INTRODUCTION

Biopreservation refers to extended storage life and enhanced safety of foods using the natural microflora and their antibacterial products (Stiles, 1996). Food fermentation has been used for centuries as a method to preserve perishable food products (Geisen and Holzapfel, 1996; Hansen, 2002). Lactic acid fermentation serves as affordable and manageable techniques for food preservation in absence of facilities for home refrigeration and freezing (Steinkraus, 1983; Nout and Sarkar, 1999). It represents a distillation of knowledge and wisdom gained by experience and based on trial and error. People might not be able to explain what is going on during fermentation, but they certainly know what do to get the desired products. They knew how to provide favourable conditions for fermentation and thus to promote the beneficial microorganisms for fermentative production of foods (Lee, 2001). However, scientific knowledge on the biochemical changes and on the identity of these microorganisms were unknown, partly unto the recent time. Numerous types of traditional fermented foods all over the world have already been microbiologically explored.

Bacteria, mostly lactic acid bacteria (LAB), yeasts and filamentous moulds constitute the microbiota of fermented foods and beverages, which are present in or on the ingredients, utensils, environment, and are selected through adaptation to the substrates (Hesseltine, 1983, Steinkraus, 1997). In the Indian sub-continent, mostly due to wide variation in agro-climatic conditions and diverse form of food culture of different ethnic people, a diversity of microorganisms is associated with traditional fermented foods and beverages (Soni and Sandhu, 1990;

Tamang, 1998). Microorganisms change the chemical composition of raw materials during food fermentation enhancing the nutritive value, enriching the blend diet with improved flavour and texture, preserving the perishable foods, fortifying the products with essential amino acids, vitamins, minerals, degrading the undesirable components and antinutritive factors, improving the digestibility, and stimulating the probiotic functions (Steinkraus, 1994; Stiles and Holzapfel, 1997; Adams and Nout, 2001). Among the various microorganisms present in food fermentation, LAB are the dominant microfloras in many traditional fermented foods such as vegetable products (Kim and Chun, 2005), fermented milk products (Shah, 2005), cereal products (Nout, 2001), meat products (Rantsiou and Cocolin, 2006), and fish products (Adams, 1998). Lactic acid bacteria are Gram-positive, nonsporing, catalase-negative, devoid of cytochromes, of nonaerobic habit but aerotolerant, fastidious, acid-tolerant, and strictly fermentative with lactic acid as the major end-product during sugar fermentation (Axelsson, 1998; Klein *et al.*, 1998). LAB produce lactic acid during traditional fermentation, the characteristic fermentative product, which reduce pH of the substrate to a level where growth of putrefactive, pathogenic and toxinogenic bacteria are inhibited (Holzapfel *et al.*, 1995). The LAB which are usually designated as "GRAS" (generally recognised as safe) status in foods (Donohue and Salminen, 1996), can also exert as 'biopreservative' (Holzapfel *et al.*, 2003). The genera of LAB mostly present in foods are *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus*, *Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus*, *Vagococcus* and *Weissella* (Stiles and Holzapfel, 1997;

Carr *et al.*, 2002). The advantages of lactic acid food fermentations include resistant to spoilage and food toxins; they make the foods less likely to transfer pathogenic microorganisms; they generally preserve the foods between the time of harvest and consumption, and they modify the flavour of the original ingredients and often improve the nutritional value (Nout and Ngoddy, 1997; Salminen and Wright, 1998).

North East India has eight states; most of them are situated in hills and foothills. Samples of fermented vegetables and bamboo shoots were collected from three states viz. Sikkim, Arunachal Pradesh and Manipur, respectively, for the study materials in this thesis. Sikkim is a mountainous state of India in the Eastern Himalayas with an area of 7096 sq. km and altitudes ranging from 300 m to 8,500 m. The state comprises four districts. A total population of Sikkim is 5,40,493 (Census of India, 2001) and is populated by three major ethnic groups, Nepali, Bhutia and Lepcha. Arunachal Pradesh is also in the Eastern Himalayan range with an area of 83,743 sq. km. The state comprises thirteen districts with a total population of 10,96,702 (Census of India, 2001) and is populated by many tribes including Nyishing, Adi, Abors, Khampti, Apatami, etc. Manipur has an area of 22,327 sq. km with a population of 23,88,634 (Census of India, 2001). It has nine districts and is populated by many ethnic communities mostly Meiteis, Kuki, etc.

Traditional fermented foods constitute an important part of the local diet in the Sikkimese culture (Tamang, 2005a). Such traditional foods are closely associated with socio-economic development status, religious and cultural practices, and have been evolved as the result of tradition and

empirical experiences of generations over a period of time. The daily per capita consumption of fermented foods in the Sikkim Himalayas is 87.6 g representing 10 % of the total daily food consumed in the local diet (Yonzan and Tamang, 1998). A variety of fermented vegetable products are prepared and consumed in the Eastern Himalayan regions of India (the Darjeeling hills, Sikkim, Manipur, Nagaland, Meghalaya), Nepal and Bhutan (Tamang *et al.*, 1988; Tamang, 2001). These traditional products are usually prepared by rural women using their indigenous knowledge of fermentation for the biopreservation of perishable vegetables for storage and consumption.

Food culture in North East India has been reflected in the pattern of food production in a mixed farming system. Depending on the altitudinal variation, seasonal vegetables such as cabbage, cauliflower, leafy mustard 'rayo-saag', young tendrils and fruits of squash 'iskus', brinjal, chili, cucumber, young tendrils and fruits of pumpkin, sponge gourd, tomato, tree tomato, radish, carrot, etc. are cultivated and eaten in Sikkim (Annual Report, 2005), and also in other hill regions of North East India. Preparation of wild edible plants including young bamboo shoots, ferns, stinging nettles, and their parts such as seeds, fruits, roots, leaves, flowers in local diet are important components of food culture (Rai *et al.*, 2005).

About 78 indigenous and exotic species of bamboo belonging to 19 genera are reported in the biodiversity-rich regions of North East India (Hore, 1998). Bhatt *et al.* (2003) reported that about 266.4 quintal, 4420.3 quintal and 4336.3 quintal of bamboo shoots are harvested annually in

Sikkim, Meghalaya and Mizoram, respectively. Young succulent shoots of these 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). Some popular fermented bamboo tender shoots of North East India are mesu of Sikkim, soidon, soibum and soijim of Manipur, ekung, eup and hiring of Arunachal Pradesh, kharisa and bah gazar of Assam, syrwa of Meghalaya, zusem and zutsuk of Nagaland and rep of Mizoram (Singh, 2002; Bhatt *et al.*, 2005; Tamang, 2005b).

Varieties of ethnic fermented foods and beverages are traditionally prepared and consumed, and even marketed within the region in North East India (Tamang, 2001). Some of the traditional food products of North East India were extensively studied (Tamang, 2005b), however, majority of them are yet to be investigated scientifically. Though, there have been no published papers or reports on many traditional fermented vegetable and bamboo shoot products of North East India except few vegetable-based fermented products which were reported earlier. Tamang and Sarkar (1993) reported the microbiology of sinki, a traditional fermented radish tap-root product of the Darjeeling hills and Sikkim. Tamang and Sarkar (1996) also reported the microbiology and biochemistry of mesu, a traditional fermented bamboo shoot product of the Darjeeling hills and Sikkim. Karki *et al.* (1983a, 1983b, 1983c, 1983d) studied the microbiology and biochemistry of gundruk from Nepal. Pravabati and Singh (1986), Giri and Janmejay (1987, 1994, 2000) studied some parameters of soibum, fermented bamboo shoot of Manipur. To the best of our knowledge, no microbial systematic of predominant lactic acid

bacteria isolated from most of the traditional fermented perishable vegetables and wild bamboo shoot products of North East India, and their technological properties were studied.

Objectives

The present thesis was aimed to document the indigenous knowledge of biopreservation methods of perishable vegetables including edible bamboo shoots of North East India; and to isolate, characterise and identify the predominant lactic acid bacteria associated with the various traditional fermented vegetable and bamboo shoot products. The objective of the thesis was also to study the technological properties of the identified strains of functional lactic acid bacteria such as acidifying capacity, enzymatic profiles, their antimicrobial and bacteriocin activities against potential strains, ability to produce biogenic amines, degradation of antinutritive factors such as phytic acid and oligosaccharides and to determine the degree of hydrophobicity of such strains. The LAB Strains were selected on the basis of technological properties and tested for production of fermented vegetable products under laboratory conditions, and subjected to sensory evaluation. Microbial population dynamics of LAB and other microbial groups during fermentation were also examined. Proximate composition of samples of fermented vegetables and bamboo shoots was also calculated.