Sikkim	Rongli (n = 2)	6.1 ± 0.1	2.3 ± 0.1	4.4 ± 0.1	4.9 ± 0.1	1.1 ± 0.1	5.4 ± 0.1
	East Sikkim	Tadong (n = 2)	6.4 ± 0.1	2.0 ± 0.1	5.2 ± 0.1	3.6 ± 0.1	2.0 ± 0.1	5.4 ± 0.3

n = number of samples.

Data represents the means (± SD) of number of samples.

LAB, lactic acid bacteria; TVC, total viable count; DL, Less than detection limit (10 cfu / g).

Micrococcaceae includes species of *Micrococcus* and *Staphylococcus.*

Table 4. Microbiological populations of meat products collected from different places of the Kumaun Himalaya

Product	Region	Place of collection	Log cfu / g sample					
			Bacteria					
			LAB	Bacilli	Micrococcaceae	Yeast	Moulds	TVC
Charta-yshya	Dharch-ula district	Pangu (n = 2)	7.4 ± 0.2	3.0 ± 0.1	6.9 ± 0.1	5.1 ± 0.1	2.4 ± 0.1	9.0 ± 0.1
	Dharch-ula district	Rumjum (n = 2)	6.3± 0.1	2.3 ± 0.1	5.9 ± 0.1	5.3 ± 0.1	1.3 ± 0.1	7.8 ± 0.1
	Dharch-ula district	Marchal (n = 2)	7.2 ± 0.1	3.2 ± 0.1	6.9 ± 0.1	4.2 ± 0.1	1.1 ± 0.1	7.1 ± 0.1
Jamma	Dharch-ula district	Dharchula (n = 2)	8.6 ± 0.1	3.6 ± 0.1	6.0 ± 0.1	6.2 ± 0.1	3.8 ± 0.1	9.2 ± 0.1
	Dharch-ula district	Sosa (n = 2)	7.3 ± 0.1	3.5 ± 0.1	5.3 ± 0.3	5.5 ± 0.1	3.4 ± 0.1	7.5 ± 0.3

	Dharch-ula district	Rumjum (n = 2)	6.3 ± 0.3	3.3 ± 0.1	5.3 ± 0.1	5.4 ± 0.1	3.3 ± 0.1	7.4 ± 0.1
Arjia	Dharch-ula district	Dharchula (n = 2)	8.3 ± 0.1	3.3 ± 0.1	6.5 ± 0.1	5.1 ± 0.1	<DL	9.1 ± 0.1
	Dharch-ula district	Rumjum (n = 2)	7.2 ± 0.1	4.2 ± 0.1	6.3 ± 0.1	4.3 ± 0.1	<DL	9.0 ± 0.1

Data represents the means (± SD) of number of samples.

n = number of samples. LAB, lactic acid bacteria; TVC, total viable count; DL, Less than detection limit (10 cfu / g). Micrococcaceae includes species of *Micrococcus* and *Staphylococcus.*

Occurrence of pathogenic bacteria

Samples of meat products were examined for the presence of *Listeria* sp., *Salmonella* sp., and *Shigella* sp. using the selective media (Table 5 and 6). None of these pathogenic bacteria were detected in 68 samples analysed. The count of enterobacteriaceae was recorded at the level of 10^4 cfu/g. The detection level of *Bacillus cereus* was less than 10 cfu/g in few samples (data not shown).

Table 5. Occurence of pathogenic bacteria in meat products collected from different places of the Sikkim Himalaya

Product	Region	Place of collection	Log cfu / g sample			
			Enterobacteriaceae	*Salmonella*	*Shigella*	*Listeria monocytogenes*
Lang kargyong	North Sikkim	Mangan (n = 3)	3.5 ± 0.6	0	0	0
	North Sikkim	Pangthang (n = 3)	4.1 ± 0.8	0	0	0
	North Sikkim	Sankalan (n = 2)	4.2 ± 0.6	0	0	0
	North Sikkim	Pakshyak (n = 2)	2.8 ± 0.4	0	0	0
	East Sikkim	Lingtam (n = 2)	3.0 ± 0.7	0	0	0
	East Sikkim	Rongli (n = 2)	0	0	0	0
	East Sikkim	Zuluk (n = 3)	0	0	0	0
Yak kargyong	East Sikkim	Gnathang (n = 2)	0	0	0	0
	East Sikkim	Kupup (n = 2)	0	0	0	0
	North Sikkim	Lachen (n = 3)	0	0	0	0

Continued (Table 5)

Product	Region	Place of collection	Log cfu / g sample			
			Enterobacteriaceae	*Salmonella*	*Shigella*	*Listeria monocytogenes*
Faak kargyong	East Sikkim	4th mile (n = 2)	2.8 ± 0.4	0	0	0
	East Sikkim	Ranka (n = 2)	3.0 ± 0.2	0	0	0
	West Sikkim	Tashiding (n = 2)	3.0 ± 0.5	0	0	0
	North Sikkim	Mangan (n = 2)	2.0 ± 0.4	0	0	0
Lang satchu	East Sikkim	Ranka (n = 2)	2.8 ± 0.4	0	0	0
	East Sikkim	Tadong (n = 2)	3.1 ± 0.4	0	0	0
	West Sikkim	Rincenpong (n = 2)	3.0 ± 0.1	0	0	0
	West Sikkim	Kewzing (n = 2)	3.3 ± 0.5	0	0	0
Yak satchu	East Sikkim	Gnathang (n = 2)	0	0	0	0
	East Sikkim	Kupup (n = 2)	0	0	0	0
	North	Lachen	0	0	0	0

	Sikkim	(n = 2)				

Continued (Table 5)

Product	Region	Place of collection	Log cfu / g sample			
			Enterobacteriaceae	*Salmonella*	*Shigella*	*Listeria monocytogenes*
Suka ko masu	South Sikkim	Namchi (n = 2)	2.5 ± 0.5	0	0	0
	East Sikkim	Rongli (n = 2)	2.4 ± 0.1	0	0	0
	East Sikkim	Tadong (n = 2)	3.3 ± 0.1	0	0	0

n = number of samples.

Data represents the means (± SD) of 3 sets of experiment.

Table 6. Occurance of pathogenic bacteria in meat products collected from different places of the Kumaun Himalaya

Product	Region	Place of collection	Log cfu / g sample			
			Enterobacteriaceae	*Salmonella*	*Shigella*	*Listeria monocytogenes*
Chartayshya	Dharchula district	Pangu (n = 2)	0	0	0	0
	Dharchula district	Rumjum (n = 2)	0	0	0	0
	Dharchula district	Marchal (n = 2)	0	0	0	0
Jamma	Dharchula district	Dharchula (n = 2)	5.0 ± 0.1	0	0	0
	Dharchula district	Sosa (n = 2)	4.3 ± 0.4	0	0	0
	Dharchula district	Rumjum (n = 2)	4.5 ± 0.1	0	0	0
Arjia	Dharchula district	Dharchula (n = 2)	4.4 ± 0.1	0	0	0
	Dharchula district	Rumjum (n = 2)	5.0 ± 0.1	0	0	0

n = number of samples.

Data represents the means (± SD) of 3 sets of experiment.

Grouping of representative LAB strains

A total of 440 bacterial isolates were isolated from meat products of different places of the Sikkim Himalaya. Similarly, 182 bacterial isolates were also isolated from the samples of different place of Dharchula of Pithoregarh district of the Kumaun Himalaya. All isolates were purified in MRS broth, and their cell morphology and preliminary taxonomical tests were performed. All bacterial isolates were considered lactic acid bacteria (LAB) because: (i) they grew well in anaerobic agar, (ii) formed clear halo in Calcium carbonate supplemented MRS agar plates, (iii) Gram-positive, (iv) catalase-negative, (v) non-motile and (vi) non-sporing bacteria.

A grouping of all LAB isolates was done on the basis of cell morphology, gas production from glucose and production of ammonia from arginine (Tables 7 and 8). The representative strains of LAB were selected randomly from each grouped strains based on similar morphology, gas production from glucose and hydrolysis of arginine. A total of 157 representative strains of LAB (108 strains from the Sikkim Himalaya and 49 strains from the Kumaun Himalaya) were grouped for further identifications. Representative strains were assigned the strain code number indicating the sample names and source.

Table 7. Grouping of representative strains of the LAB isolated from meat products of the Sikkim Himalaya

Product[a]	Cell shape	Gas from glucose	Arginine hydrolysis	Grouped Strains	Representative strains	
					Total No.	Strain code
Lang kargyong (92)	Rod	–	–	13	4	KP:L3, KP:L8, KP:L13, KP:L30
	Rod	+	+	5	2	ZK:L5, KM:L32
	Rod	–	+	5	2	KM:L31, ZK:L7
	Rod	+	–	35	6	KM:L1, KM:L10, LK:L2, KP:L5, KP:L14, ZK:L4
	Coccus	–	+	14	3	KM:L19, KM:L34, KM:L35
	Coccoid rod	+	–	12	4	KP:L7, KP:L11, KP:L18, LK:L4
	Coccoid rod	–	+	8	3	ZK:L1, ZK:L3, ZK:L6
Yak Kargyong (53)	Rod	–	–	29	8	YKK:L2, YKK:L3, YKK:L4, YKK:L8, YKK:L11, YKg:L3, YKg:L7, YKg:L8,
	Rod	+	+	1	1	YK:L11
	Rod	–	+	7	3	YKK:L1, YKK:L5, YKg:L9
	Rod	+	–	6	2	YK:L1, YK:L3
	Coccus	–	+	4	2	YK:L2, YK:L15

	Coccoid rod	+	–	6	2	YK:L5, YK:L9

Continued (Table 7)

Product[a]	Cell shape	Gas from glucose	Arginine hydrolysis	Grouped Strains	Total No.	Representative strains
	Coccoid rod	–	+	69	12	KS:L1, KS:L18, KS:L26, SR:L7, SR:L6, TS:L9, TS:L23, TS:L20, TS:L7, TS:L8, KS:L22, KS:L17
Faak kargyong (78)	Rod	+	+	2	1	FK:L5
	Coccoid rod	+	–	41	8	FK:L1, FK:L8, FK:L11, FK:L3, FK:L2, FK:L14, FK:L25, FK:L6
	Coccoid rod	–	+	3	3	FK:L4, FK:L10, FK:L17
	Coccoid	–	+	28	4	FK:L13, FK:L14, FK:L15, FK:L16
	Coccoid rod	–	–	4	2	FK2:L7, FK1:L15
Yak satchu (58)	Coccus / tetrad	–	+	58	14	YS:L2, YS:L1, YS:L13, YS:L7, YS:L4, YS:L3, YS:L10, YS:L8, YS:L6, YS:L18, YS:L16, YS:L5, YS:L20, YS:L9
Lang Satchu (93)	Rod	–	–	1	1	SR:L4
	Rod	–	+	1	1	SR:L8
	Coccus / tetrad	–	+	22	5	KS:L2, KS:L6, KS:L8, KS:L10, KS:L15

Continued (Table 7)

Product[a]	Cell shape	Gas from glucose	Arginine hydrolysis	Grouped Strains	Representative strains	
					Total No.	Strain Code
Suka ko masu (66)	Rod	–	+	4	2	BS:L25, BS:L4
	Coccoid	–	–	14	3	BS:L18, BS:L17, BS;L9
	Coccoid	–	+	48	10	BS:L1, BS:L2, BS;L5, BS:L7, BS:L11, BS;L16, BS:L19, BS:L21, BS:L23, BS:L13

[a]Total number of isolates in each product are given in parenthesis.

All strains of LAB were Gram-positive, catalase-negative, non-motile and non-sporing.

Table 8. Grouping of representative strains of the LAB isolated from meat products of the Kumaun Himalaya

Product[a]	Cell shape	Gas from glucose	Arginine hydrolysis	Grouped Strains	Representative strains	
					Total No.	Strain Code
Chartayshya (65)	Rod	+	+	9	3	CD:L3, CD:L21, CD:L6
	Coccoid rod	–	+	19	6	CD:L5, CD:L2, CD:L22, CD:L14, CD:L7, CD:L23
	Coccus / tetrad	–	+	37	7	CD:L13, CD:L15, CD:L1, CD:L8, CD:L10, CD:L20, CD:L26
Jamma (69)	Rod	+	–	4	2	KJ:L1, KJ:L15
	Coccoid rod	+	+	9	3	KJ:L8, KJ:L6, KJ:L7
	Coccoid rod	+	–	33	7	KJ:L2, KJ:L14, KJ:L18, KJ:L21, KJ:L23, KJ:L25, KJ:L29
	Coccoid	–	–	3	1	KJ:L9
	coccoid	–	+	5	2	KJ:L4, KJ:L5
	Coccus / tetrad	–	+	15	5	KJ:L10, KJ:L13, KJ:L31, KJ:L3, KJ:L16

Arjia (48)	Coccoid	–	+	26	7	KA:L3, KA:L5, KA:L8, KA:L16, KA:L20, KA:L13, KA:L15
	Ccccus / tetrad	–	+	22	6	KA:L1, KA:L2, KA:L4, KA:L11, KA:L17, KA:L21

[a]Total number of isolates in each product are given in parenthesis.

All strains of LAB were Gram-positive, catalase-negative, non-motile and non-sporing.

Characterization and identification of LAB

All representative strains of LAB were phenotypically characterised including growth at different temperatures (8° C, 10° C, 15 °C, 45° C); pH (3.9 and 9.6); tolerance in different concentrations of salts (6.5 %, 10 % and 18 %), following the taxonomic keys described by Schillinger and Lücke, (1987), Wood and Holzapfel (1995) and Dykes *et al.* (1994). The configuration of lactic acid produced was determined enzymatically using D-lactate and L-lactate dehydrogenase test kits (Tamang *et al*., 2005). The presence of *meso*-diaminopimelic acid in the cell walls of LAB was determined using thin-chromatography on cellulose plate (Tamang *et al*., 2000). Sugar fermentation of LAB isolates were determined by the API 50 CHL test strips (bioMérieux, France) and the identification was interpreted using APILAB PLUS database software (bioMérieux, France).

Out of 92 strains of LAB isolated from *lang kargyong*, 40 were heterofermentative rods, 18 were homofermentative rods, 14 were homofermentative cocci, 12 were gas-producing coccoid rods and 8 were gas-negative coccoid rods (Table 9). Of the 53 strains of LAB isolated from *yak kargyong*, 7 were heterofermentative rods, 36 were homofermentative rods, 4 were homofermentative cocci and 6 were gas-producing coccoid rods (Table 10). Out of 78 strains of LAB isolated from *faak kargyong*, 2 strains were heterofermentative rods, 41 were gas-producing coccoid

rods, 28 were coccoid and 7 were non-gas producing coccoid rods (Table 11). Out of 93 strains of LAB isolated from *lang satchu,* 2 were homofermentative rods, 22 were tetrad-forming cocci and 69 were non-gas producing coccoid rods (Table 12). Of the 58 strain isolated from *yak satchu*, all the strains were tetrad-forming cocci indicating its predominance in the sample (Table 13). Of the 66 strains of LAB isolated from *suka ko masu*, 62 strains were non-gas producing coccoids and 4 were homofermentative rods (Table 14). Out of 65 strains of LAB isolated from *chartayshya*, 9 strains were heterofermentative rods, 19 non-gas producing coccoid rods and 37 were tetrad-forming cocci (Table 15). Of the 69 strains of LAB isolated from *jamma*, 4 were heterofermentative rods, 42 were gas-producing coccoid rods, 8 were coccoids and 15 were tetrad-forming cocci (Table 16). Out of 48 strains of LAB from *arjia*, 26 were coccoids and 22 were tetrad-forming cocci (Table 17). All cocci forming tetrads were presumptively grouped as pediococci. Based on the key proposed by Simpson and Taguchi (1995), all 14 tetrads isolated from *yak satchu* (YS:L2, YS:L1, YS:L13, YS:L7, YS:L4, YS:L3, YS:L10, YS:L8, YS:L6, YS:L18, YS:L16, YS:L5, YS:L20, YS:L9), 5 strains from *lang satchu* (KS:L2, KS:L6, KS:L8, KS:L10, KS:L15), 7 tetrads from *chartayshya* (CDL13, CD:L15, CD:L1, CD:L8, CD:L10, CD:L20, CD:L26), 5 tetrad strains (KJ:L10, KJ:L13, KJ:L31, KJ:L3, KJ:L16) from *jamma* and 6 tetrads (KA:L1, KA:L2, KA:L4, KA:L11, KA:L17, KA:L21) isolated from *arjia* were identified as *Pediococcus*

pentosaceous (Tables 12, 13, 15, 16 and 17). The sugar fermentation profiles using API identification profile and APILAB PLUS database software, also confirmed the identity of *Pediococcus pentosaceus* (Plate a).

The three cocci strains (YK:L19, KM:L34, KM:L35) isolated from *lang kargyong*, 2 cocci strains (YK:L2, YK:L15) from *yak kargyong* and 4 coccoid strains (FK:L13, FK:L14, FK:L15, FK:L16) from *faak kargyong*, 12 coccoid strains (KS:L1, KS:L18, KS:L26, SR:L6, SR:L7, TS:L9, KS:L17, TS:L23, TS:L20, TS:L7, TS:L6, KS:L22) from *lang satchu* and 10 coccoid strains (BS:L1, BS:L2, BS:L5, BS:L7, BS:L11, BS:L16, BS:L19, BS:L21, BS:L23, BS:L13) from *sheakua* of the Sikkim Himalaya were identified as *Enterococcus faecium* (Tables 9, 10, 11, 12 and 14). Similarly, 6 strains (CD:L5, CD:L2, CD:L22, CD:L4, CD:L7, CD:L12) from *chartayshya*, 2 strains (KJ:L4, KJ:L5) from *jamma* and 7 strains (KA:L3, KA:L5, KA:L8, KA:L16, KA:L20, KA:L13, KA:L15) from *arjia* of the Kumaun Himalaya were identified as *Enterococcus faecium* (Tables 15, 16 and 17) (Plate b). All the cocci / coccoid strains isolated both from the Sikkim and the Kumaun Himalayas showed the similar characteristics on the basis of sugar fermentation profiles and also growth in 6.5 % NaCl, at 45° C. Interestingly, strain KJ:L9, isolated from *jamma* did not hydrolyse arginine, grew well at pH 3.9 and did not ferment mannitol. This strain was tentatively identified as *Enterococcus cecorum* based on the taxonomical key of Wood and Holzapfel (1995).

Photo 7. *Chartayshya*

Photo 8. *Jamma*

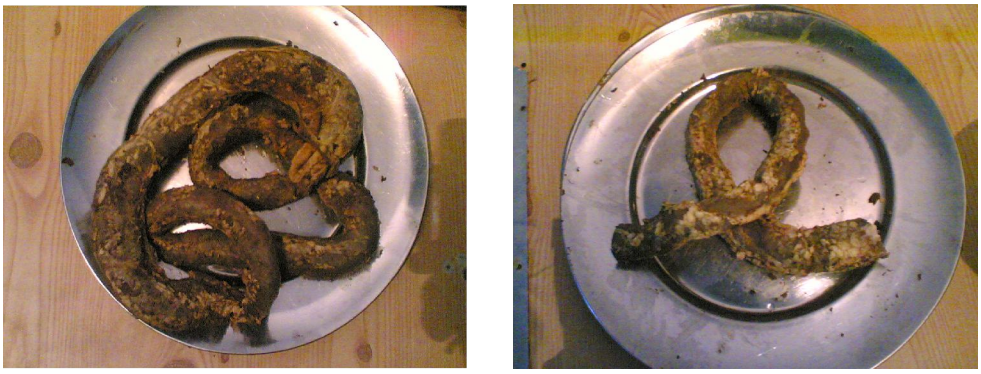


Photo 9. *Arjia*

Photo 10. Traditional meat processing in Kumaun Himalaya: (a) Cleaning of intestine; (b) Seasoning; (c) Mixing and kneading; (d) Semi-liquid ingredients; (e) Stuffing into the intestine; (f) Pressing ingredients into the intestine; (g) Cooking; (h) Freshly prepared *Jamma.*

All coccoid rod strains (KP:L7, KP:L11, KP:L18, LK:L4, YK:L5, YK:L9, FK:L1, FK:L8, FK:L11, FK:L3, FK:L2, FK:L14, FK:L25, FK:L6, KJ:L2, KJ:L14 KJ:L18, KJ:L21, KJ:L23 KJ:L25, KJ:L29) isolated from meat products produced D(-) lactate from glucose, did not hydrolyse arginine, produced gas from glucose and showed the typical leuconostoc-like ovoid cells. All these coccoid strains produced dextran when grown on 5 % sucrose agar, and fermented sucrose, galactose, maltose, mannose and xylose. Based on above mentioned characteristics and sugar fermentation profiles using API confirmed their identity as *Leuconostoc mesenteroides* (Tables 9, 10, 11 and 16) (Plate d).

Based on the detailed characterizations and identification profiles, the following genera and species of functional lactic acid bacteria isolated from traditionally prepared meat products were identified as *Lactobacillus curvatus, Lb. sake, Lb. divergens, Lb. carnis, Lb. casei, Lb. sanfrancisco, Lb. brevis, Lb. plantarum* (Plate c), *Leuconostoc mesenteroides, Enterococcus faecium,* and *Pediococcus pentosaceus* according to the taxonomical keys proposed by Schillinger and Lücke (1987).

Prevalence of LAB

The most dominant LAB in *lang kargyong* were lactobacilli represented by 71.7 % followed by *Enterococcus* (15.2 %) and *Leuconostoc* (13.1) (Fig 18a). Similarly, in case of *yak kargyong* lactobacilli were the dominant microflora representing 81.1 % followed by *Leuconostoc* and *Enterococcus* comprising 11.3 % and 7.6 %, respectively (Fig 18a). *Leuconostoc* was the dominant LAB (52.6 %) in *faak kargyong*, whereas *Enterococcus* was represented by 35.9 % and lactobacilli (11.5 %) (Table 18) (Fig 18a). In *lang satchu*, *Enterococcus* represented the most dominant microflora representing 74.2 % followed by *Pediococcus* (23.7) and lactobacilli (2.1 %) (Fig 18b). *Pediococcus* was the most dominant lactic microflora representing 100 % in *yak satchu* whereas in *suka ko masu*, the dominant LAB was *Enterococcus* (72.7 %), followed by lactobacilli (27.3 %) (Table 18) (Fig 18b). In meat products of the Kumaun Himalaya (Table 19), the most dominant LAB recovered was *Pediococcus* representing 56.9 % followed by *Enterococcus* (29.3 %) and lactobacilli (13.8 %) in *chartayshya*. About 47.8 % of LAB was represented by *Leuconostoc,* 21.7 % by *Pediococcus,* 18.9 % by lactobacilli and 11.6 % by *Enterococcus* in *jamma*. The dominant LAB in *arjia* was *Enterococcus* representing 54.2 % of the total microorganisms followed by 45.8 % of *Pediococcus*. (Fig 19).

Table 18. Prevalence of LAB isolated from meat products of the Sikkim Himalaya

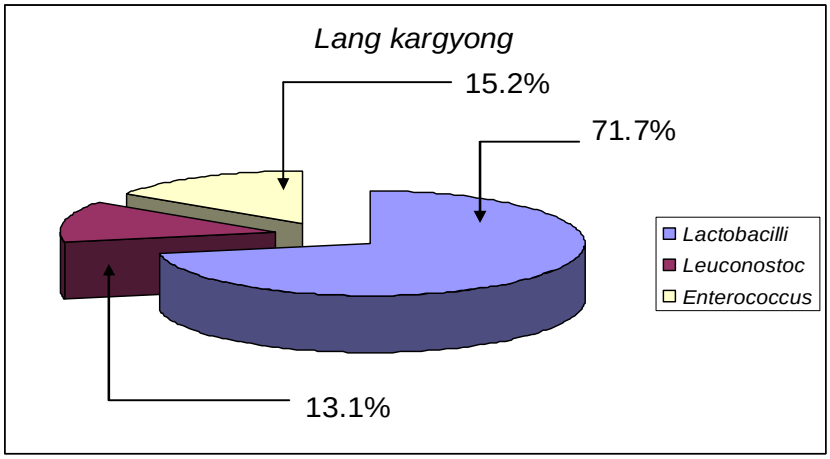
Product[a]	Functional LAB	% of Prevalence					
		Homoferment. lactics	Heteroferment. lactics	Total lactobacilli	*Leuconostoc*	*Enterococcus*	*Pediococcus*
Lang kargyong (92)	*Lb.sake, Lb.curvatus, Lb.divergens, Lb.carnis, Lb.sanfrancisco, E. faecium, Leuc.mesenteroides*	28.2	43.5	71.7	13.1	15.2	0
Yak kargyong (53)	*Lb.sake, Lb.plantarum, Lb.curvatus, Lb.casei, Lb.carnis, Lb.sanfrancisco, E. faecium, Lb.divergens, Leuc.mesenteroides*	67.9	13.2	81.1	11.3	7.6	0
Faak kargyong (78)	*Lb.brevis, Lb.plantarum, Lb.carnis, E. faecium, Leuc.mesenteroides*	9.0	2.5	11.5	52.6	35.9	0
Lang satchu (93)	*P.pentosaceous, Lb.casei, Lb.carnis, E. faecium,*	2.1	0	2.1	0	74.2	23.7
Yak satchu (58)	*P.pentosaceous*	0	0	0	0	0	100
Suka ko masu (66)	*Lb.plantarum, Lb.carnis, E. faecium,*	27.3	0	27.3	0	72.7	0

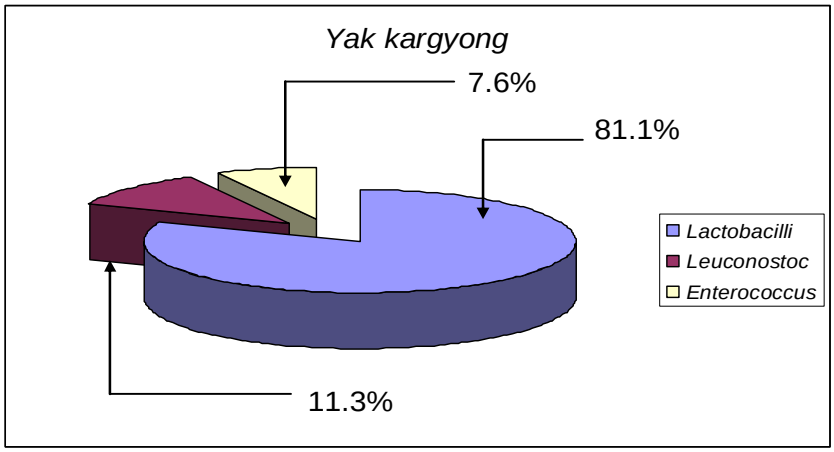
[a]Total number of LAB isolates of each products are given in parenthesis

Table 19. Prevalence of LAB isolated from meat products of the Kumaun Himalaya

Product[a]	Functional LAB	% of Prevalence					
		Homoferment. lactics	Heterofermen t. lactics	Total lactobacilli	*Leuconostoc*	*Enterococcus*	*Pediococcus*
Chartayshya (65)	*Lb.divergens, E. faecium, P.pentosaceous*	0	13.8	13.8	0	29.3	56.9
Jamma (69)	*E. faecium, Lb.divergens, Lb.sanfrancisco, Leuc.mesenteroides, E.cecorum, P. pentosaceous*	0	18.9	18.9	47.8	11.6	21.7
Arjia (48)	*P.pentosaceous, E. faecium,*	0	0	0	0	54.2	45.8

[a]Total number of LAB isolates of each products are given in parenthesis


Lang kargyong
15.2%
71.7%
13.1%
Lactobacilli
Leuconostoc
Enterococcus


Yak kargyong
7.6%
81.1%
11.3%
Lactobacilli
Leuconostoc
Enterococcus

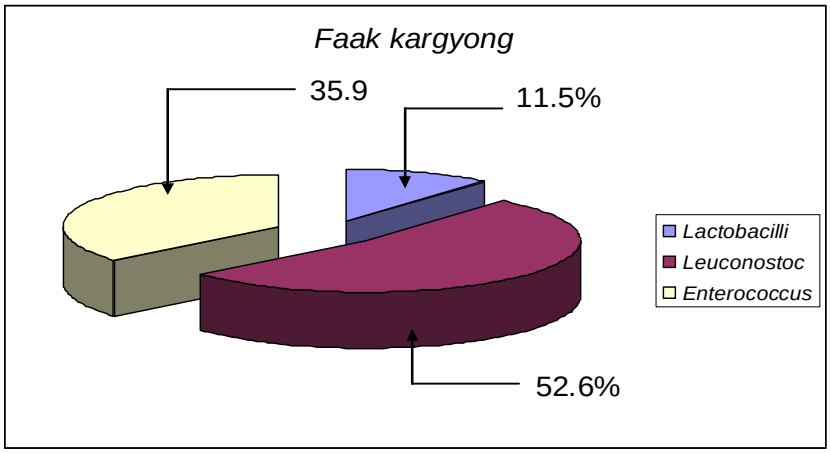
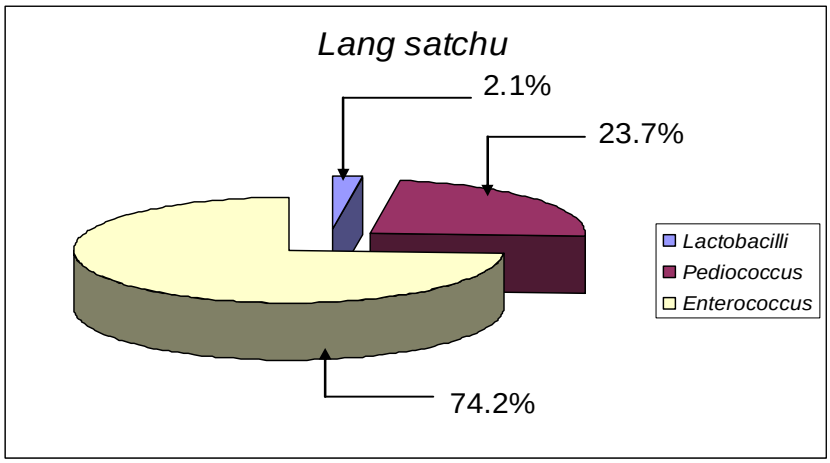
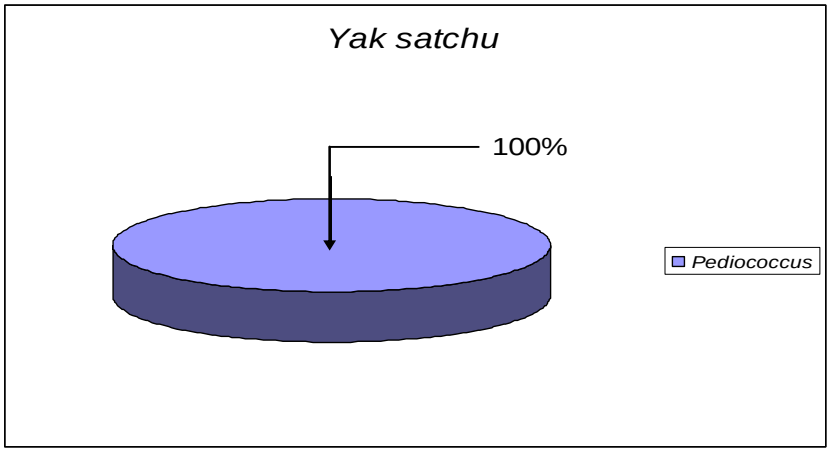

Faak kargyong
35.9
11.5%
52.6%
Lactobacilli
Leuconostoc
Enterococcus

Fig 18a. Graphic representation of prevalence of LAB in meat products of the Sikkim Himalaya.


Lang satchu
2.1%
23.7%
74.2%
Lactobacilli
Pediococcus
Enterococcus


Yak satchu
100%
Pediococcus

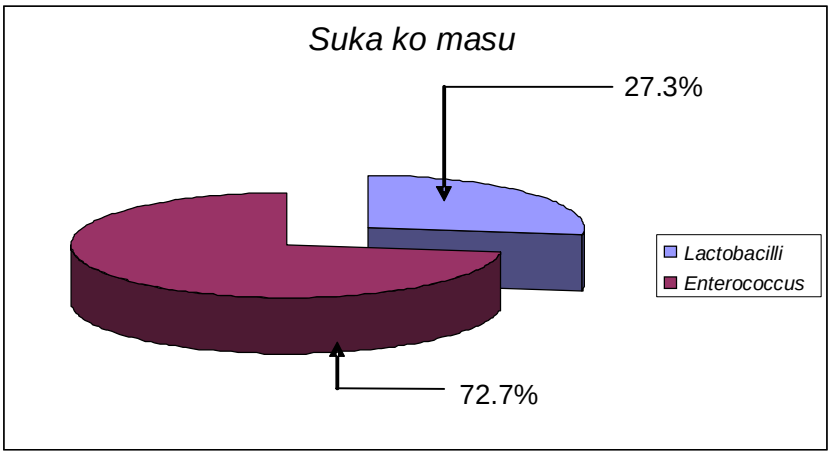
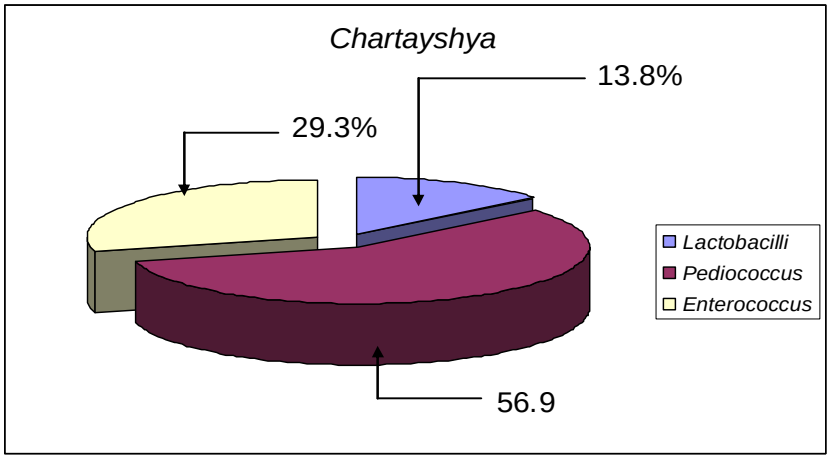
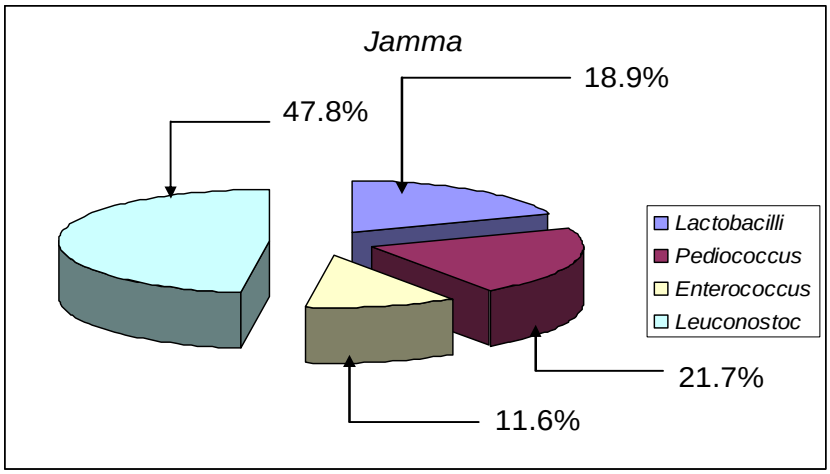

Suka ko masu
27.3%
72.7%
Lactobacilli
Enterococcus

Fig 18b. Graphic representation of prevalence of LAB in meat products of the Sikkim Himalaya.





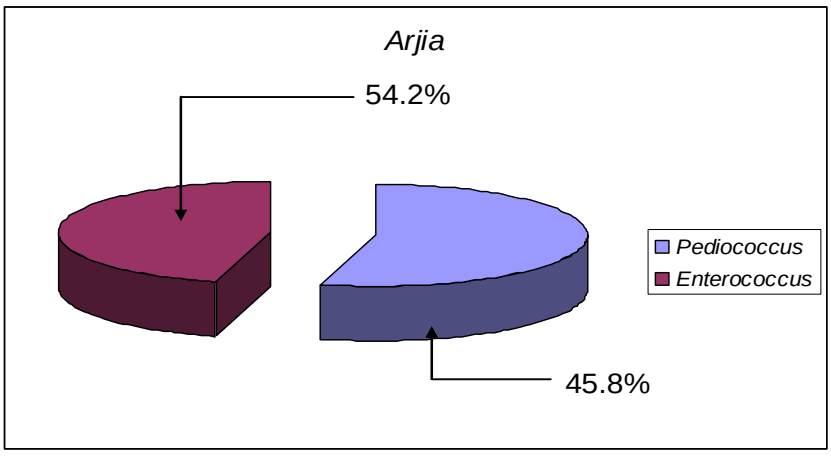


Fig 19. Graphic representation of prevalence of LAB in meat products of the Kumaun Himalaya.

Grouping and identity of representative strains of bacilli

A total of 238 strains of endospore-forming rods were isolated from 69 samples from different places of the Sikkim and the Kumaun Himalayas. All the strains isolated were Gram-positive, catalase positive, aerobic and motile (Table 20). Based on the taxonomical keys of Claus and Berkeley (1986); Slepecky and Hemphill (1992), 32 representative strains were grouped for further identification. Spore-former rod strains KM:B1, KP:B1 (*Lang kargyong*); YK:B1, YK:B1, YK:B2 (*Yak kargyong*); TS:B1 (*Lang satchu*); YS:B8 (*Yak satchu*); BS:B2 (*suka ko masu*); CD:B2, CD:B9 (*Chartayshya*); KJ:B1, KJ:B5 (*Jamma*); KA:B2 (*Arjia*) were identified as *Bacillus subtilis* (Plate e). Similarly, strains ZK:B2 (*Lang kargyong*); YK:B8 (*yak kargyong*); TK:B9, FK:B2 (*Faak kargyong*); TS:B3, SR:B2 (*Lang satchu*); YS:B3, YS:B2 (*Yak satchu*); BS:B7 (*Suka ko masu*); CD:B3 (*Chartayshya*); KA:B8 (*Arjia*) were identified as *Bacillus mycoides* (Plate f). Strain KM:B2 (*Lang kargyong*); BS:B1 (*Suka ko masu*); CD:B7 (*Chartayshya*); KA:B4 (*Arjia*) were identified as *Bacillus thuringiensis*. Strains SR:B6 isolated from *lang satchu* was identified as *Bacillus lentus*. Strains FK:B6 (*Faak kargyong*); strains YS:B1 (*Yak satchu*) were identified as *Bacillus licheniformis* and KJ:B3 (*Jamma)* was identified as *Bacillus sphaericus* (Table 21).

Table 20. Grouping of representative strains of the Bacilli isolated from meat products of the Sikkim and the Kumaun Himalaya

Product	Cell shape	Catalase	Starch hydrolysis	Grouped Strains	Representative strains	
					Total No.	Strain code
Lang kargyong (n = 17)	Rod	+	+	51	4	KM:B1, KP:B1, KM:B2, ZK:B2
Yak Kargyong (n = 7)	Rod	+	+	22	3	YK:B1, YK:B8, YK:B2
Faak Kargyong (n = 8)	Rod	+	+	25	4	TK:B4, TK:B9, FK:B2, FK:B6
Lang Satchu (n = 9)	Rod	+	+	27	4	TS:B1, TS:B3, SR:B6, SR:B2
Yak satchu (n = 6)	Rod	+	+	23	4	YS:B3, YS:B1, YS:B8, YS:B2
Suka ko masu (n = 6)	Rod	+	+	24	3	BS:B1, BS:B2, BS:B7
Chartayshya (n = 6)	Rod	+	+	24	4	CD:B3, CD:B2, CD:B9, CD:B7
Jamma (n = 6)	Rod	+	+	24	4	KJ:B1, KJ:B3, KJ:B5
Arjia (n = 4)	Rod	+	+	18	3	KA:B2, KA:B8, KA:B4

n = number of samples.
All isolates were Gram-positive, catalase positive, aerobic, motile and spore-formers

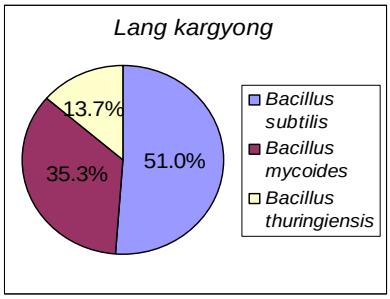
Prevalence of bacilli

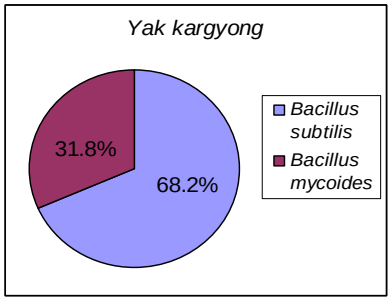
The prevalence of bacilli in *lang kargyong* was *Bacillu subtilis* (51.0 %) followed by *B. mycoides* (35.3 %) and *B. thuringiensis* (13.7 %) (Table 22). Similarly, in *yak kargyong* 68.2% of the total bacilli was represented by *Bacillus subtilis*, whereas *B. mycoides* represented 31.8 %. In *faak kargyong* the prevalence of bacilli was *B. mycoides* (48.0 %), *B. subtilis* (40 %) and *B. licheniformis* (12 %). In *lang satchu B. subtilis* was represented by 48.1 %, followed by *B. mycoides* (37.1 %) and *B. lentus* (14.8 %). In *yak satchu* 47.8 % comprised *B. mycoides* followed by *B. subtilis* (39.1 %) and *B. licheniformis* (13.1 %). *B. subtilis* (50.0 %) was the most dominant bacilli in *suka ko masu* followed by *B. mycoides* (33.3 %) and *B. thuringiensis* (16.7 %). In traditionally processed meat products of the Kumaun Himalaya, the most dominant bacilli recovered were *B. subtilis* in all the samples analyzed: *B. subtilis* (54.1 %), *B. mycoides* (29.2 %) and *B. thuringiensis* (16.7 %) in *chartayshya*; *B. subtilis* (85.0 %), *B. sphaericus* (15.0 %) in *jamma* and *B. subtilis* (77.8 %), *B. thuringiensis* (11.1 %) and *B. mycoides* (11.1 %) in *arjia* (Fig 20).

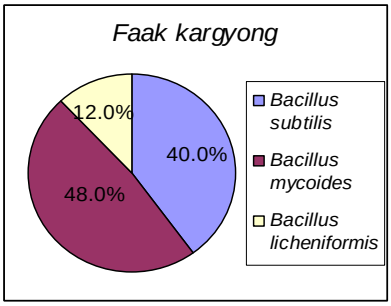
Table 22. Prevalence of Bacilli isolated from meat products of the Sikkim and the Kumaun Himalayas

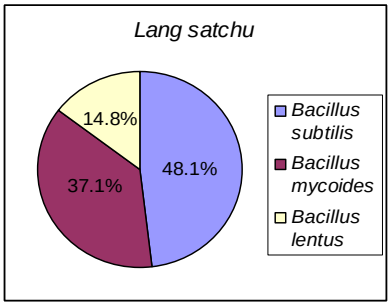
Product[a]	% of Prevalence					
	Bacillus subtilis	*Bacillus mycoides*	*Bacillus thuringiensis*	*Bacillus sphaericus*	*Bacillus licheniformis*	*Bacillus lentus*
Lang kargyong (51)	51.0	35.3	13.7	0	0	0
Yak kargyong (22)	68.2	31.8	0	0	0	0
Faak kargyong (25)	40.0	48.0	0	0	12.0	0
Lang satchu (27)	48.1	37.1	0	0	0	14.8
Yak satchu (23)	39.1	47.8	0	0	13.1	0
Suka ko masu (24)	50.0	33.3	16.7	0	0	0
Chartayshya (24)	54.1	29.2	16.7	0	0	0
Jamma (20)	85.0	0	0	15.0	0	0
Arjia (18)	77.8	11.1	11.1	0	0	0

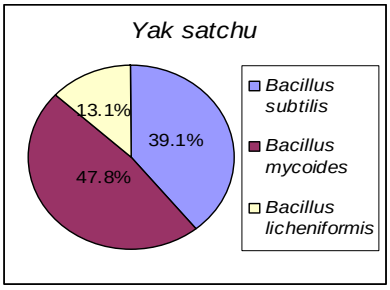
[a]Total number of spore-former from each product are given in parenthesis

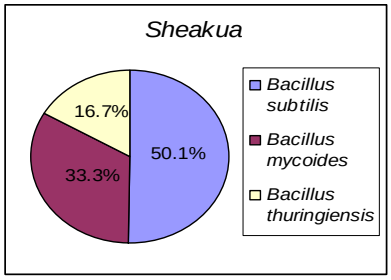

Lang kargyong
13.7%
35.3%
51.0%
Bacillus subtilis
Bacillus mycoides
Bacillus thuringiensis

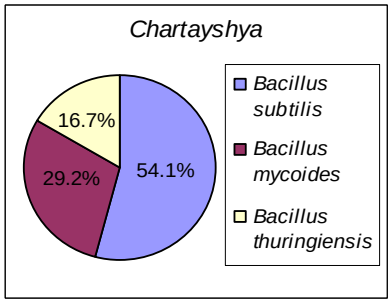

Yak kargyong
31.8%
68.2%
Bacillus subtilis
Bacillus mycoides

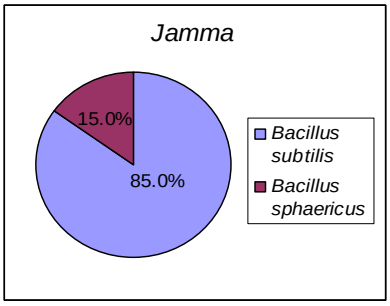

Faak kargyong
12.0%
48.0%
40.0%
Bacillus subtilis
Bacillus mycoides
Bacillus licheniformis


Lang satchu
14.8%
37.1%
48.1%
Bacillus subtilis
Bacillus mycoides
Bacillus lentus


Yak satchu
13.1%
47.8%
39.1%
Bacillus subtilis
Bacillus mycoides
Bacillus licheniformis


Sheakua
16.7%
33.3%
50.1%
Bacillus subtilis
Bacillus mycoides
Bacillus thuringiensis


Chartayshya
16.7%
29.2%
54.1%
Bacillus subtilis
Bacillus mycoides
Bacillus thuringiensis


Jamma
15.0%
85.0%
Bacillus subtilis
Bacillus sphaericus

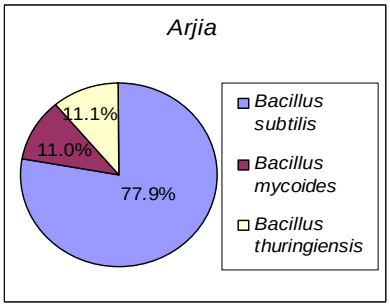

Arjia
11.1%
11.0%
77.9%
Bacillus subtilis
Bacillus mycoides
Bacillus thuringiensis

Fig 20. Graphic representation of prevalence of spore-former in meat products of the Sikkim and the Kumaun Himalaya.

Aerobic cocci

A total of 203 strains of aerobic cocci were isolated from 52 samples of the Sikkim Himalaya (Table 23) and a total of 81 strains were isolated from 16 samples of the Kumaun Himalaya (Table 24). All the samples were analysed for the microbiological population (Table 3 and 4). In all the samples, the predominance of *Staphylococcus* sp. over *Micrococcus* sp. was recorded. Micrococcaceae strains were characterized and identified following the keys described by von Rheinbaben and Hadlok (1981) and Bergey's Mannual of Systematic Bacteriology by Sneath *et al.* (1986). About 91.0 % of the total isolates were identified and characterized as *Staphylococcus* sp. (Plate g) and remaining 9.0 % of the isolates were *Micrococcus* sp. (Plate h) in all the samples analysed (Tables 23 and 24). The strains were further confirmed as *Micrococcus* sp. showing growth in furazolidone (FTO) agar and *Staphylococcus* sp. did not grow in FTO agar. Non-FTO strains were also identified as *Staphylococcus aureus* by using selective media Baird Parkar Agar (M043 HiMedia).

Table 23. Differential characteristics of *Micrococcus* and *Staphylococcus* isolated from meat products of the Sikkim Himalaya

Product[a]	Cell size	Gram	Catalase	Arginie hydrolysis	Tetrads	Growth on NaCl 10%	Growth on FTO agar	Grouped strains	% of prevalance	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*

[a]Total number of micrococcaceae from each products are given in parenthesis.
FTO, Furazolidone agar

Table 24. Differential characteristics of *Micrococcus* and *Staphylococcus* isolated from meat products of the Kumaun Himalaya

Product[a]	Cell size	Gram strain	Catalase	Arginie hydrolysis	Tetrads	Growth on NaCl 10%	Growth on FTO agar	Grouped strains	% of prevalance	Identity
Chartayshya (32)	1.6 (0.5-2.4)	+	+	-	+	-	+	2	6.3	*Micrococcus*
	1.1 (0.5-1.6)	+	+	+	-	+	-	30	93.7	*Staphylococcus*
Jamma (24)	1.5 (0.5-2.4)	+	+	-	+	-	+	2	8.3	
	1.0 (0.5-1.6)	+	+	+	-	+	-	22	91.7	*Staphylococcus*
Arjia (25)	1.0 (0.5-2.4)	+	+	-	+	-	+	2	8.0	*Micrococcus*
	1.1 (0.5-1.6)	+	+	+	-	+	-	23	92.0	*Staphylococcus*

[a]Total number of Micrococcaceae from each products are given in parenthesis.
Furazolidone (FTO) agar

Grouping of representative of yeasts

Though the dominant microflora in all samples of meat products was LAB, a sizable number of yeasts were also recovered in the samples analysed. A total of 298 yeast strains were isolated from meat products collected from different places of the Sikkim and the Kumaun Himalayas. The representative strains of yeast were selected randomly from each grouped strains having similar colony appearance, cell shape, type of

mycelia and ascospore for detailed identification (Table 25). Representative strains were assigned the strain code number indicating the sample names and source.

Table 25. Grouping of representative strains of Yeast isolated from meat products of the Sikkim and the Kumaun Himalaya

[a]Product	Colony	Cell shape	Mycelium	Ascospore	Grouped strains	Representative strains	
						Total no.	Strains code
Lang kargyong (59)	Ss	S-O	Pseudo	Spheroidal	52	2	KP:Y5, PK:Y1
	Ss	O-E	Pseudo	Spheroidal	7	2	ZK:Y10, ZK:Y2
Yak Kargyong (35)	Ss	S-O	Pseudo	Spheroidal	35	2	YK:Y2, YK:Y1
Faak kargyong (41)	Ss	S-O	Pseudo	Spheroidal	30	2	FK:Y1, FK:Y2
	Ss	O-E	Pseudo	Hat	11	2	TK:Y2, TK:Y3
Lang Satchu (32)	Ss	S-O	Pseudo	Spheroidal	25	1	TS:Y3
	Ss	O-E	Pseudo	Hat	7	2	TS:Y4, SR:Y2
Yak satchu (25)	Ss	S-O	Pseudo	Spheroidal	25	2	YS:Y1, YS:Y3
Suka ko masu (23)	Ss	S-O	Pseudo	Spheroidal	16	2	BS:Y3, BS:Y6
	Ds	O-C	True and Pseudo	Hat	7	2	BS:Y8, BS:Y9

Continued (Table 25)

[a]Product	Colony	Cell shape	Mycelium	Ascospore	Grouped strains	Representative strains	
						Total no.	Strains code
Chartayshya (33)	Ss	S-O	Pseudo	Spheroidal	25	2	CD:Y2, CD:Y1
	Ss	O-E	Pseudo	Hat	8	1	CD:Y14
Jamma (22)	Ss	S-O	Pseudo	Spheroidal	16	1	KJ:Y6
	Ds	O-C	True and Pseudo	Hat	6	1	KJ:L13
Arjia (28)	Ss	S-O	Pseudo	Spheroidal	18	1	KA:Y1
	Ds	O-C	True and Pseudo	Hat	10	2	KA:Y3, KA:Y7

[a]Total number of isolates in each product are given in parenthesis.
All isolates reproduced by multilateral budding.
Ds, dusty surface; Ss, smooth surface; O-C, oval to cylindrical; O-E, oval to ellipsoidal; S-O, spherical to oval.

Characteristics and identity of yeasts

Following the taxonomical keys of Kreger-van Rij (1984), and Kurtzman and Fell (1998), Sugar fermentation and assimilation tests of randomly selected representative strains of yeasts were carried out (Table 26). Strains (KP:Y5, PK:Y1, TS:Y3, BS:Y6, BS:Y3, CD:Y1, CD:Y2, KJ:Y6, KA:Y1) from *lang kargyong, lang satchu, suka ko masu, chartayshya, jamma* and *arjia* showed smooth surfaced colonies with spheroidal ascospores and fermented glucose weakly were identified as *Debaryomyces hansenii* (Table 26) (Plate i). FK:Y1, FK:Y2, YS:Y1, YS:Y3 from *faak kargyong* and *yak*

satchu were identified as *Debaryomyces polymorphus* and YK:Y2, YK:Y1 from *yak kargyong* were identified as *Debaryomyces pseudopolymorphus* (Table 26). Strains (ZK:Y10, ZK:Y2, TS:Y4, SR:Y2) were identified as *Pichia anomala* on the basis of sugar fermentation and assimilation tests (Table 26). They formed 1-4 hat-shaped ascospores per ascus. BS:Y8, BS:Y9 had dusty, dry and powdery surfaced 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 to 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 and were identified as *Pichia burtonii* (Table 26) (Plate j). Strains (TK:Y2, TK:Y3, CD:Y14) from *faak kargyong* and *chartayshya* were identified as *Candida famata*. Strains (KJ:Y13) isolated from *jamma* were identified as *Candida albicans* (Plate k) whereas strains (KA:Y7, KA:Y3) from *arjia* were identified as *Candida humicola* on the basis of sugar fermentation and assimilation tests (Table 26) (Plate l).

Prevalence of yeasts

The most dominant yeast recovered in all the samples isolated from meat products of the Sikkim and the Kumaun Himalaya were *Debaryomyces* sp. Out of 59 strains of yeasts isolates, 88.1 % were *Debaryomyces hansenii* followed by *Pichia anomala* comprised 11.9 % in *lang kargyong* (Table 27). In *yak kargyong* all isolates were *Debaryomyces pseudopolymorphus*. Of the 41 isolates from *faak kargyong* 73.2 % were *Debaryomyces polymorphus* and remaining 26.8 % were *Candida famata*. In *lang satchu* prevalence of yeast was *Debaryomyces hansenii* (78.1 %) followed by *Pichia anomala* comprising 21.9 %. Out of 25 strains of isolates, isolated from *yak satchu*, all the yeast strains were identified as *Debaryomyces polymorphus* representing 100 %. The most dominant yeast isolates recovered in *suka ko masu* were *Debaryomyces hansenii* representing 69.6 % followed by *Pichia burtonioi* having 30.4 %. In *chartayshya*, 75.8 % were *Debaryomyces hansenii* followed by *Candida famata* (24.2 %). Out of 22 strains of yeasts isolated from *jamma*, 72.7 % were *Debaryomyces hansenii* followed by *Candida albicans* comprised 27.3 %. *Debaryomyces hansenii* was dominant in *arjia* having 64.3 % of the total yeast isolates followed by *Candida humicola* representing 35.7 % (Fig 21). The result shows that the yeast belonging to the genus *Debaryomyces* sp. was dominant in all the samples analysed.

Table 27. Prevalence of Yeast in meat product from the Sikkim and the Kumaun Himalaya

Product[a]	% of Prevalence							
	Debaryomyces hansenii	*Debaryomyces polymorphus*	*Debaryomyces pseudopolymorphus*	*Candida albicans*	*Candida humicola*	*Candida famata*	*Pichia burtonii*	*Pichia anomala*
Lang kargyong (59)	88.1	0	0	0	0	0	0	11.9
Yak kargyong (35)	0	0	100	0	0	0	0	0
Faak kargyong (41)	0	73.2	0	0	0	26.8	0	0
Lang satchu (32)	78.1	0	0	0	0	0	0	21.9
Yak satchu (25)	0	100	0	0	0	0	0	0
Suka ko masu (23)	69.6	0	0	0	0	0	30.4	0
Chartayshya (33)	75.8	0	0	0	0	24.2	0	0
Jamma (22)	72.7	0	0	27.3	0	0	0	0
Arjia (28)	64.3	0	0	0	35.7	0	0	0

[a]Total number of yeast isolates of each products are given in parenthesis

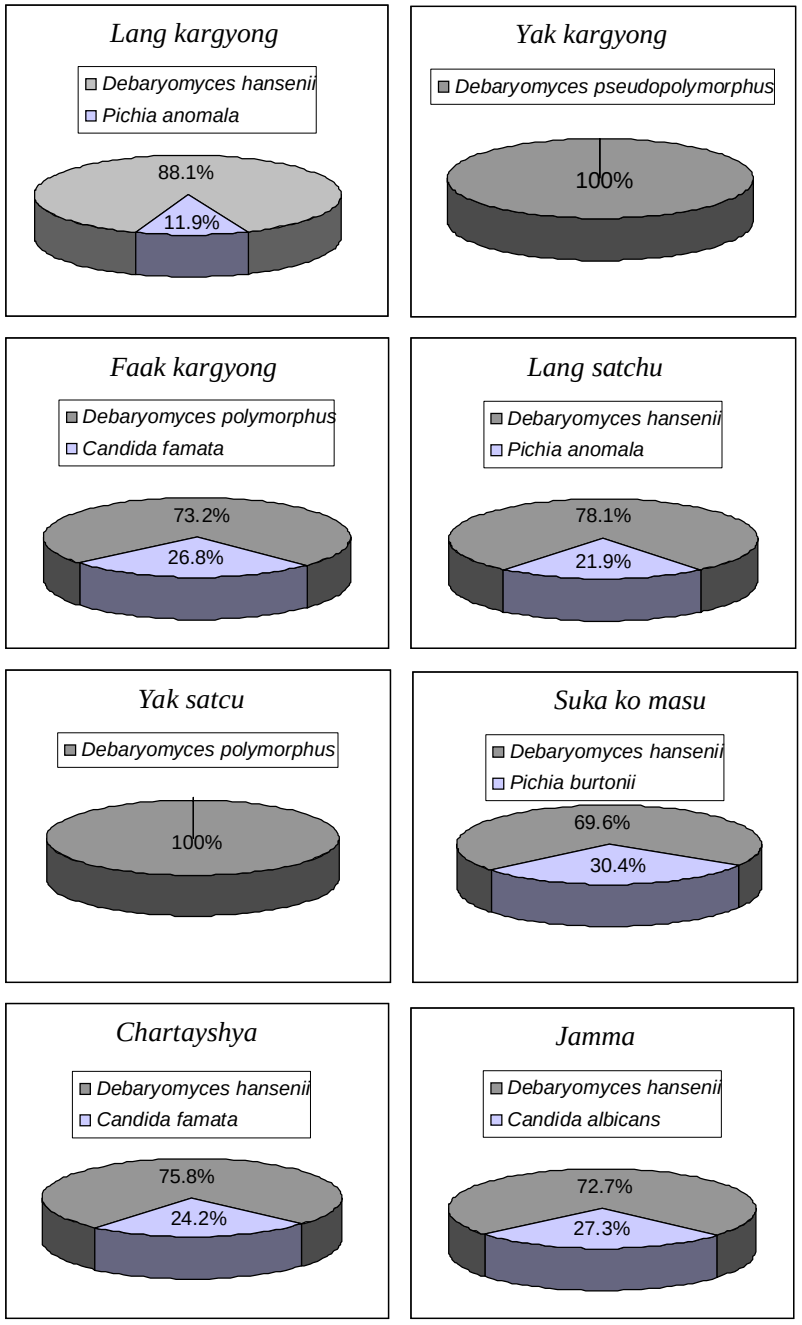


Fig 21. Graphic representation of prevalence of Yeasts in meat products of the Sikkim and the Kumaun Himalaya.

Profiles of microorganisms

Compiling the identification profiles of the bacteria isolated from traditionally processed meat products of the Sikkim and the Kumaun Himalayas, it was revealed that the 7 genera with 18 species were recovered. These were *Lactobacillus sake, Lb. curvatus, Lb. plantarum Lb. brevis, Lb. casei, Lb. divergens Lb. carnis, Lb. sanfrancisco, Enterococcus faecium, Leuconostoc mesenteroides, Pediococcus pentosaceous, Bacillus subtilis, B. mycoides, B. thuringiensis, B. licheniformis, B. lentus, B. sphaericus, Staphylococcus aureus* and *Micrococcus* sp. The following yeast strains were recovered comprising 3 genera with 8 species, *Debaryomyces hansenii, Debaryomyces pseudopolymorphus, D. polymorphus, Pichia anomala, P. burtonii, Candida albicans, C. famata* and *C. humicola* (Tables 28 and 29).

Table 28. Profile of microorganisms isolated from meat products of the Sikkim Himalaya

Product	Microorganisms	
	Bacteria	Yeast
Lang kargyong	*Lb. sake, Leuc. mesenterioides, Lb. divergens, Lb. carnis, Lb. Sanfransisco, E.faecium, Lb. curvatus, B.subtilis, B.mycoides, B.thuringiensis, S.aureus, Micrococcus*	*D. hansenii, P. anomala*
Yak kargyong	*Leuc. mesenterioides, Lb. casei, Lb. plantarum, Lb. sake, Lb. divergens, Lb. carnis, Lb. sanfrancisco, E. faecium, Lb. curvatus, B.subtilis, B.mycoides, S.aureus, Micrococcus*	*D. pseudopolymorphus*
Faak kargyong	*Lb. brevis, Leuc. mesenterioides, E. faecium, Lb. carnis, Lb. plantarum, B.subtilis, B.mycoides, B.licheniformis, S.aureus, Micrococcus*	*C. famata, D. polymorphus*

Continued (Table 28)

Product	Microorganisms	
	Bacteria	Yeast
Lang satchu	*P. pentosaceous,* *Lb. casei,* *Lb. carnis,* *E. faecium,* *B.subtilis,* *B.mycoides,* *B. lentus,* *S.aureus,* *Micrococcus*	*D. hansenii,* *P. anomala*
Yak satchu	*P. pentosaceous,* *B.subtilis,* *B.mycoides,* *B. licheniformis,* *S.aureus,* *Micrococcus*	*D. polymorphus*
Suka ko masu	*Lb. carnis,* *E. faecium,* *Lb. plantarum,* *B.subtilis,* *B.mycoides,* *B.thuringiensis,* *S.aureus,* *Micrococcus*	*D. hansenii,* *P. burtonii*

DL, Less than detection limit (10 cfu / g).

Table 29. Profile of microorganisms isolated in meat products of the Kumaun Himalaya

Product	Microorganisms	
	Bacteria	Yeast
Chartayshya	*Lb. divergen,s* *E. faecium,* *P. pentosaceous,* *B.subtilis,* *B.mycoides,* *B.thuringiensis,* *S.aureus,* *Micrococcus*	*D. hansenii,* *C. famata*
Jamma	*Leuc. mesenterioides,* *P. pentosaceous,* *E cecorum,* *Lb. sanfrancisco,* *E. faecium,* *Lb. ivergens*, *B. subtilis,* *B. sphaericus,* *S.aureus,* *Micrococcus*	*D. hansenii,* *C. albicans*
Arjia	*E. faecium* *P. pentosaceous,* *B.subtilis,* *B.mycoides,* *B.thuringiensis,* *S.aureus,* *Micrococcus*	*D. hansenii,* *C. humicola*

DL, Less than detection limit (10 cfu / g).

Technological properties of LAB

Enzymatic profiles

Enzymatic profiles of LAB strains were assayed using the API-zym (bioMérieux, France) galleries (Table 30). Each of the predominant LAB strain produced a wide spectrum of enzymes. These strains showed relatively weak esterase (C4) and strong arylamidase and phosphatase activities. However, *Lactobacillus carnis* ZK:L7; *Lb. plantarum* YKK:L2, BS:L17; *Pediococcus pentosaceous* CD:L26 showed weak proteinase activity whereas, phosphohydrolase activity was shown by all strains tested with the methods applied. Acid phosphatase activity was detected in all the strains tested among which >40 nanomole activities was showed by *Lb. plantarum* YKK:L2; *Lb. casei* YKK:L3. Phosphohydrolase activity was shown by all strains tested. The α-glucosidase activity was highest (>40 nanomoles) in *Lactobacillus curvatus* KP:L13; *Lactobacillus sanfrancisco* LK:L2; *Lb. brevis* FK:L5; *Leuconostoc mesenteroides* KJ:L18; *Lb. divergens* KJ:L6. Relatively moderate glucosaminidase activity was shown by *Leuc. mesenteroides* FK:L3; *Pediococcus pentosaceous* KS:L6, YS:L13, CD:L26; *Lb. casei* SR:L4; *Lb. carnis* BS:L25; *Lb. divergens* CD:L3; *Enterococcus cecorum* KJ:L9. The α-galactosidase activity was shown by few strains of LAB tested whereas β-galactosidase activity was highest (>40 nanomoles) in

Leuconostoc mesenteroides KP:L11, FK:L3; *Lb. sake* YKg:L8; *Lb. brevis* FK:L5;

Pediococcus pentosaceous YS:L13, CD:L26 KA:L4; *Lb. divergens* CD:L3;

Table 30. Enzymatic profiles of LAB strains from Meat products of the Sikkim and the Kumaun Himalaya using API-zym

Enzyme	Strain (Activity in nanomoles)								
	KP:L8	KP:L13	ZK:L5	ZK:L7	LK:L2	KM:L19	KP:L11	YKK:L2	YKK:L3
Control (without enzyme)	0	0	0	0	0	0	0	0	0
Alkaline phosphatase	5	5	5	0	5	5	0	5	10
Esterase (C4)	5	0	0	0	0	0	0	0	0
Esterase lipase (C8)	5	5	0	5	5	0	0	0	0
Lipase (C14)	0	0	0	0	0	0	0	5	0
Leucine arylamidase	5	5	5	5	5	5	30	>40	>40
Valine arylamidase	0	0	0	0	0	0	5	30	>40
Cystine arylamidase	0	0	0	0	0	0	5	>40	5
Trypsin	0	0	0	0	0	0	0	0	0
α-chymotrypsin	0	0	0	0	0	0	0	0	0
Acid phosphatase	5	10	10	5	20	20	20	>40	>40
Napthol-AS-BI-phosphohydrolase	10	10	10	20	10	10	10	10	30
α-galactosidase	0	0	5	0	20	5	30	0	0
β-galactosidase	0	0	30	5	0	10	>40	30	5
β-glucuronidase	0	0	0	0	0	0	0	0	0
α-glucosidase	30	>40	0	5	>40	0	30	0	0
β-glucosidase	5	5	0	0	0	0	>40	0	0
N-acetyl-β-	0	0	0	0	0	0	0	0	0

glucosaminidase									
α-mannosidase	0	0	0	0	0	0	0	0	0
α-fucosidase	0	0	0	0	0	0	0	0	0

Data represents the means of 3 sets of experiment.

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. Mesenteroides* (Lang kargyong); YKK:L2 *Lb. plantarum*, YKK:L3 *Lb. casei* (Yak kargyong).

Continued (Table 30)

Enzyme	Strain (Activity in nanomoles)								
	YKg:L8	FK:L5	FK:L3	KS:L6	SR:L4	YS:L13	YS:L20	BS:L17	BS:L16
Control (without enzyme)	0	0	0	0	0	0	0	0	0
Alkaline phosphatase	5	0	0	0	5	0	0	0	0
Esterase (C4)	0	0	0	0	0	0	5	0	0
Esterase lipase (C8)	0	0	0	0	5	0	5	5	5
Lipase (C14)	0	0	0	0	5	5	5	0	0
Leucine arylamidase	30	10	>40	>40	>40	>40	30	30	30
Valine arylamidase	>40	0	>40	>40	>40	>40	5	5	5
Cystine arylamidase	5	0	5	30	5	10	5	5	5
Trypsin	0	0	0	0	0	0	0	0	0
α-chymotrypsin	0	0	0	0	0	0	0	0	0
Acid phosphatase	30	0	10	5	10	10	20	30	10
Napthol-AS-BI-phosphohydrolase	5	10	10	10	20	30	20	10	10
α-galactosidase	0	20	0	0	0	0	0	0	0
β-galactosidase	>40	>40	>40	20	10	>40	0	0	0
β-glucuronidase	0	0	0	0	0	0	0	0	0
α-glucosidase	0	>40	0	0	10	0	0	0	0
β-glucosidase	0	30	0	10	10	5	0	0	0
N-acetyl-β-glucosaminidase	0	0	10	20	30	5	0	0	0
α-mannosidase	0	0	0	0	0	0	0	0	0
α-fucosidase	0	0	0	0	0	0	0	0	0

Data represents the means of 3 sets of experiment.

Ykg:L8 *Lb. sake* (Yak kargyong); FK:L5 *Lb. brevis*, FK:L3 *Leuc. mesenteroides* (Faak kargyong); KS:L6 *P. pentosaceous*, SR:L4 *Lb. casei* (lang satchu); YS:L13 *P. pentosaceous,* YS:L20 *P. pentosaceous* (Yak satchu); BS:L17 *Lb. plantarum,* BS:L16 *E faecium* (Suka ko masu / Sheakua).

Continued (Table 30)

Enzyme	Strain (Activity in nanomoles)							
	BS:L25	CD:L3	CD:L26	KJ:L9	KJ:L18	KJ:L6	KA:L4	KA:L16
Control (without enzyme)	0	0	0	0	0	0	0	0
Alkaline phosphatase	5	5	0	0	0	0	0	0
Esterase (C4)	0	0	5	0	0	0	0	0
Esterase lipase (C8)	0	0	5	0	0	0	0	0
Lipase (C14)	5	5	5	5	0	0	5	5
Leucine arylamidase	>40	>40	>40	>40	10	10	>40	>40
Valine arylamidase	>40	>40	>40	>40	0	0	>40	>40
Cystine arylamidase	5	5	5	5	0	5	5	5
Trypsin	0	0	0	0	0	0	0	0
α-chymotrypsin	0	0	0	0	0	0	0	0
Acid phosphatase	20	30	5	10	5	10	10	10
Napthol-AS-BI-phosphohydrolase	20	20	20	10	10	20	10	20
α-galactosidase	0	0	0	0	30	20	0	0
β-galactosidase	5	>40	>40	>40	0	0	>40	10
β-glucuronidase	0	0	0	0	0	0	0	0

α-glucosidase	0	0	0	0	>40	>40	0	0
β-glucosidase	10	20	20	20	>40	>40	5	20
N-acetyl-β-glucosaminidase	10	20	20	30	0	0	5	30
α-mannosidase	0	0	0	0	0	0	0	0
α-fucosidase	0	0	0	0	0	0	0	0

BS:L25 *Lb. carnis* (Suka ko masu / Sheakua); CD:L3 *Lb. divergens,* CD:L26 *P.pentosaceous* (Chartayshya); KJ:L9 *E. cecorum,* KJ:L18 *Leuc.mesenteroides*, KJ:L6 *Lb. divergens* (Jamma); KA:L4 *P. pentosaceous,* KA:L16 *E. faecium* (Arjia)).

Enterococcus cecorum KJ:L9. None of the strains tested showed β-glucuronidase, α-mannosidase and α-fucosidase activities (Table 30).

Antimicrobial activities

Tables 31 and 32 showed the antimicrobial activities of LAB strains, isolated from meat products of the Sikkim and the Kumaun Himalayas against different indicator strains used such as *Listeria innocua* DSM 20649, *Listeria monocytogenes* DSM 20600, *Bacillus cereus* CCM 2010, *Staphylococcus aureus* S1, *Klebsiella pneumoniae* subsp. *pneumoniae* BFE 147, *Enterobacter agglomerans* BFE 154 and *Pseudomonas aeruginosa* BFE 162. Most of the LAB strains showed the clear inhibition zones in agar-spot plates against the used indicator strains, showing antagonisms. The LAB strains showing the inhibition zones of measurements by scale of more than 4 mm in agar-spot plates were selected for bacteriocin assay against the respective pathogenic bacteria (Tables 31 and 32).

Table 31. Antimicrobial activity of the LAB strains isolated from meat products of the Sikkim Himalaya

Strains	Indicator strains						
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Lang kargyong							
KP:L3	–	–	–	–	+	–	–
KP:L8	–	–	–	–	+	+	–
KP:L13	–	–	–	–	+	–	–
KP:L30	–	–	–	–	+	–	–
ZK:L5	–	–	–	–	+	+++	–
KM:L32	–	–	–	–	–	–	–
KM:L31	–	–	–	–	–	–	–
ZK:L7	–	–	–	–	–	++	–
KM:L1	–	–	–	–	–	–	–
KM:L10	–	–	–	–	–	–	–
LK:L2	–	–	–	–	++	+	–
KP:L5	–	–	–	–	++	+	–
KP:L14	++	–	–	–	–	–	–
ZK:L4	+	–	–	–	+	+++	–
KM:L19	–	–	–	–	+	+++	–
KM:L34	–	–	–	–	+	–	–
KM:L35	–	–	–	–	++	+++	–
KP:L7	–	–	–	–	+	+++	–
KP:L11	–	–	–	–	+++	+	–
KP:L18	–	–	–	–	+	+++	–
LK:L4	–	–	–	–	+	+++	–
ZK:L1	–	–	–	–	++	+++	–

Continued (Table 31)

Strains	Indicator strains						
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Lang kargyong							
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:L3	–	–	–	–	+	+	–
YK:L2	–	–	–	–	+	++	–
YK:L15	–	–	–	–	+	++	–
YK:L5	–	–	–	–	+	+	–
YK:L9	–	–	–	–	+	–	–

Continued (Table 31)

Strains	Indicator strains						
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Faak kargyong							
FK:L5	–	–	–	–	+	–	–
FK:L1	–	–	–	–	++	–	–
FK:L8	–	–	–	–	+	–	–
FK:L11	–	–	–	–	+	–	–
FK:L13	–	–	–	–	++	–	–
FK:L4	–	–	–	–	–	–	–
FK:L10	–	–	–	–	++	–	–
FK:L14	–	–	–	–	+	–	–
FK:L15	–	–	–	–	–	–	–
FK:L16	–	–	–	–	–	–	–
FK:L17	–	–	–	–	–	–	–
FK:L3	–	–	–	–	–	–	–
FK:L2	–		–	–	–	–	–
FK1:L14	–	–	–	–	–	–	–
FK:L25	–	–	–	–	–	–	–
FK:L6	–	–	–	–	–	–	–
FK:L7	–	–	–	–	–	–	–
FK:L15	–	–	–	–	–	–	–
Lang Satchu							
KS:L2	–	–	–	–	–	–	–
KS:L6	–		–	–	–	–	–
KS:L8	–	–	–	–	–	–	–

Continued (Table 31)

Strains	Indicator strains						
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Lang Satchu							
KS:L10	–	–	–	–	+	–	–
KS:L15	–	–	–	–	+	–	–
SR:L4	–	–	–	–	++	–	–
SR:L8		–	–	–	–	–	–
KS:L1	–	–	–	–	+	–	–
KS:L18	–		–	–	+	++	–
KS:L26	–	–	–	–	+	++	–
SR:L7	–	–	–	–	+	–	–
SR:L6	–	–	–	–	+	–	–
TS:L9	–	–	–	–	+	+	–
TS:L23		–	–	–	++	–	–
TS:L20	–	–	–	–	+		–
TS:L7	–		–	–	++	–	–
TS:L6	–	–	–	–	++	–	–
KS:L22	–	–	–	–	+	–	–
KS:L17	–	–	–	++	+	+	–
Yak satchu							
YS:L2	–	–	–	–	++	–	–
YS:L1	–	–	–	–	+	–	–
YS:L13	–	–	–	–	+	–	–
YS:L7	–	–	–	–	++	–	–
YS:L4	–	–	–	–	+	–	–

Continued (Table 31)

Strains	Indicator strains						
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Yak satchu							
YS:L3	–	–	–	–	–	–	–
YS:L10	–	–	–	–	–	–	–
YS:L8	–	–	–	–	–	–	–
YS:L6	–	–	–	–	–	–	–
YS:L18	–	–	–	–	–	–	–
YS:L16	–	–	–	–	–	–	–
YS:L5	–	–	–	–	–	–	–
YS:L20	–	–	–	–	++	–	–
YS:L9	–	–	–	–	–	–	–
Sukako masu							
BS:L25	–	–	–	–	–	–	–
BS:L4	–	–	–	–	+	–	–
BS:L18	–	–	–	–	++	–	–
BS:L17	–	–	–	–	++	–	–
BS:L9	–	–	–	–	+	–	–
BS:L1	–	–	–	–	+	–	–
BS:L2	–	–	–	–	–	–	–
BS:L5	–	–	–	–	–	–	–

Continued (Table 31)

Strains	Indicator strains						
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Suka ko masu							
BS:L7	–	–	–	–	–	–	–
BS:L11	–	–	–	–	–	–	–
BS:L16	–	–	–	–	–	–	–
BS:L19	–	–	–	–	–	–	–
BS:L21	–	–	–	–	+++	–	–
BS:L23	–	–	–	–	+	–	–
BS:L13	–	–	–	–	+	–	–

Data shows 3 sets of experiments.

–, no zone of inhibition; +, 1mm - 3mm; ++, 4mm -6mm; +++, >7mm

Table 32. Antimicrobial activity of the LAB strains isolated from meat products of the Kumaun Himalaya

Strains	Indicator strains						
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Chartayshya							
CD:L3	–	–	–	–	+	–	–
CD:L21	–	–	–	–	+	–	–
CD:L6	–	–	–	–	+	–	–
CD:L5	–	–	–	–	++	–	–
CD:L2	–	–	–	–	+	–	–
CD:L22	–	–	–	–	+	–	–
CD:L14	–	–	–	–	+	–	–
CD:L7	–	–	–	–	+	–	–
CD:L23	–	–	–	–	+	–	–
CD:L13	–	–	–	–	+	–	–
CD:L15	–	–	–	–	+	–	–
CD:L1	–	–	–	–	++	–	–
CD:L8	–	–	–	–	+	–	–
CD:L10	–	–	–	–	+	–	–
CD:L20	–	–	–	–	+	–	–
CD:L26	–	–	–	–	++	–	–

Continued (Table 32)

Strains	Indicator strains						
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Jamma							
KJ:L1	–	–	–	–	+++	–	–
KJ:L15	–	–	–	–	+++	–	–
KJ:L8	–	–	–	–	+	–	–
KJ:L6	–	–	–	–	++	–	–
KJ:L7	–	–	–	–	++	–	–
KJ:L2	–	–	–	–	+	–	–
KJ:L14	–	–	–	–	++	–	–
KJ:L18	–	–	–	–	++	–	
KJ:L21	–	–	–	–	++	–	–
KJ:L23	–	–	–	–	+	–	–
KJ:L25	–	–	–	–	++	–	–
KJ:L29	–	–	–	–	+	–	–
KJ:L10	–	–	–	–	++	–	–
KJ:L13	–	–	–	–	+	–	–
KJ:L31	–	–	–	–	+	–	–
KJ:L3	–	–	–	–	+	–	–
KJ:L16	–	–	–	–	+	–	–
KJ:L9	–	–	–	–	+	–	–
KJ:L4	–	–	–	–	+	–	–
KJ:L5	–	–	–	–	+	–	–

Continued (Table 32)

Strains	Indicator strains						
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Arjia							
KA:L1	–	–	–	–	++	–	–
KA:L2	–	–	–	–	+	–	–
KA:L4	–	–	–	–	++	–	–
KA:L11	–	–	–	–	++	–	–
KA:L17	–	–	–	–	+	–	–
KA:L21	–	–	–	–	+	–	–
KA:L3	–	–	–	–	+	–	–
KA:L5	–	–	–	–	+	–	–
KA:L8	–	–	–	–	++	–	–
KA:L16	–	–	–	–	++	–	–
KA:L20	–	–	–	–	++	–	–
KA:L13	–	–	–	–	+	–	–
KA:L15	–	–	–	–	+	–	–

Data shows 3 sets of experiments.

–, no zone of inhibition; +, 1mm - 3mm; ++, 4mm - 6mm; +++, >7mm

Bacteriocin Assay

Lactic acid bacteria (LAB) strains showing the inhibition zones of >4 mm against indicator strains in agar-spot plates, were selected for bacteriocin assay against the respective indicator strains. Cell-free supernatant extract of 24 h culture of selected LAB strains were spotted onto the plates containing indicator organisms. None of the tested strains of LAB from meat products of the Sikkim and the Kumaun Himalayas produced bacteriocin against used pathogenic bacteria under the applied condition (Tables 33 and 34).

Table 33. Screening of bacteriocin-producing strains of LAB from meat products by cell-free culture supernatants of the Sikkim Himalaya

Strains	Indicator strains			
	L. innocua DSM 20649	*S. aureus* S1	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. agglomerans* BFE 154
Lang kargyong				
ZK:L5	–	–	–	–
ZK:L7	–	–	–	–
LK:L2	–	–	–	–
KP:L5	–	–	–	–
ZK:L4	–	–	–	–
KP:L14	–	–	–	–
KM:L19	–	–	–	–
KM:L35	–	–	–	–
KP:L7	–	–	–	–
KP:L11	–	–	–	–
KP:L18	–	–	–	–
LK:L4	–	–	–	–
ZK:L1	–	–	–	–
ZK:L3	–	–	–	–
ZK:L6	–	–	–	–
Yak kargyong				
YKK:L2	–	–	–	–
YKK:L3	–	–	–	–
YKK:L4	–	–	–	–
YKK:L8	–	–	–	–
YKK:L11	–	–	–	–

Continued (Table 33)

Strains	Indicator strains			
	L. innocua DSM 20649	*S. aureus* S1	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. agglomerans* BFE 154
Yak kargyong				
YKg:L3	–	–	–	–
YKg:L7	–	–	–	–
YKg:L8	–	–	–	–
YK:L11	–	–	–	–
YKK:L1	–	–	–	–
YKK:L5	–	–	–	–
YKg:L9	–	–	–	–
YK:L1	–	–	–	–
YK:L2	–	–	–	–
YK:L15	–	–	–	–
Faak kargyong				
FK:L1	–	–	–	–
FK:L13	–	–	–	–
FK:L10	–	–	–	–
Lang Satchu				
SR:L4	–	–	–	–
KS:L18	–	–	–	–
KS:L26	–	–	–	–
TS:L23	–	–	–	–
TS:L7	–	–	–	–
TS:L6	–	–	–	–

Continued (Table 33)

Strains	Indicator strains			
	L. innocua DSM 20649	*S. aureus* S1	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. agglomerans* BFE 154
Yak Satchu				
YS:L2	YS:L2	YS:L2	YS:L2	YS:L2
YS:L7	YS:L7	YS:L7	YS:L7	YS:L7
YS:L20	YS:L20	YS:L20	YS:L20	YS:L20
Sukako masu				
BS:L17	BS:L17	BS:L17	BS:L17	BS:L17
BS:L21	BS:L21	BS:L21	BS:L21	BS:L21
BS:L18	BS:L18	BS:L18	BS:L18	BS:L18

Data shows 3 sets of experiment.

[a]Strains showing ≥ ++ inhibition zone in the agar spot assay for antagonism were selected for bacteriocin assay. –, no zone of inhibition

Table 34. Screening of bacteriocin-producing strains of LAB from meat products by cell-free culture supernatants of the Kumaun Himalaya

Strains	Indicator strains			
	L. innocua DSM 20649	*S. aureus* S1	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. agglomerans* BFE 154
Chartayshya				
CD:L5	–	–	–	–
CD:L1	–	–	–	–
CD:L26	–	–	–	–
Jamma				
KJ:L1	–	–	–	–
KJ:L15	–	–	–	–
KJ:L6	–	–	–	–
KJ:L7	–	–	–	–
KJ:L14	–	–	–	–
KJ:L18	–	–	–	–
KJ:L21	–	–	–	–
KJ:L23	–	–	–	–
KJ:L25	–	–	–	–
KJ:L10	–	–	–	–
Arjia				
KA:L20	–	–	–	–
KA:L8	–	–	–	–
KA:L1	–	–	–	–
KA:L16	–	–	–	–
KA:L11	–	–	–	–

KA:L4	–	–	–	–

Data shows 3 sets of experiment.

[a]Strains showing ≥ ++ inhibition zone in the agar spot assay for antagonism were selected for bacteriocin assay.

–, no zone of inhibition

Screening of biogenic amines-producing LAB

Strains of LAB isolated from meat products collected from different places of the Sikkim and the Kumaun Himalayas were screened for their ability to produce biogenic amines (Tables 35 and 36). None of the strains produced biogenic amines in the biogenic screening medium containing precursor amino acids (tyrosine, lysine, histidine, ornithine) in the method applied.

Table 35. Screening of biogenic amine producing LAB strains from meat products of the Sikkim Himalaya

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
Lang kargyong	*Lb. sake* KP:L3	–	–	–	–
	Lb. sake KP:L8	–	–	–	–
	Lb. curvatus KP:L13	–	–	–	–
	Lb. sake KP:L30	–	–	–	–
	Lb. divergens ZK:L5	–	–	–	–
	Lb. divergens KM:L32	–	–	–	–
	Lb. carnis KM:L31	–	–	–	–
	Lb. carnis ZK:L7	–	–	–	–
	Lb. sanfransisco KM:L1	–	–	–	–
	Lb. sanfransisco KM:L10	–	–	–	–
	Lb. sanfransisco LK:L2	–	–	–	–
	Lb. sanfransisco KP:L5	–	–	–	–
	Lb. sanfransisco KP:L14	–	–	–	–
	Lb. sanfransisco ZK:L4	–	–	–	–
	E. faecium KM:L19	–	–	–	–
	E. faecium KM:L34	–	–	–	–
	E. faecium KM:L35	–	–	–	–
	Leuc. mesenterioides KP:L7	–	–	–	–
	Leuc. mesenterioides KP:L11	–	–	–	–
	Leuc. mesenterioides KP:L18	–	–	–	–
	Leuc.mesenterioides LK:L4	–	–	–	–
	Lb. carnis ZK:L1	–	–	–	–
	Lb. carnis ZK:L3	–	–	–	–
	Lb. carnis ZK:L6	–	–	–	–

Continued (Table 35)

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
	Lb. plantarum YKK:L2	–	–	–	–
	Lb. casei YKK:L3	–	–	–	–
	*Lb. curvatus*YKK:L4	–	–	–	–
	Lb. casei YKK:L8	–	–	–	–
	Lb. plantarum YKK:L11	–	–	–	–
	Lb. plantarum YKg:L3	–	–	–	–
	Lb. sake YKg:L7	–	–	–	–
	Lb. sake YKg:L8	–	–	–	–
Yak kargyong	*Lb. divergens* YK:L11	–	–	–	–
	Lb. carnis YKK:L1	–	–	–	–
	Lb. carnis YKK:L5	–	–	–	–
	Lb. sake YKg:L9	–	–	–	–
	Lb. sanfrancisco YK:L1	–	–	–	–
	Lb. sanfrancisco YK:L3	–	–	–	–
	E. faecium YK:L2	–	–	–	–
	E. faecium YK:L15	–	–	–	–
	Leuc. mesenterioides YK:L5	–	–	–	–
	Leuc. mesenterioides YK:L9	–	–	–	–
Faak kargyong	*Lb. brevis* FK:L5	–	–	–	–
	Leuc. mesenterioides FK:L1	–	–	–	–
	Leuc. mesenterioides FK:L8	–	–	–	–
	Leuc. mesenterioides FK:L11	–	–	–	–
	E. faecium FK:L13	–	–	–	–
	Lb. carnis FK:L4	–	–	–	–
	Lb. carnis FK:L10	–	–	–	–
	E. faecium FK:L14	–	–	–	–
	E. faecium FK:L15	–	–	–	–
	E. faecium FK:L16	–	–	–	–

	Lb. carnis FK:L17	–	–	–	–
	Leuc. mesenterioides FK:L3	–	–	–	–

Continued (Table 35)

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
Faak kargyong	*Leuc. mesenterioides* FK:L2	–	–	–	–
	Leuc. mesenterioides FK:L14	–	–	–	–
	Leuc. mesenterioides FK:L25	–	–	–	–
	Leuc. mesenterioides FK:L6	–	–	–	–
	Lb. plantarum FK:L7	–	–	–	–
	Lb. plantarum FK:L15	–	–	–	–
Lang satchu	*P. pentosaceous* KS:L2	–	–	–	–
	P. pentosaceous KS:L6	–	–	–	–
	P. pentosaceous KS:L8	–	–	–	–
	P. pentosaceous KS:L10	–	–	–	–
	P. pentosaceous KS:L15	–	–	–	–
	Lb. casei SR:L4	–	–	–	–
	Lb. carnis SR:L8	–	–	–	–
	E. faecium KS:L1	–	–	–	–
	E. faecium KS:L18	–	–	–	–
	E. faecium KS:L26	–	–	–	–
	E. faecium SR:L7	–	–	–	–
	E. faecium SR:L6	–	–	–	–
	E. faecium TS:L9	–	–	–	–
	E. faecium TS:L23	–	–	–	–
	E. faecium TS:L20	–	–	–	–
	E. faecium TS:L7	–	–	–	–
	E. faecium TS:L6	–	–	–	–
	E. faecium KS:L22	–	–	–	–
	E. faecium KS:L17	–	–	–	–
Yak satchu	*P. pentosaceous* YS:L2	–	–	–	–
	P. pentosaceous YS:L1	–	–	–	–

	P. pentosaceous YS:L13	–	–	–	–
	P. pentosaceous YS:L7	–	–	–	–
	P. pentosaceous YS:L4	–	–	–	–
	P. pentosaceous YS:L3	–	–	–	–

Continued (Table 35)

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
Yak satchu	*P. pentosaceous* YS:L10	–	–	–	–
	P. pentosaceous YS:L8	–	–	–	–
	P. pentosaceous YS:L6	–	–	–	–
	P. pentosaceous YS:L18	–	–	–	–
	P. pentosaceous YS:L16	–	–	–	–
	P. pentosaceous YS:L5	–	–	–	–
	P. pentosaceous YS:L20	–	–	–	–
	P. pentosaceous YS:L9	–	–	–	–
Suka ko masu	*Lb. carnis* BS:L25	–	–	–	–
	Lb. carnis BS:L4	–	–	–	–
	Lb. plantarum BS:L18	–	–	–	–
	Lb. plantarum BS:L17	–	–	–	–
	Lb. plantarum BS:L9	–	–	–	–
	E. faecium BS:L1	–	–	–	–
	E. faecium BS:L2	–	–	–	–
	E. faecium BS:L5	–	–	–	–
	E. faecium BS:L7	–	–	–	–
	E. faecium BS:L11	–	–	–	–
	E. faecium BS:L16	–	–	–	–
	E. faecium BS:L19	–	–	–	–
	E. faecium BS:L21	–	–	–	–
	E. faecium BS:L23	–	–	–	–
	E. faecium BS:L13	–	–	–	–

Tyr = Tyrosine, tyramine precursor; Lys = Lysine, cadaverine precursor; His = Histidine, histamine precursor; Orn = Ornithine, putrescine precursor

Table 36. Screening of biogenic amine producing LAB strains from meat products of the Kumaun Himalaya

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
Chartayshya	*Lb. divergens* CD:L3	–	–	–	–
	Lb. divergens CD:L21	–	–	–	–
	Lb. divergens CD:L6	–	–	–	–
	E. faecium CD:L5	–	–	–	–
	E. faecium CD:L2	–	–	–	–
	E. faecium CD:L22	–	–	–	–
	E. faecium CD:L14	–	–	–	–
	E. faecium CD:L7	–	–	–	–
	E. faecium CD:L23	–	–	–	–
	P. pentosaceous CD:L13	–	–	–	–
	P. pentosaceous CD:L15	–	–	–	–
	P. pentosaceous CD:L1	–	–	–	–
	P. pentosaceous CD:L8	–	–	–	–
	P. pentosaceous CD:L10	–	–	–	–
	P. pentosaceous CD:L20	–	–	–	–
	P. pentosaceous CD:L26	–	–	–	–
Jamma	*Leuc. mesenterioides* KJ:L2	–	–	–	–
	Leuc. mesenterioides KJ:L14	–	–	–	–
	Leuc. mesenterioides KJ:L18	–	–	–	–
	Leuc. mesenterioides KJ:L21	–	–	–	–
	Leuc. mesenterioides KJ:L23	–	–	–	–
	Leuc. mesenterioides KJ:L25	–	–	–	–
	Leuc. mesenterioides KJ:L29	–	–	–	–
	P. pentosaceous KJ:L10	–	–	–	–
	P. pentosaceous KJ:L13	–	–	–	–
	P. pentosaceous KJ:L31	–	–	–	–
	P. pentosaceous KJ:L3	–	–	–	–

Continued (Table 36)

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
Jamma	*P. pentosaceous* KJ:L16	–	–	–	–
	E cecorum KJ:L9	–	–	–	–
	E. faecium KJ:L4	–	–	–	–
	E. faecium KJ:L5	–	–	–	–
	Lb. sanfrancisco KJ:L1	–	–	–	–
	Lb. sanfrancisco KJ:L15	–	–	–	–
	Lb. divergens KJ:L8	–	–	–	–
	Lb. divergens KJ:L6	–	–	–	–
	Lb. divergens KJ:L7	–	–	–	–
Arjia	*P. pentosaceous* KA:L1	–	–	–	–
	P. pentosaceous KA:L2	–	–	–	–
	P. pentosaceous KA:L4	–	–	–	–
	P. pentosaceous KA:L11	–	–	–	–
	P. pentosaceous KA:L17	–	–	–	–
	P. pentosaceous KA:L21	–	–	–	–
	E. faecium KA:L3	–	–	–	–
	E. faecium KA:L5	–	–	–	–
	E. faecium KA:L8	–	–	–	–
	E. faecium KA:L16	–	–	–	–
	E. faecium KA:L20	–	–	–	–
	E. faecium KA:L13	–	–	–	–
	E. faecium KA:L15	–	–	–	–

Tyr = Tyrosine, tyramine precursor; Lys = Lysine, cadaverine precursor; His = Histidine, histamine precursor; Orn = Ornithine, putrescine precursor.

Hydrophobicity of the LAB strains

Tables 37 and 38 show the percentage hydrophobicity of the LAB strains. Out of 108 strains tested for the percentage of hydrophobicity of the Sikkim Himalaya, 95 strains showed less than 30 % hydrophobicity, 11 strains showed 30-70 %, and only 2 strains had more than 70 % hydrophobicity. *Lb. brevis* FK:L5 isolated from *faak kargyong* showed 71.7 % of hydrophobicity and *Lb. plantarum* isolated from *yak kargyong* showed 70.3 % of hydrophobicity. Similarly, the percentage of hydrophobicity from meat products of the Kumaun Himalaya was also tested, none of the strains were found to be hydrophobic.

Table 37. Percentage hydrophobicity of LAB strains from meat products of the Sikkim Himalaya

Product	Strain	% Hydrophobicity
Lang kargyong	*Lb. sake* KP:L3	50. ± 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
	Lb. divergens ZK:L5	2.3 ± 0.40
	Lb. divergens KM:L32	2.6 ± 0.09
	Lb. carnis KM:L31	2.0 ± 0.84
	Lb. carnis ZK:L7	3.4 ± 5.31
	Lb. sanfransisco KM:L1	5.2 ±6.20
	Lb. sanfransisco KM:L10	6.7 ± 4.30
	Lb. sanfransisco LK:L2	2.2 ± 1.00
	Lb. sanfransisco KP:L5	4.2 ± 1.72
	Lb. sanfransisco KP:L14	4.2 ± 2.1
	Lb. sanfransisco ZK:L4	4.5 ± 2.5
	E. faecium KM:L19	7.0 ± 1.39
	E. faecium KM:L34	6.1 ± 1.39
	E. faecium KM:L35	6.8 ± 1.90
	Leuc. mesenterioides KP:L7	3.1 ± 0.43
	Leuc. mesenterioides KP:L11	1.9 ± 0.37
	Leuc. mesenterioides KP:L18	7.7 ± 2.81
	Leuc.mesenterioides LK:L4	4.5 ± 2.42
	E. faecium ZK:L1	6. 3 ± 2.23

Continued (Table 37)

Product	Strain	% Hydrophobicity
Lang kargyong	*E. faecium* ZK:L3	3.2 ± 1.91
	E. faecium ZK:L6	5.3 ± 1.84
Yak kargyong	*Lb. plantarum* YKK:L2	70.3 ± 1.03
	Lb. casei YKK:L3	8.2 ± 2.1
	*Lb. curvatus*YKK:L4	4.8 ± 0.9
	Lb. casei YKK:L8	3.5 ± 1.20
	Lb. plantarum YKK:L11	62.8 ± 1.25
	Lb. plantarum YKg:L3	36.4 ± 1.22
	Lb. sake YKg:L7	22.7 ± 9.81
	Lb. sake YKg:L8	42.5 ± 1.09
	Lb. brevis YK:L11	61.9 ± 0.54
	Lb. carnis YKK:L1	3.0 ± 1.08
	Lb. carnis YKK:L5	7.5 ± 1.23
	Lb. sake YKg:L9	46.5 ± 1.61
	Lb. sanfrancisco YK:L1	2.8 ± 0.38
	Lb. sanfrancisco YK:L3	5.6 ± 1.12
	E. faecium YK:L2	4.3 ± 1.13
	E. faecium YK:L15	2.5 ± 1.4
	Leuc. mesenterioides YK:L5	6.2 ± 1.2
	Leuc. mesenterioides YK:L9	2.6 ± 0.89
Faak kargyong	*Lb. brevis* FK:L5	71.7 ± 1.48
	Leuc. mesenterioides FK:L1	2.7 ± 1.45
	Leuc. mesenterioides FK:L8	3.1 ± 1.75
	Leuc. mesenterioides FK:L11	2.1 ± 1.52
	E. faecium FK:L13	2.5 ± 0.29
	Lb. carnis FK:L4	0.5 ± 0.08
	Lb. carnis FK:L10	1.8 ± 0.23
	E. faecium FK:L14	1.3 ± 0.16

	E. faecium FK:L15	2.3 ± 0.34

Continued (Table 37)

Product	Strain	% Hydrophobicity
Faak kargyong	*E. faecium* FK:L16	1.1 ± 0.76
	Lb. carnis FK:L17	1.6 ± 0.26
	Leuc. mesenterioides FK:L3	3.3 ± 0.68
	Leuc. mesenterioides FK:L2	3.2 ± 0.34
	Leuc. mesenterioides FK:L14	1.5 ± 0.22
	Leuc. mesenterioides FK:L25	8.9 ± 4.38
	Leuc. mesenterioides FK:L6	2.6 ± 1.03
	Lb. plantarum FK:L7	25.5 ± 140
	Lb. plantarum FK:L15	30.9 ± 1.7
Lang satchu	*P. pentosaceous* KS:L2	4.1 ± 0.82
	P. pentosaceous KS:L6	2.1 ± 062
	P. pentosaceous KS:L8	3.1 ± 151
	P. pentosaceous KS:L10	5.3 ± 1.45
	P. pentosaceous KS:L15	3.7 ± 149
	Lb. casei SR:L4	6.5 ± 0.46
	Lb. carnis SR:L8	2.4 ± 2.12
	E. faecium KS:L1	6.6 ± 1.26
	E. faecium KS:L18	3.0 ± 0.09
	E. faecium KS:L26	1.2 ± 0.24
	E. faecium SR:L7	2.1 ± 0.23
	E. faecium SR:L6	4.0 ± 1.52
	E. faecium TS:L9	3.3 ± 1.29
	E. faecium TS:L23	2.2 ± 0.89
	E. faecium TS:L20	2.1 ± 0.23
	E. faecium TS:L7	1.9 ± 0.34
	E. faecium TS:L6	4.0 ± 0.18
	E. faecium KS:L22	2.1 ± 0.25
	E. faecium KS:L17	3.0 ± 0.03

Continued (Table 37)

Product	Strain	% Hydrophobicity
Yak satchu	*P. pentosaceous* YS:L2	1.1 ± 0.87
	P. pentosaceous YS:L1	1.6 ± 1.88
	P. pentosaceous YS:L13	2.3 ± 0.86
	P. pentosaceous YS:L7	1.3 ± 0.28
	P. pentosaceous YS:L4	4.5 ± 3.05
	P. pentosaceous YS:L3	2.6 ± 1.28
	P. pentosaceous YS:L10	2.9 ± 1.02
	P. pentosaceous YS:L8	1.4 ± 0.30
	P. pentosaceous YS:L6	1.3 ± 0.27
	P. pentosaceous YS:L18	2.2 ± 0.56
	P. pentosaceous YS:L16	2.2 ± 1.69
	P. pentosaceous YS:L5	1.6 ± 0.87
	P. pentosaceous YS:L20	1.5 ± 0.45
	P. pentosaceous YS2:L9	1.2 ± 0.42
Suka ko masu	*Lb. carnis* BS:L25	1.3 ± 0.45
	Lb. carnis BS:L4	1.8 ± 0.12
	Lb. plantarum BS:L18	2.5 ± 1.22
	Lb. plantarum BS:L17	23.1 ± 1.04
	Lb. plantarum BS:L9	32.2 ± 0.86
	E. faecium BS:L1	1.2 ± 0.52
	E. faecium BS:L2	1.2 ± 0.33
	E. faecium BS:L5	1.5 ± 0.66
	E. faecium BS:L7	2.0 ± 0.45
	E. faecium BS:L11	5.1 ± 0.23
	E. faecium BS:L16	6.9 ± 0.34
	E. faecium BS:L19	2.6 ± 0.18
	E. faecium BS:L21	3.1 ± 0.25
	E. faecium BS:L23	3.0 ± 3.03

	E. faecium BS:L13	2.1 ± 2.13

Data represents the means (± SD) of 4 sets of experiment.

Table 38. Percentage hydrophobicity of LAB strains from meat products of the Kumaun Himalaya

Product	Strain	% Hydrophobicity
Chartayshya	*Lb. divergens* CD:L3	7.1 ± 2.4
	Lb. divergens CD:L21	1.6 ± 0.45
	Lb. divergens CD:L6	2.5 ± 0.52
	E. faecium CD:L5	10.1 ± 1.6
	E. faecium CD:L2	3.1 ± 0.34
	E. faecium CD:L22	2.0 ± 0.33
	E. faecium CD:L14	2.2 ± 0.24
	E. faecium CD:L7	9.1 ± 0.34
	E. faecium CD:L23	4.3 ± 0.58
	P. pentosaceous CD:L13	1.8 ± 0.7
	P. pentosaceous CD:L15	1.3 ± 0.34
	P. pentosaceous CD:L1	1.2 ± 0.71
	P. pentosaceous CD:L8	3.1 ± 0.32
	P. pentosaceous CD:L10	2.5 ± 0.33
	P. pentosaceous CD:L20	3.7 ± 1.80
	P. pentosaceous CD:L26	3.4 ± 0.34
Jamma	*Lb. sanfrancisco* KJ:L1	3.5 ± 1.80
	Lb. sanfrancisco KJ:L15	2.2 ± 1.07
	Lb. divergens KJ:L8	2.1 ± 1.21
	Lb. divergens KJ:L6	7.0 ± 3.70
	Lb. divergens KJ:L7	2.3 ± 1.27

Continued (Table 38

Product	Strain	% Hydrophobicity
	Leuc. mesenterioides KJ:L2	1.3 ± 0.27
	Leuc. mesenterioides KJ:L14	1.4 ± 1.03
	Leuc. mesenterioides KJ:L18	1.2 ± 0.11
	Leuc. mesenterioides KJ:L21	1.3 ± 0.13
	Leuc. mesenterioides KJ:L23	1.2 ± 0.22
	Leuc. mesenterioides KJ:L25	2.0 ± 0.67
	Leuc. mesenterioides KJ:L29	3.0 ± 1.13
Jamma	*P. pentosaceous* KJ:L10	3.4 ± 1.01
	P. pentosaceous KJ:L13	1.1 ± 0.25
	P. pentosaceous KJ:L31	1.6 ± 1.03
	P. pentosaceous KJ:L3	2.2 ± 0.39
	P. pentosaceous KJ:L16	2.8 ± 0.18
	E cecorum KJ:L9	5.7 ± 1.94
	E. faecium KJ:L4	4.0 ± 1.1
	E. faecium KJ:L5	3.4 ± 0.16
	P. pentosaceous KA:L1	2.3 ± 0.34
	P. pentosaceous KA:L2	3.2 ± 0.36
	P. pentosaceous KA:L4	8.3 ± 0.78
Arjia	*E. faecium* KA:L8	0.9 ± 0.04
	E. faecium KA:L16	2.9 ± 2.10
	E. faecium KA:L20	2.0 ± 0.21
	E. faecium KA:L13	1.2 ± 0.22
	E. faecium KA:L15	1.4 ± 0.21

Continued (Table 38)

Product	Strain	% Hydrophobicity
Arjia	*P. pentosaceous* KA:L11	1.1 ± 0.96
	P. pentosaceous KA:L17	3.9 ± 0.82
	P. pentosaceous KA:L21	2.5 ± 1.06
	E. faecium KA:L3	2.7 ± 1.32
	E. faecium KA:L5	4.2 ± 0.72

Data represents the means (± SD) of 4 sets of experiment.

Proximate composition

The proximate composition of traditionally prepared meat products collected from different places of the Sikkim and the Kumaun Himalayas were analyzed (Tables 39 and 40). The pH of all these products was in between 5.3 and 6.9 with the titrable acidity ranging from 0.3-2.6 %. In all samples analysed, food value ranged from 400.0-634.5 kcal / 100g dry matter.

Table 39. Proximate composition of meat products collected from different places in the Sikkim Himalaya

Parameter	Product					
	Lang kargyong[1] (n = 17)	*Yak kargyong*[2] (n = 7)	*Faak kargyong*[3] (n = 8)	*Lang satchu*[4] (n = 9)	*Yak satchu*[5] (n = 6)	*Suka ko masu*[6] (n = 6)
pH	6.7 ± 0.3	6.9 ± 0.2	6.5 ± 0.3	6.1 ± 0.2	5.7 ± 0.2	5.3 ± 0.3
Titratable acidity % (as lactic acid)	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	2.6 ± 0.2	2.5 ± 0.2	1.1 ± 0.2
Moisture %	59.8 ± 0.8	21.9 ± 0.7	41.0 ± 1.0	22.8 ± 0.7	23.7 ± 1.5	23.2 ± 0.3
Ash (% DM)	3.8 ± 0.6	2.8 ± 0.4	2.8 ± 0.3	5.4 ± 0.6	7.3 ± 0.4	1.8 ± 0.4
Fat (% DM)	10.3 ± 1.1	49.1 ± 0.7	27.1 ± 0.8	5.9 ± 0.6	4.7 ± 0.5	2.0 ± 0.1
Protein (% DM)	8.4 ± 1.2	16.0 ± 0.8	11.5 ± 0.8	57.7 ± 1.2	51.0 ± 1.6	44.8 ± 0.8
Carbo-hydrate (% DM)	77.5 ± 2.7	32.0 ± 1.7	58.6 ± 2.1	31.0 ± 2.3	37.0 ± 2.4	51.4 ± 1.5
Food value (Kcal /100g DM)	436.2 ± 3.2	634.5 ± 2.3	501.4 ± 4.8	407.7 ± 2.0	405.8 ± 0.9	403.1 ± 0.5

n, total number of samples (n) collected from each source is given in parenthesis.

DM, dry matter.

Data represents the means (± SD) of triplicate of each sample.

[1]Samples collected from Mangan (3), Pangthang (3), Sankalan (2), Zuluk (3), Lingtam (2), Rongli (2) and Pakshyak (2).

[2]Samples collected from Gnathang (2), Kupup (2) and Lachen (3).

[3]Samples collected from 4th mile (2), Tashiding (2), Mangan (2) and Ranka (2).

[4]Samples collected from Ranka (2), Kewzing(3), Rinchenpong (2) and Tadong (2).

[5]Samples collected from Gnathang (2), kupup (2) and Lachen (2).

[6]Samples collected from Namchi (2), Rongli (2) and Tadong (2).

Table 40. Proximate composition of meat products collected from different places in the Kumaun Himalaya

Parameter	Product		
	Chartayshya[1] (n = 6)	*Jamma*[2] (n = 6)	*Arjia*[3] (n = 4)
pH	6.5 ± 0.1	5.5 ± 0.2	6.3 ± 0.1
Titratable acidity % (as lactic acid)	2.1 ± 0.1	1.5 ± 0.1	1.1 ± 0.1
Moisture %	17.4 ± 0.2	65.1 ± 0.6	60.2 ± 0.2
Ash (% DM)	7.8 ± 1.2	5.2 ± 0.5	3.5 ± 0.4
Fat (% DM)	17.0 ± 0.2	4.2 ± 0.5	5.5 ± 0.4
Protein (% DM)	36.6 ± 3.0	7.8 ± 1.0	6.4 ± 0.9
Carbo-hydrate (% DM)	38.6 ± 4.3	82.8 ± 2.0	84.6 ± 1.7

Food value (Kcal /100g DM)	454.0 ± 5.9	400.0 ± 0.7	413.5 ± 1.2

n, total number of samples (n) collected from each source is given in parenthesis.

DM, dry matter.

Data represents the means (± SD) of triplicate of each sample.

[1]Samples collected from Pangu (2), Rumjum (2) and Marchal (2).

[2]Samples collected from Dharchula (2), Sosa (2) and Rumjum (2).

[3]Samples collected from Dharchula (2) and Rumjum (2)

Table 28. Profile of microorganisms isolated in meat products of the Sikkim Himalaya

Product	Microorganisms		
	Bacteria	Yeast	Moulds
Lang kargyong	*Lb. sake, Leuc. mesenterioides, Lb. divergens, Lb. carnis, Lb. sanfransisc,o E. faecium, Lb. curvatus, B.subtilis, B.mycoides, B.thuringiensis, S.aureus, Micrococcus*	*D. hansenii, P. anomala*	2.1 ± 0.8
Yak kargyong	*Leuc. mesenterioides, Lb. casei, Lb. plantarum, Lb. sake, Lb. divergens, Lb. carnis, Lb. sanfrancisco, E. faecium, Lb. curvatus, B.subtilis, B.mycoides, S.aureus, Micrococcus*	*D.pseudopolymorphus*	2.4 ± 0.1
Faak kargyong	*Lb. brevis, Leuc. mesenterioides, E. faecium, Lb. carnis, Lb. plantarum, B.subtilis, B.mycoides,*	*C. famata, D. polymorphus*	<DL

	B.licheniformis, S.aureus, Micrococcus		

Continiud (Table 28)

Lang satchu	*P. pentosaceous, Lb. casei, Lb. carnis, E. faecium, B.subtilis, B.mycoides, B. lentus, S.aureus, Micrococcus*	*D. hansenii, P anomala*	1.0 ± 0.4
Yak satchu	*P. pentosaceous, B.subtilis, B.mycoides, B. licheniformis, S.aureus, Micrococcus*	*D. polymorphus*	<DL
Suka ko masu	*Lb. carnis, E. faecium, Lb. plantarum, B.subtilis, B.mycoides, B.thuringiensis, S.aureus, Micrococcus*	*D. hansenii, P. burtonii*	2.0 ± 0.1

DL, Less than detection limit (10 cfu / g).

Table 29. Profile of microorganisms isolated in meat products of the Kumaun Himalaya

Product	Microorganisms		
	Bacteria	Yeast	Moulds
Chartayshya	*Lb. divergen,s E. faecium, P. pentosaceous, B.subtilis, B.mycoides, B.thuringiensis, S.aureus, Micrococcus*	*D. hansenii, C. famata*	2.4 ± 0.1
Jamma	*Leuc. mesenterioides, P. pentosaceous, E cecorum, Lb. sanfrancisco, E. faecium, Lb. ivergens, B. subtilis, B. sphaericus, S.aureus, Micrococcus*	*D. hansenii, C. albicans*	3.4 ± 0.1
Arjia	*E. faecium P. pentosaceous, B.subtilis, B.mycoides, B.thuringiensis, S.aureus, Micrococcus*	*D. hansenii, C. humicola*	<DL

DL, Less than detection limit (10 cfu / g).

Documentation of traditional knowledge

The diverse ethnic groups of the Himalayan people of Sikkim and Kumaun prepare and consume a variety of traditionally processed smoked/sun-dried/air-dried/fermented meat products for centuries. They use their indigenous knowledge of preservation of perishable meats without using starter culture and chemicals. Information were sought directly from the local people of the respective places on the types of indigenous meat products, they prepare and consume, their traditional method of preparation, culinary skills and socio-economy of the products. About 88.3 % of the people of the Sikkim Himalaya are non-vegetarians (Tamang *et al.,* 2007). The Bhutias and the Lepchas usually prefer beef, yak and pork. Beef is taboo to majority of Nepalis except the Tamang and Sherpa castes. The Newar caste of Nepalis prefers to eat buffalo meat. Regular consumption of meat is expensive for majority of poor rural people. They slaughter domestic animals usually on special occasions, like festival and marriages. After the ceremony, the fresh meat is cooked and eaten. The remaining flesh of meat is preserved by smoking or drying to make *suka ko masu* or dried meat for future consumption. The Bhutia and the Lepcha of North Sikkim, slaughter yaks occasionally, consume the fresh meat and the rest are preserved by smoking or drying or by fermenting. Traditional sausage like meat product such as *lang kargyong,*

yak kargyong and *faak kargyong* are also made with the leftover parts of the animal. *Kargyong* consists of ground meat, fat, salt, spices, sometimes blood and other ingredients such as herbs. These ingredients are then stuffed into the animal intestine with both ends tied up and are cooked. This product can be kept for future consumption.

The indigenous skills of the Himalayan people in preservation methods of raw meat can be justified by making sausage-like products using unappealing animal parts such as scraps, organ meats, fat, blood etc. Hence, *kargyong* making may be first concerned as a use for leftovers of meat. Some of the common as well as lesser-known traditionally processed meat products of the Sikkim Himalaya are *suka ko masu* (beef, chevon, buff), *kargyong* (beef, pork, yak), *satchu* (yak, beef), *kheuri* (yak, beef), *chilu* (yak, beef) etc. *Kheuri* is a typical type of meat product of the Sikkim Himalaya confined to Bhutias of North Sikkim. *Chilu* is another type of traditionally prepared meat product. It is stored animal fat inside the stomach of sheep which is stitched and pressed by heavy loads. It is used as a substitute of edible oil. The ethnic people use the stomach of sheep (locally called *Vyangloong*) in case of preparation of both *kheuri* and *chilu* because the size of the stomach of yak is too large to fill with the minimum ingredients. Now a day, this traditional method of preparation of *chilu* and *kheuri* is vanishing due to the extinction of the sheep locally known as *Vyangloong* species and also on the ban order of Government of

its slaughtering. The indigenous knowledge of cutting of fresh meat into a strand-like and dried in an open air or in the wooden kitchen is a common practice among the ethnic people of the Sikkim Himalaya. Both the fried *kargyong* and fried *satchu* are popular side dish servwd with *rakshi*, distilled liquor or *chyang/kodo ko jaanr*, mild alcoholic finger-millet-based beverage in Sikkim (Tamang, 2005a).

There are three types of common meat products generally prepared by the Bhutias of the Kumaun region, known as *chartayshya, jamma and arjia*. *Jamma* and *arjia* are the similar traditionally prepared chevon meat product similar to *kargyong* of the Sikkim Himalaya but the raw material varies. *Chartayshya* is a dried meat product. People offer cooked *chartayshya* to worship the ancestral spirit and only after that, they serve in meal. Although chicken, duck, fish, pork, beef and eggs are popular meats served in the south-east Asian countries but the serving of meats in these countries are greatly influenced by religious dictates of the dominant religions (McWilliams, 2007). Ethnic people might have invented such preservation technique to feed themselves during the scarcity of food. Though antiquity of using meat products by the ethnic people of the Himalayas is yet to be studied; documentation on traditional processing, preservation of highly perishable raw meats provides a vital information for further studies on meat products. All nine

meat products are naturally cured without using starter cultures or addition of sodium nitrites/nitrates.

Microorganisms

The microbial population of traditionally prepared meat products collected from different places of the Sikkim and the Kumaun Himalayas revealed that lactic acid bacteria (LAB) comprising lactobacilli, pediococci, leuconostoc and enterococci were the dominant microorganisms present in a viable number above 10^8 cfu/g, followed by micrococcaceae and yeasts with the population not exceeding 10^7 cfu/g in both. Micrococcaceae, LAB, yeasts are the typical microflora in meat products with the involvement of micrococcaceae being the most important Vilar *et al.,* (2000). Taxonomically diverse species of LAB have been isolated from *lang kargyong, yak kargyong, faak kargyong, lang satchu, yak satchu, sheakua, chartayshya, jamma and arjia* samples of the Sikkim and the Kumaun Himalayas. The lactic acid bacteria represent four major genera-*Lactobacillus, Leuconostoc, Pediococcus* and *Enterococcus.* Classification of LAB into different genera is largely based on morphology, gas production from glucose (Kandler, 1983), mode of glucose fermentation, and growth at different temperatures (Mundt, 1986; Dykes, *et al.,* 1994). LAB are the excellent candidate for reducing pathogenic levels on foods because they inhibit the growth of pathogenic

bacteria through various mechanisms without causing unacceptable sensory changes (Kostrzynska, 2006).

On the basis of a combination of phenotypic properties and the API sugar profiles data, strains of LAB isolated from meat products of the Sikkim and the Kumaun Himalaya were identified as *Lb. sake, Lb. curvatus*, *Lb. divergens, Lb. carnis, Lb. sanfransisco, E. faecium, E. cecorum, Leuc. mesenterioides, Lb. plantarum, Lb. casei, Lb. brevis, P. pentosaceous.* Schillinger and Lücke (1986) reported that *Lb. divergens, Lb. carnis* as well as *Lb. sake, Lb. curvatus* are frequently found in vaccum packaged meat. *Leuc. mesenteroides, Pd. pentosaceous, Lb. brevis, E. faecium,* have also been isolated from meat product (Samelis *et al.,*1994). *Lactobacillus sake* isolated from *lang kargyong* and *yak kargyong* did not ferment maltose and lactose; this has also been observed by other workers (Schillinger and Lücke, 1987; Hastings and Holzapfel (1987a,b); Korkeala and Mäkeliä, 1989; Grant and Patterson, 1991). Enterococci play a beneficial role in production of many fermented foods (Bouton *et al.,* 1998; Cintas *et al.,* 2000). *E. faecium* appears to pose a low risk for use in foods, because these strains generally harbour fewer recognised virulence determinants than *E. faecalis* (Franz *et. al*., 2003). Santos *et al.* (1998) isolated *Pediococcus* sp. from a Spanish dry fermented sausage, *Chorizo.* Several species and strains of pediococci are used as starter cultures in fermentation of meat (Diebel *et al.*, 1961; Smith and Palumbo, 1983) and sausage products (Tagg *et al.,* 1976).

Osmanagaoglu *et al.* (2001) reported the occurrence of *Pediococcus pentosaceous* Pep 1 isolated from vacuum-packed sausage, which produce a potentially novel antimicrobial agent against several food spoilage and health hazards associated species of food origin. The 4 strains of LAB identified (*Lb. plantarum, P. pentosaceus, P. pentosaceus* and *P. pentosaceus*) would be useful as the starter and protective culture in the processing of the *sucuk* and similar fermented products (Yaman *et al.,* 1998).

A sizable number of yeasts were also recovered in meat products. Based on the detailed characterization and identification profiles, the following yeasts were isolated and identified as *Debaryomyces hansenii, D. polymorphus D. pseudopolymorphus, P. burtonii, P. anomala, Candida famata, C. albicans and C. humicola*. *Debaryomyces* spp. were the dominant among the yeast isolated from traditionally prepared meat products of the Himalayas. Although bacteria are considered to have the dominant role in meat fermentation, the contribution of yeasts nevertheless is significant (Romano *et al.,* 2006). A diversity of yeast species has been isolated from fermented sausages and cured *hams* produced in different countries with little exception (Martin *et al.,* 2006; Tamang and Fleet, 2008). The findings correlate with the work of Leistner and Bem (1970), Comi and Cantoni (1980) that the yeast of the genus *Debaryomyces* predominates on dry sausages. Simmoncini *et al.* (2007) have reported the occurrence of *D. hansenii, Candida famata* from Italian dry cured *ham*. *Debaryomyces hansenii*

is reported from a traditional South Italian processed sausages along with bacteria (Baruzzi *et al.,* 2006). Rossmanith *et al.* (1972) observed that the curing colour and flavour could be improved by addition of selected *Debaryomyces* strains to the sausage mixture. The prevalence of *D. hansenii* in all batches of Greek dry salami suggested that its potential use as a starter culture in Greek dry salami (Metaxopoulos *et al*., 1996). Coretti (1977) reported that the contamination of *D. hansenii*, lactobacilli and micrococci gave best result in the production of fermented sausages. The sausage inoculation of yeast strains resulted at the end of ripening, in more pronounced proteolysis and lipolysis (Patrignani *et al.,* 2007). Among micrococcaceae, about 91 % of the isolates were identified as *Staphylococcus* sp. and only 9 % were *Micrococcus* sp. in the 68 samples analysed, the findings proves that the dominance of *Staphylococcus* sp. over *Micrococcus* sp. *Staphylococcus aureus* was reported in several meat products (Sinell and Baumgart, 1966; Barber and Deibel, 1972; Kuschfeldt, 1980). There was the predominance of staphylococci over other micrococcaceae in almost all the data reported on the characterization of microbial flora of fermented meats (Montel *et al.,* 1992*;* Samelis *et al.,* 1998). The predominance of isolates of the *Staphylococcus* genus in comparison with those of *Micrococcus* genus appears as common phenomenon in the majority of studies on the characterization of the microbial flora in raw-cured meat products (Graham and Blumer, 1971;

Francisco *et al.,* 1981; Von Rheinbaben and Seipp, 1986; Molina *et al.,* 1989; Kotzekidou, 1992; Rodriguez *et al.,* 1994; Garcia *et al.,* 1995) and fermented sausages (Comi *et.al.,* 1992). Vilar *et al.* (2000) and Papamanoli *et al.* (2002) also reported the same result in dry fermented sausage. Micrococcaceae species are used to enrich fermentative microorganisms during ageing of the products in order to enhance the colour stability of the cured meat and prevent rancidity (Papamanoli *et al.,* 2002). The activities of the micrococcaceae group reduce spoilage, decreases processing time and contribute to flavour development (Montel *et al.,* 1998). Bacilli were also detected at the level of $<10^4$ cfu/g. in the samples analysed from the Sikkim and the Kumaun Himalaya. The results for the technological properties of *Bacillus* strains isolated from southern Italian sausage made without a selected starter, suggest that *Bacillus* strains, always present in meat curing could play a role in the development of texture and organoleptic characteristics of the sausages (Baruzzi *et al*., 2006). López-Díaz *et al.* (2001) reported the presence of moulds in meat products.

Isolation, enrichment, purification, characterization and proper identification and authentic nomenclature of microorganisms involved in fermented foods are important aspects of microbial systematic which also ensure the quality control and nomalised production of fermented foods (Tamang and Holzapfel, 1999). The isolated, identified and preserved

microorganisms from lesser-known meat products may contribute significant information on unknown microbial gene pool as genetic resources of the Himalayan region.

Occurrence of pathogenic bacteria

Meat is highly or extremely susceptible to microbial spoilage (Farnworth, 2003). Certain faults in the manufacture and storage of the meat products may lead to outbreaks of food-borne pathogen (Lücke, 2003). Fermented meat products usually do not undergo a physical treatment to eliminate pathogenic microorganisms, the meat has to be high quality with regard to hygienic and microbial counts and the control of pathogens is achieved by appropriate fermentation technology, including the use of starter cultures (Farnworth, 2003). Food-borne pathogens *Bacillus cereus, Salmonella, Shigiella, Listeria monocytogenes* were not detected in any of the samples analysed whereas enterobacteriaceae was found in the few samples at the level of $<10^4$ cfu/g. fermented meat and sausages are considered safe for consumption due to low pH and water activity which inhibit the growth of pathogenic bacteria (Ferreira *et al.,* 2006).

Technological or Functional properties

Technological or functional properties of LAB strains isolated from fermented foods are considered as one of the important criteria for selection of starter culture(s) to be used in the manufacture of functional foods (Durlu-Ozkaya, 2001; Badis, 2004). The use of the API-zym technique has been reported (Arora *et al*., 1990) as a rapid and simple means of evaluating and localising 19 different hydrolases of microorganisms associated with dairy fermentations. This method is also 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 other fermented products (Tamang *et al*., 2000). LAB strains isolated from traditional meat products showed very weak lipolytic activity. Weak lipolytic activity of LAB strains has been shown by Montel *et al.* (1998) during *nham* fermentation. Absence of proteinases (trypsin and chymotrypsin) and presence of strong peptidase (leucine-, valine-, and cystine-arylamidase) activities produced by the predominant LAB strains isolated from meat products of the Sikkim and the Kumaun Himalayas are possible traits of desirable quality for their use in production of typical flavour and aroma. Proteolytic events that take place during the processing of dry sausages result in an increase in small peptides and free amino acids, similar to that

which occurs during cheese ripening (Henriksen *et al.,* 1997). The composition and concentration of these compounds contribute to the overall flavor in cured meat products such as dry sausages (Henriksen *et al.,* 1997) and *ham* (Aristoy *et al.,* 1995; Toldrá *et al.,* 1997). In sausages, microbial peptidases have an important role in the hydrolysis of oligopeptides (Molly *et al.,* 1997). Exopeptidases from *Lb. sake* are responsible for the generation of free amino acids and contribute to flavour improvement of dry fermented sausage (Demeyer *et al.,* 2000). Most of the studies on proteases of meat microorganisms have been carried out with lactobacilli (Fadda *et al.,* 1999) and, especially, with *Lactobacillus sakei* (Sanz and Toldra, 2001, 2002). Herreros *et al*. (2003) reported the highest aminopeptidase activity in *Lb. plantarum, Lb. casei, Leuc. mesenteroides* sub.sp. *dextranicum* isolated from armada cheese. Some of the strains isolated from meat product exhibit high activity of β-galactosidase.

All the strains of LAB isolated from meat products except few showed strong antimicrobial activities against a number of potentially pathogenic Gram-positive and Gram-negative bacteria in the method applied. However, the cell free supernatant fluid extracts of LAB strains isolated from meat products could not produce bacteriocin under the applied condition. Lactic acid bacteria compete with other

microorganisms by screening antagonistic compounds and modifying the micro-environment by their metabolism (Lindgren and Dobrogosz, 1990; Tagg, 1992). Production of bacteriocin depends on a number of intrinsic and extrinsic factors including redox potential, water activity, pH and temperature (Yang and Ray, 1994; Delgado *et al*., 2005). It could be speculated that the antimicrobial activities showed by the strains isolated from the traditional meat products may be due to the other antimicrobials such as organic acids, hydrogen perooxide etc. Ammor *et al.* (2006) explained that *Lactobacillus sake* isolate displayed an additional inhibitory effect by hydrogen peroxide against *Listeria innocua.* Jofre *et al.* (2008) studied the addition of enterocins A and B to raw-sausages can reduce the population of *Listeria monocytogenes, Staphylococcus aureus, and Salmonella* sp.

A total of 68 samples collected and analysed from the Sikkim and the Kumaun Himalaya, none of the strains were found to produce biogenic amines. Biogenic amines are the organic bases with aliphatic, aromatic or heterocyclic structures that can be found in several foods, in which they are mainly produced by microbial decarboxylation of amino acids, with the exception of physiological polyamines (Silla Santos, 1996). 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).

These are organic basic compounds which occur in different kinds of foods such as dry sausages, fishery products, cheese, wine, beer, and other fermented foods (ten Brink *et al*., 1990; Halász *et al*., 1994). The inability of most strains of LAB to produce biogenic amines in tested meat products analysed is a good indication of their acceptability and their potential for the possible development as starter culture. The production of biogenic amines by LAB to be selected as starter cultures is not a desirable property (Buchenhüskes, 1993; Holzapfel, 1997). Samples with moderate, high or very high levels of biogenic amines could be considered as products of less quality and their consumption could be unhealthy for sensitive individuals (Latorre-Moratalla *et. al.,* 2007).

Bacterial adherence to hydrocarbons such as hexadecane, proved to be a simple and rapid method to determine cell surface hydrophobicity (Rosenberg *et. al*., 1980; van Loosdrecht *et al*., 1987; Ding and Lämmler, 1992; Vinderola *et al*., 2004). Only two strains, *Lb. brevis* and *Lb. plantarum* isolated from *faak kargyong* and *yak kargyong* respectively had more than 70 % hydrophobicity indicating their hydrophobic nature. 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 culture (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 (Bernet *et al*., 1993; Salminen *et al*., 1996). The behaviour of LAB could be dependent on interfacial processes and thus on cell surfaces physicochemical properties and composition (Gatti *et al*., 1997; Boonaert and Rouxhet, 2000; Gómez-Zavaglia *et al*., 2002).

Proximate composition

The proximate composition of meat products collected from the different places of the Sikkim and the Kumaun Himalayas were analysed (Tables 39 and 40). The pH values ranged from 5.3-6.9. The pH of all the products was slightly acidic in nature due to the predominance of the LAB flora. Anifantaki *et al.* (2002) reported the pH values of *frankfurters* ranged from 6.3-6.6 and in some cases the value was 5.7. Due to the production of lactic acid, the final pH of cooked meat products reached pH values of 5.0-5.3 (Gardner, 1983). Sun/smoke drying of most of the products, such as *yak kargyong, faak kargyong, lang satchu, yak satchu, Sheakua* and *Chartayshya* as a result of dehydration, have < 41 % of the moisture content. This data corresponds with the work reported by Garcia Fontan *et al*. (2007) on Spanish traditional pork sausage *androlla.* Due to the low moisture content and slightly acidic nature of some of the

meat products like *Kargyong, satchu, sheakua, chartayshya* etc., can be kept for longer period and are safe for consumption.

Conclusion

Meat is the flesh (muscle tissue) of warm blooded animal and it is highly susceptible to microbial spoilage. Drying or smoking or fermentation of perishable meat is a remarkable step in the traditional meat processing as bipreservation in the Himalayas. These meat products are preserved for several months without refrigeration and can be consumed at anytime.

Species of bacteria associated with nine meat products (*lang kargyong, yak kargyong, faak kargyong, lang satchu, yak satchu, sheakua, chartayshya, jamma* and *arjia*) of the Sikkim and the Kumaun Himalayas were Lactic acid bacteria - *Lactobacillus sake, Lb. curvatus, Lb. plantarum, Lb. brevis, Lb. casei, Lb. divergens, Lb. carnis, Lb. sanfrancisco, Enterococcus faecium, Leuconostoc mesenteroides, Pediococcus pentosaceous*; Bacilli – *Bacillus subtilis, B. mycoides, B. thuringiensis, B. sphaericus, B. licheniformis* and *B. lentus*; Micrococcaceae – staphylococci and micrococci. Yeasts isolated from nine meat products were *Debaryomyces hansenii, D. polymorphus, D. pseudopolymorphus, Candida albicans, C. humicola, C. famata, Pichia burtonii* and *P. anamola*. The dominant microflora in traditionally prepared meat products were the lactic acid bacteria followed by yeasts, micrococcaceae and bacilli. This study showed that strains of LAB and yeast play

important and partly complex role in this traditional fermentation process by their technological or functional properties related to a specific and partly a wide enzyme spectrum, their acidifying capacity and antimicrobial activities of LAB, non-production of biogenic amines and some of the strains showed presumptive probiotic properties. Due to possession of superior functional properties, some of the lactic acid bacteria isolated from traditional meat products can be used as starter culture(s) for controlled and optimized production of meat products typical of the Sikkim and the Kumaun Himalayas.

The major objective of this thesis was to document indigenous knowledge of ethnic people of the Central (Kumaun) and the Sikkim Himalayas on meat processing and to access the microbiological profiles and proximate composition of various traditional meat products. This thesis also emphasised to study some functional properties of lactic acid bacteria. During the first phase, an extensive field survey was conducted in different regions of Sikkim and Kumaun Himalayas. Based on personal observation and interviews with the producers, eleven types of indigenous meat products (*lang kargyong, yak kargyong, faak kargyong, lang satchu, yak satchu* and *suka ko masu*) from the Sikkim Himalaya and three

major types of meat products (*chartayshya, jamma and arjia*) from Uttarakhand were documented.

A total of 68 samples (52 from the Sikkim Himalaya and 16 from the Kumaun Himalaya) of various types of meat products were analyzed for the microbiological profile. In all traditionally prepared meat products, the population of lactic acid bacteria (LAB) was 10^5 cfu/g to 10^8 cfu/g; yeasts was 10^3 to 10^7 cfu/g; bacilli was 10 to 10^4 cfu/g; micrococcaceae was 10^5 to 10^7 cfu/g; and total viable count was 10^5 to 10^9 cfu/g. Filamentous moulds were also detected in few samples of the meat products at the level of less than 10^4 cfu/g. The following bacteria were isolated and identified from the traditional meat products: *Lactobacillus sake, Lb. curvatus, Lb. plantarum Lb. brevis, Lb. casei, Lb. divergens Lb. carnis, Lb. sanfrancisco, Enterococcus faecium, Leuconostoc mesenteroides, Pediococcus pentosaceous, Bacillus subtilis, B. mycoides, B. thuringiensis, B. licheniformis, B. lentus, B. sphaericus, Staphylococcus aureus* and *Micrococcus* sp. The following yeast strains were recovered: *Debaryomyces hansenii, Debaryomyces pseudopolymorphus, D. polymorphus, Pichia anomala, P. burtonii, Candida albicans, C. famata* and *C. humicola*.

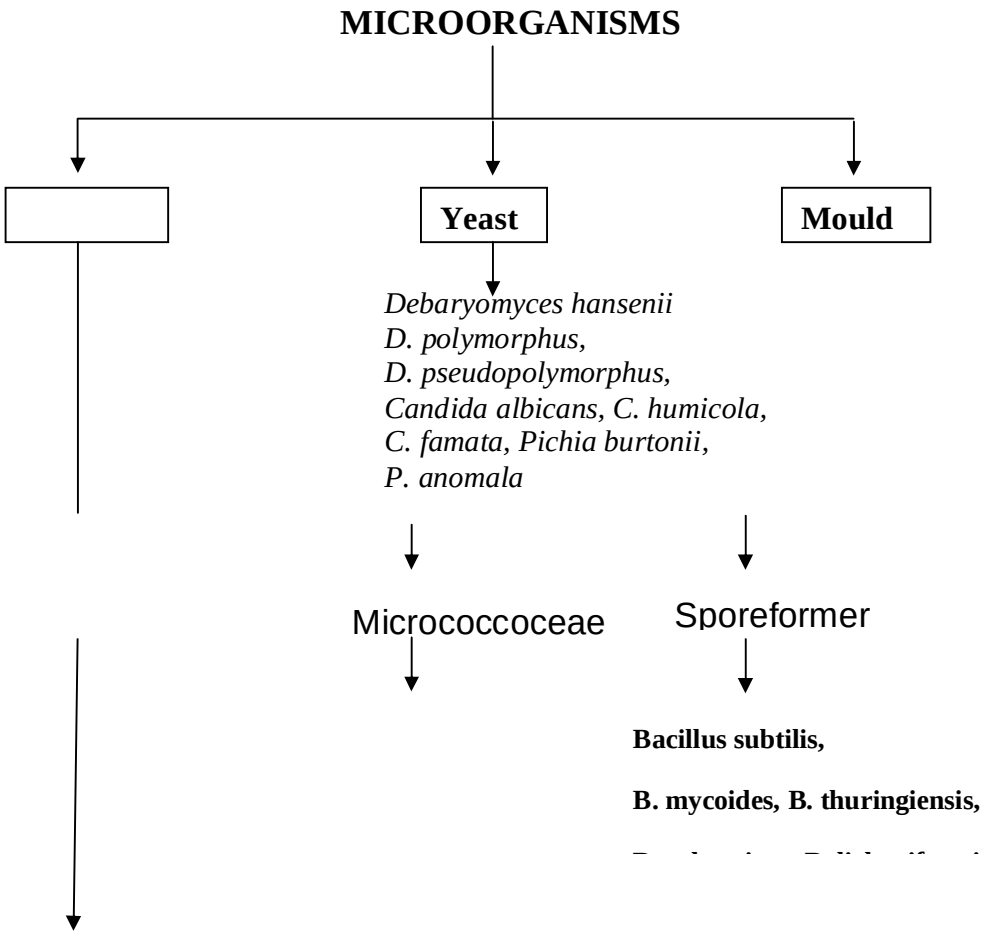
Enzymatic profiles of LAB strains were assayed using the API-zym (bioMérieux, France) galleries. Absence of proteinases (trypsin and chymotrypsin) and presence of strong peptidase (leucine-, valine-, and cystine-arylamidase) activities produced by the predominant LAB strains

isolated from meat products of the regions are possible traits of desirable quality for their use in production of typical flavour and aroma. All the strains of LAB isolated from meat products except few showed strong antimicrobial activities against a number of potentially pathogenic Gram-positive and Gram-negative bacteria in the method applied. However, the cell free supernatant fluid extracts of LAB strains isolated from meat products could not produce bacteriocin under the applied condition. None of the strains were found to produce biogenic amines from the traditional meat products analysed. Bacterial adherence to hydrocarbons such as hexadecane, proved to be a simple and rapid method to determine cell surface hydrophobicity. Only two strains, *Lb. brevis* and *Lb. plantarum* isolated from *faak kargyong* and *yak kargyong*, respectively had more than 70 % hydrophobicity indicating their hydrophobic nature. The proximate composition of meat products collected from the different places showed the nutritional value essential for local people in their diet.

The experimental data showed that LAB isolated from traditional meat products had remarkable functional properties such as diverse enzymatic activities, antimicrobial activities, non-production of biogenic amines and even probiotic properties. Traditional meat processing prolongs the self life of highly perishable raw meats without refrigeration. The general impression among the meat consumers that sausages are prepared by the Europeans is absolute due to first report on production of

various ethnic sausages like *kargyong, satchu, chartayshya, arjia* etc. in the Himalaya. The difference is the ethnic sausages are unnoticed, confined to particular community. This thesis would explore the possibility of upgrading traditional sausages into national and global markets.

Profile of microorganisms isolated from traditional meat products of the Sikkim Himalaya and Kumaun Himalaya



Aksu, M.I., Kaya, M. and Ockerman, H.W. (2005). Effect of modified atmosphere packaging and temperature on the shelf life of sliced Pastirma produced from frozen/thawed meat. *Journal of Muscle*

Lactobacillus plantarum
Lactobacillus casei
Lactobacillus curvatus
Lactobacillus sake
Homo-fermentative rod

Lactobacillus brevis
Lactobacillus sanfrancisco
Lactobacillus divergens
Leuconostoc mesenteroides
Hetero-fermentative rod

Staphylococcus *sp.*
Enterococcus faecium
Enterococcus cecorum
Pediococcus
Coccus

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