DISCUSSION

Traditional Preservation

Indigenous knowledge of the ethnic people living in the North East states of India on production of fermented vegetable products including fermented bamboo shoots was worth documenting, both as low-cost functional foods, and for socio-cultural reasons. Gundruk and sinki are important fermented non-salted vegetable foods in the local diet of the Sikkim Himalayas, and are prepared at households. Gundruk and sinki production practiced by the ethnic people of Sikkim is a unique type of biopreservation of foods through lactic acid fermentation. It is usually prepared during winter when perishable leafy vegetables are plenty in the sub-Himalayan regions. Invention of biopreservation methods by the ethnic people of the Himalayan regions through pit fermentation or lactic acid fermentation is significant due to transforming of the availability of raw materials at a particular season to those of deficit. Moreover, there is a remarkable step to store the perishable vegetable in absence of cold-storage or refrigeration, where majority of rural people cannot afford canned or frozen foods.

Sun drying of freshly prepared gundruk, sinki and inziangsang is a traditional preserving method by which the shelf life of the products is prolonged. Dried products are preserved for several months without refrigeration and consumed during long monsoon season when fresh vegetables are scarce. Dry gundruk, sinki and inziangsang are comparatively lighter than the weight of fresh substrates and can therefore, be carried easily while traveling. People might have invented such preservation technique to feed themselves while traveling for long distances, and also to store for future use whenever vegetables were in plenty. Carrying gundruk, sinki, inziangsang and other fermented products such as dried kinema and hawaijar (fermented soybeans), hard chhurpi (yak milk cheese-like product), sidra, sukuti, karoti (dried and smoked fish), ngari and tungtap (fermented fish products), etc. is still common practice in North East India while traveling for long distances (Tamang, 2005b). Because of the acidic taste, gundruk, sinki and inziangsang are said to be good appetizers, and the ethnic people use these foods for remedies from indigestion. Goyang is a lesser-known fermented vegetable food of the Sherpas living in high altitude in the Sikkim Himalayas. Fermentation of cucumber is observed only in Sikkim among the North East states, where the lesser-known product called khalpi is consumed as pickle. All these ethnic fermented foods have important bearing in the dietary habits of the people of North East India.

Young succulent shoots of the bamboo species are mostly used as an edible delicacy by different tribes of North East India (Sharma, 1989), and some as fermented products (Tamang, 2001). Several authors while reporting on the detailed taxonomical and ecological aspects of bamboos of North East India, did mention the use of bamboos as foods, such as soidon and soibum of Manipur (Giri and Janmejay, 1987), ekung and eup of Arunachal Pradesh (Basar and Bisht, 2002), kharisa and bah gazar of Assam (Singh, 2002), syrwa of Meghalaya (Bhatt *et al.*, 2003), zusem and zutsuk of Nagaland (Bhatt *et al.*, 2005) and rep of Mizoram (Bhatt *et al.*, 2003). However, most of these traditional fermented bamboo shoot products of North East India have not been studied in regards to microbiology except mesu (Tamang, 1992; Tamang and Sarkar, 1996) and soibum (Giri and Janmejay 1987, 1994, 2000), and technological properties of functional LAB present in these products. Due to our convenience and other logistic reasons, we collected among the fermented bamboo shoots, only samples of ekung, eup and hirring from Arunachal Pradesh; soibum, soidon and soijim from Manipur, and of course, mesu from Sikkim for our study. It is worth to notice that no preservative is added during storage in all above-mentioned fermented vegetable foods.

Microorganisms

The microbial population of goyang, gundruk, inziangsang, khalpi, sinki, ekung, eup, hirring, mesu, soibum, soidon and soijim samples revealed that lactic acid bacteria (LAB), comprising lactobacilli, pediococci, leuconostocs, lactococci (in goyang and hirring samples only) and enterococcus (in soibum samples only) were the predominant microorganisms present in viable numbers above 10^7 cfu/g. The identity of the LAB seems to correspond with that of LAB typically reported for fermented vegetable products of different origin and in various regions (Steinkraus, 1996; Lee, 1997). Taxonomically diverse species of LAB have been identified from goyang, gundruk, inziangsang, khalpi, sinki, ekung, eup, hirring, mesu, soibum, soidon and soijim samples of North East India. They represented the six genera of LAB - *Lactobacillus, Leuconostoc, Lactococcus, Pediococcus, Enterococcus* and *Tetragenococcus.* 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).

On the basis of a combination of phenotypic properties and molecular techniques, strains of LAB isolated from gundruk, inziangsang, khalpi and sinki were identified as *Lb. brevis*, *Lb. plantarum, P. pentosaceus* and *P. acidilactici.* During the identification procedure, the phenotypic characterisation of the LAB strains was followed by the RAPD-PCR technique. The fingerprints obtained from this technique were used to identify clusters of closely related or even identical strains allowing selecting representative strains of these groups for further identification (Schillinger *et al.*, 2003). A rep-PCR using $(GTG)_5$ primer was an appropriate tool to separate *Lb. plantarum* from the phenotypically almost indistinguishable *Lb. pentosus* and *Lb. paraplantarum* and a species-specific PCR for *Lb. brevis* allowed the identification of the heterofermentative lactobacilli (Kostinek *et al.*, 2005).

On the basis of the RAPD profiles, the dedrogram shown in Fig 13, the pediococci strains from gundruk and inziangsang were separated into *P. pentosaceus*, and *P. acidilactici.* Randomly amplified polymorphic DNA (RAPD) is an accurate technique for separation of *P. pentosaceus and P. acidilactici*, isolated from Ethiopian fermented foods (Nigatu *et al.*, 1998).

Lb. plantarum. Lb. brevis, *Lactococcus* (*Lc.*) *lactis, P. pentosaceus* and *Enterococcus faecium*, isolated from goyang of Sikkim, and *Leuc. fallax* (khalpi and sinki) were identified on the basis of detailed phonotypic characters including the API sugar profile data. *Lb. brevis, Lb. plantarum, P. pentosaceus,* and *P. acidilactici* have been reported to be involved in many lactic acid fermented vegetable foods including sauerkraut and cucumbers (Fleming *et al.*, 1985; Font de Valdez *et al.*, 1990; Tanasupawat *et al.*, 1993; Randazzo *et al.*, 2004). Enteroccoci play 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). Finding of *Lc. lactis* in goyang supports that this species is also commonly isolated from plant materials (Sandine *et al.*, 1972), though, the most recognised habitat for the lactococci is milk products (Teuber *et al.*, 1991).

Gundruk samples of Nepal contained *Lb. plantarum, Lb. casei* subsp. *casei, Lb. casei* subsp. *pseudoplantarum, Lb. cellobiosus* (= *Lb. fermentum*) and *P. pentosaceus* (Karki, 1986). Gundruk is also an important fermented vegetable food of Nepal (Dietz, 1984). However, there is no earlier report on microbial composition of gundruk samples prepared in Sikkim. *Leuc. mesenteroides, P. pentosaceus, Lb. brevis* and *Lb. plantarum* are known to contribute the complex sauerkraut fermentation process (Pederson and Albury, 1969). Several species of LAB were taxonomically identified from kimchi of Korea, which included *Leuc. mesenteroides, Lc. lactis, Lb. plantarum, Lb. brevis, E. faecalis, P. pentosaceus* and *Weissella kimchi* (Mheen and Kwon, 1984; Choi *et al.*, 2002; Kim and Chun, 2005). Recovery of lactobacilli in sinki and the confirmation of their identity by molecular tools confirmed the earlier findings of Tamang and Sarkar (1993). There are not many reports on the occurrence of *Leuc. fallax* in foods. Strains of these species have been isolated from sauerkraut (Schillinger *et al.*, 1989), fermented rice cake (puto) in the Philippines (Kelly *et al.*, 1995) and from plant exudates of *Gerbera jamesonii* (Middelhoven and Klijn, 1997). *Leuc. fallax* has recently been recognised as one of predominating organisms in the early heterofermentative stage of sauerkraut fermentation (Barrangou *et al.*, 2002).

Strains of LAB isolated from mesu, soidon, soibum and soijim were identified using phenotypic tests and genotypic methods such as rep-PCR and RAPD-PCR, as *Lb. brevis, Lb. plantarum, Lb. curvatus* and *P. pentosaceus.* For the other LAB strains *Leuc. mesenteroides, Leuc. fallax, Leuc. lactis, Leuc. citreum* and *Enterococcus durans* and also strains from ekung, eup and hirring of Arunachal Pradesh, phenotypic charaterisation including DAP, lactate configuration and API system was performed for the identification to species level. The typical enterococci (*Enterococcus durans)* can be easily distinguished from other Gram-positive, catalase-negative, homofermentative cocci such as streptococci and lactococci by their ability to grow both at 10 and 45° C, in 6.5 % NaCl, and at pH 9.6 (Franz *et al.*, 2003). The lactic floral composition in ekung, eup and hirring was *Lb. plantarum, Lb. casei, Lc. lactis,* and *Tetragenococcus halophilus* (only in ekung). *Lb. casei* has been found the dominant species in naturally fermented Sicilian green olives (Randazzo *et al.*, 2004). Recovery of *Lb. plantarum, Lb. brevis* and *P. pentosaceus* in mesu samples collected from Sikkim confirmed the earlier report of Tamang and

Sarkar (1996). *Leuc. citreum* is reported in traditional French cheese (Cibik *et al.*, 2000). Findings of *Lb. plantarum, Lb. brevis, Leuc. fallax, Leuc. mesenteroides* and *Enterococcus durans* in soibum of Manipur has updated the earlier findings of Giri and Janmejay (1994), the identification of which was based on a limited phenotypic characters (Giri and Janmejay, 1987). Microbial composition of soidon and soijim of Manipur was reported for the first time in this thesis. Similar fermented bamboo shoot products called naw-mai-dong or nor-mai-dorng of Thailand also contained lactobacilli, leuconostocs and pediococci (Dhavises, 1972; Phithakpol *et al.*, 1995). Though, *Enterococcus durans* has been isolated from Feta cheese (Igoumenidou *et al.*, 2005), there is no published report of its presence in any fermented vegetable product. Isolation, enrichment, purification, characterisation and proper identification and authentic nomenclature of functional microorganisms involved in fermented foods are important aspects of microbial systematic, which also ensure the quality control and normalised production of fermented foods (Tamang and Holzapfel, 1999). The isolated, identified and preserved microorganisms from lesser-known fermented vegetable products may contribute significant information on unknown microbial gene pool as genetic resources of North East India.

Pathogenic contaminants

Occurrence of bacterial contaminants mostly *Staphyloccus aureus* in few finish samples of goyang, gundruk and sinki, *Bacillus cereus* in few dry samples of sinki and enterobacteriaceae in goyang and gundruk samples around 10^2 cfu/g was observed. These pathogens might have introduced during handling of raw leaves for preparation when pH was not low enough to inhibit their growth. Otherwise, no pathogenic bacteria such as *Listeria, Salmonella* and *Shigella* were detected in analysed samples, due to acidic nature of the products. Small number of *Bacillus cereus* in foods is not considered significant (Roberts *et al.*, 1996). Rapid growth of LAB could restrict the growth of other organisms simply by their physical occupation of available space and uptake of most readily assimilative nutrients (Adams and Nicolaides, 1997). Moreover, lactic acid produced by LAB may reduce pH to a level where pathogenic bacteria may be inhibited or destroyed (Holzapfel *et al.*, 1995; Tsai and Ingham, 1997).

Yeasts

Though the dominant microflora in fermented vegetable and bamboo products of North East India was lactic acid bacteria, a sizable number of yeasts mostly *Pichia, Candida, Saccharomyces* and *Rhodotorula* were also reported in some finish products of goyang, khalpi, sinki, ekung, hirring and mesu in numbers ranging between 10^4 and 10^6 cfu/g. These yeasts might be spoilage in the products or might have appeared during storage. The various genera and species of yeasts occur in kimchi (Song and Park, 1992). In cucumber pickles, *Hansenula*, *Saccharomyces* and *Torulopsis* were reported (Etchells *et al.*, 1961). Some oxidative yeasts belonging to *Candida*, *Pichia*, *Debaryomyces, Saccharomyces* and *Hansenula* were associated with gassy fermentation and softening of olives (Vaughn *et al.*, 1972). It was suggested that acidification with lactic acid alone to pH 3.7 is insufficient to prevent the growth of spoilage yeast in fermented vegetables and that the presence of other organic acids is desirable (Savard *et al.*, 2002).

Technological properties

Acidification is an important technological property in relevance of selection for starter culture among the LAB (De Vuyst, 2000). LAB strains isolated from fermented vegetable and bamboo shoot products were screened for their acidifying capacity, and found that most of the LAB strains acidified with lowering of pH to 4.0 (Table 18 and 19). These strains although originating from plant sources and not from milk, appeared to be adapted to the milk ecology, since they coagulated and acidified the skim milk used in the applied method. The casein degradation initiated with milk clotting peptidases and proteinases, which produce peptides and amino acids (Mäyra-Mäkinen and Bigret, 1998). Justifying milk coagulation by the LAB strains isolated from fermented vegetables, the enzymatic activity of the LAB (Table 24 and 25) clearly shows the absence of proteinase but showed high activity of peptidases, which, may be responsible for coagulation of skim milk in the applied method. The ability of some species of LAB particularly *Lb. plantarum* in lowering pH of the substrates is significant in food preservation (Brown and Booth, 1991).

Strains of different species of *Lactobacillus, Pediococcus and Leuconostoc*, isolated from all fermented vegetable and bamboo shoot products, showed anitimicrobial activities against a number of potentially pathogenic Gram-negative and Gram-positive bacteria (Table 20 and 21). This reveals that antimicrobial properties of functional LAB can reduce the number of other undesired microorganisms in vegetable products and simultaneously perform an essential role in the preservation of a food product for human consumption, by fermentation. In addition, LAB compete with other microbes by screening antagonistic compounds and modifying the micro-environment by their metabolism (Lindgren and Dobrogosz, 1990). However, only one strain of *Lb. plantarum* IB2 (inziangsang) was found to produce a bacteriocin against *Staphylococcus aureus* S1 (Table 22). This may be explained by the fact that also other factors are involved in antagonism, and by the influence of growth rate and competitiveness of a culture for bacteriocin production (Tagg, 1992; Yang and Ray, 1994). This is determined, amongst others, by its adaptation to a substrate and by a number of intrinsic and extrinsic factors including redox potential, water activity, pH and temperature (Holzapfel *et al.*, 1995; Ouwehand, 1998). A number of Gram-positive pathogenic bacteria including *Staphylococcus aureus* have been found sensitive to bacteriocin of *Lactobacillus* (Tichaezek *et al.*, 1992; Sudirman *et al.*, 1993; Niku-Paavola *et al.*, 1999). Our finding on bacteriocin activity of *Lb. plantarum* IB2 (inziangsang) against *Staphylococcus aureus* was justified by the recent report on the inhibitory effect of bacteriocin produced by *Lb. plantarum* in foods against *Staphylococcus aureus* and other Gram-negative bacteria (Jamuna *et al.*, 2005; Lash *et al.*, 2005). Crude bacteriocin activity of *Lb. plantarum* IB2 was quantified and calculated as 32 AU/ml. Quantification is necessary to observe the quotient between the inhibition zone area and the sensitivity of the indicator bacterium used to compare different bacteriocins (Delgado *et al.*, 2005). Species of LAB strains isolated from several fermented vegetable products have the antimicrobial activities including bacteriocins and nisin production such as fermented olives (Cordeiro *et al.*, 2002; Rubia-Soria *et al.*, 2006), sauerkraut (Tolonen *et al.*, 2004), fermented carrots (Uhlman *et al.*, 1992), fermented cucumbers (Daeschel and Fleming, 1987) and cabbage juice (Kyung and Fleming, 1994).

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 fermented products (Tamang *et al.*, 2000). Absence of proteinases (trypsin and chymotrypsin) and presence of strong peptidase (leucine-, valine-, and cystine-arylamidase) and esterase-lipase (C4 and C8) activities produced by the predominant LAB strains isolated from fermented vegetable products of North East India are possible traits of desirable quality for their use in production of typical flavour. Herreros *et al.* (2003) reported the highest aminopeptidase activity in *Lb. plantarum, Lc. casei* and *Leuc. mesenteroides* subsp. *dextranicum* isolated from Armada cheese.

Antinutritive factors such as phytic acids and oligosaccharides are of particular significance in unbalanced cereal-based diets (Holzapfel, 2002; Fredrikson *et al.*, 2002). Oligosaccharides such as raffinose, stachyose and verbascose cause flatulence, diarrhea and indigestion (Abdel Gawad, 1993; Holzapfel, 1997). Due to these nutritional consequences, the degradation of antinutritive factors in food products by fermentation is desirable as reported for a number of foods of plant origin (Chavan and Kadam, 1989; Mbugua *et al.*, 1992). High activity of phosphatase by LAB strains showed their role in phytic acid degradation in fermented vegetable products, as proved in Tables 26 and 27. It was also shown that strain of *Lb. plantrum*, *Lb. brevis* and *Leuc. lactis* had moderate to high α-galactosidase activity. This indicated their ability to hydrolyse oligosaccharides of raffinose family (Holzapfel, 2002). About 76.7 % of *Lb. plantarum* strains isolated from fermented vegetable and fermented bamboo shoot products of North East India degraded raffinose while none of the *P. pentosaceus* strains and *P. acidilactici* were able to degrade raffinose. This is in conformity with that report of Holzapfel (2002) where *Lb. plantarum* strains isolated from fermented Ghanaian maize products were able to ferment raffinose, while strains of *P. pentosaceus* and *P. acidilactici* were unable to do so. Several reports support our findings of phytate reduction in fermented vegetables by lactic fermentation, as shown in maize (Lopez *et al.*, 1983), idli, a fermented rice-black gram product (Reddy *et al.*, 1986); white sorghum (Svanberg and Sandberg, 1988), pearl millet (Mahajan and Chauhan, 1987; Khetarpaul and Chauhan, 1989) and non-tannin containing cereals (Svanberg *et al.*, 1993).

Biogenic amines are organic basic compounds which occur in different kinds of foods such as sauerkraut (Taylor *et al.*, 1978), fishery products, cheese, wine, beer, dry sausages and other fermented foods (ten Brink *et al.*, 1990; Halász *et al.*, 1994), fruits and vegetables (Suzzi and Gardini, 2003). Leafy vegetables usually contain low level of biogenic amines but it may increase during fermentation due to decarboxylase activity of microorganisms (Simon-Sarkadi *et al.*, 1994; Silla-Santos, 2001). In foods, biogenic amines are mainly generated by decarboxylation of the corresponding amino acids through substrate specific enzymes of the microorganisms present in foods (ten Brink *et al.*, 1990; Straub *et al.*, 1995). The inability of most strains of LAB to produce biogenic amines in tested fermented vegetable products of North East India 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). Histaminine, precursor of biogenic amine has been recognised as the causative agent of scombroid poisoning (histamine intoxication), whereas tyramine has been related to food induced migraines and hypertensive (Taylor, 1986; Bover-cid and Holzapfel, 1999).

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). A few strains of LAB isolated from fermented vegetable products of North East India had more than 75 %

hydrophobicity (Table 30 and 31), indicating their hydrophilic nature. Percent of hydrophobicity greater than 70 % was arbitrarily classified as hydrophobic (Nostro *et al.*, 2004). The high degree of hydrophobicity of the LAB strains, isolated from fermented vegetable products of North East India, probably indicates the potential of adhesion to gut epithelial cells of human intestine, advocating their 'probiotic' character (Holzapfel *et al.*, 1998), provided these strains are consumed in a viable state. The ability to adhere to the intestinal mucosa is considered one of the main criteria in the selection of potential probiotic 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 chemical composition (Gatti *et al.*, 1997; Boonaert and Rouxhet, 2000; Gómez-Zavaglia *et al.*, 2002).

Fermentation dynamics

During natural fermentation of both 'rayo-saag' gundruk and 'mula-saag' gundruk, indigenous lactic acid bacteria changed spontaneously and at the end of the process *Lactobacillus* species, mainly *Lb. plantarum* was involved (Table 32 and 33). Spontaneous change in LAB population during several vegetables fermentation involving lactobacilli was reported (Fernandez Gonzalez *et al.*, 1993; Harris, 1998). As expected in a typical lactic fermentation (Vaughn, 1985; Lee, 1997; Lu *et al.*, 2001), the pH of the fermenting substrates decreased and the titratable acidity increased as the gundruk fermentation progressed due to growth of LAB which, converts fermentable sugars into lactic acid (Buono *et al.*, 1990). Some yeasts were detected in initial stage of both types of gundruk fermentation, and then disappeared. The population of pathogenic contaminants disappeared during fermentation because of dominance of LAB. By averting the invasion of these potential contaminants, lactic acid fermentation imparts attributes of robust stability and safety in the product like gundruk. There has been no report of any food poisoning or infectious disease infestation by consuming gundruk. The LAB produced sufficient acid for inhibition of pathogenic microorganisms in foods (Adam and Nicolaides, 1997). The LAB also preclude proliferation of contaminating acid-intolerant species of bacteria and fungi (Mensah *et al.*, 1990; Nout, 1991, 1994).

In situ fermentation of khalpi also revealed that significant decrease in pH accompanied by increase in titratable acidity with fermentation time (Table 34). After exponential increase, the population of LAB dropped slightly. Yeast, *Staphylococcus aureus* and enterobacteriaceae were associated with initial fermentation and finally disappeared during khalpi fermentation. Fresh vegetables, like cucumbers, contain a numerous and varied epiphytic microflora, including many spoilage microorganisms, and an extremely small population of LAB (Etchells *et al.*, 1961; Mundt and Hammer, 1968). Numerous chemical and physical factors influence the rate and extent of growth of various microorganisms, as well as their sequence of appearance during the fermentation (Fleming and McFeeters, 1981). Acidity, pH and buffer capacity greatly influence establishment and extent of growth of LAB during cucumber fermentation (McDonald *et al.*, 1991).

Starter culture

Use of standard starter culture is not a practice in North East India except in alcoholic beverage production (Thapa and Tamang, 2004). An attempt was made to produce gundruk and khalpi under optimised condition using selected strains of LAB. Gundruk was selected since it is widely consumed in the various regions, while khalpi was selected since cucumber is available in plenty. It was found that gundruk produced under optimised conditions using a mixture of pure culture strain of *Lb. plantarum* GLn:R1 and *P. pentosaceus* GLn:R1, selected on the superior technological property as mentioned in the result section, at 20° C for 6 days, had highest score of general acceptability (Table 35). Similarly, khalpi was prepared using a mixture of pure culture strain of *Lb. plantarum* KG:B1, *Lb. brevis* KG:B2 and *Leuc. fallax* KB:C1 (Table 36). Khalpi produced at 20° C for 72 hour had organoleptically scored the highest acceptability among the consumers; whereas khalpi made at 25° C scored the highest acceptability within 48 hour and acceptability decreased as sour taste develops further. Application of starter cultures may appear appropriate in gundruk and khalpi production at household level since it is cost-effective and may contribute to effective control and safeguarding of the fermentation process. Gundruk prepared by using a starter culture had thus advantages over the traditional method, which resulted in a shorter fermentation time that eliminates the chance of growth of contaminants, hygienic conditions, maintaining consistency with better quality and flavour. Similarly, using a mixed pure culture strains in khalpi preparation takes less time, while in natural condition it takes more than 3 days for cucumber fermentation to complete.

The final product is not always consistent in natural fermentation; the use of a mixed lactic starter culture could provide more consistent fermentations and products of higher quality (Daeschel and Fleming, 1987; Gardner *et al.*, 2001). Modern starter cultures are selected, either as single or multiple strains, especially for their adaptation to a substrate or raw material, for example milk, meat, cereals, legumes, roots, and tubers (Holzapfel *et al.*, 2003). The authentic identity of indigenous LAB isolated from traditional fermented vegetable products and their detailed technological characters should be considered for development of starter culture. Though, optimised process condition is always superior and advantageous than the conventional method, however, introduction and replacement of natural and easily operated traditional technology may be difficult to change for the producers or rural populace. Authentic identity of functional microbes in fermented foods is necessary to develop the starter cultures isolated from conventionally prepared foods (Tamang and Holzapfel, 1999). Preservation and safeguarding of foods are still major objectives of fermentation (Holzapfel, 2002). Yet, other aspects such as wholesomeness, acceptability and overall quality have become increasingly important and valued features to account of the substrate, technical properties of the strain, food safety requirements and quality expectations (Holzapfel *et al.*, 2003).

Proximate composition

Moisture content in gundruk, inziangsang, sinki and eup was low due to drying after fermentation; other products were freshly prepared with high moisture content. Samples of fermented vegetable collected from North East India had comparatively higher mineral content. Higher contents of mineral are observed in Indian vegetables (Gopalan *et al.*, 1995). Proximate and food value of fermented vegetable and bamboo shoot products of North East India are almost same as reported in other fermented vegetable products such as kimchi (Cheigh and Park, 1994), sauerkraut (Campbell-Platt, 1987), and fermented cucumber (Fleming, 1984).

Conclusion

Scientific knowledge on fermented vegetable and bamboo shoot products of North East India is sparse outside this region. Species of predominant lactic acid bacteria associated with biopreservation of perishable vegetables and some wild bamboo shoots were summarised as follows: *Lb. plantarum, Lb. brevis, Lc. lactis, E. faecium, P. pentosaceus* (goyang); *Lb. plantarum, P. pentosaceus* (gundruk); *Lb. plantarum, Lb. brevis, P. acidilactici* (inziangsang); *Lb. plantarum, Lb. brevis, Leuc. fallax* (khalpi); *Lb. plantarum, Lb. brevis, Lb. casei, Leuc. fallax* (sinki); *Lb. plantarum, Lb. brevis, Lb. casei, Tetragenococcus* *halophilus* (ekung); *Lb. plantarum, Lb. fermentum* (eup); *Lb. plantarum, Lc. lactis* (hirring); *Lb. plantarum, Lb. brevis, Lb. curvatus, Leuc. citreum, P. pentosaceus* (mesu); *Lb. plantarum, Lb. brevis, Leuc. fallax, Leuc. lactis, Leuc. mesenteroides, E. durans* (soibum); *Lb. brevis, Leuc. fallax, Leuc. lactis* (soidon); *Lb. brevis, Leuc. fallax, Leuc. lactis* (soijim).

About 65.4 %, out of 547 LAB strains isolated from traditional fermented vegetable and bamboo shoots of North East India, belonged to *Lactobacillus.* Homofermenter lactobacilli were represented by 45.4 %, followed by heterofermenter lactobacilli representing 20 %; and leuconostoc 16.3%, pediococci 12.7%, lactococci 1.7% and *Tetragenococcus* 0.7 % represented remaining lactic flora.

This study showed that strains of LAB play an important and partly complex role in these traditional fermentation processes by their functional properties related to a specific and partly a wide enzyme spectrum, their acidifying capacity, degradation of antinutritive factors, by their antimicrobial activities against potential pathogens, probiotic properties (adherence potential indicated by a high degree of hydrophobicity), and even as non-producers of biogenic amines. Some of these LAB strains possess interesting protective and functional properties, which render them interesting candidates for use as starter culture for controlled and optimised production of fermented vegetable products typical of North East India. Gundruk and khalpi prepared by a mixed pure culture strains of LAB had many advantages over the conventionally prepared products.