# INTRODUCTION

Ancient people discovered traditional technology for preservation of perishable plant and animal foods through fermentation, smoking, drying, and salting and developed the culinary skills to make food recipes based on several biological, geographical and physical factors as well as individual sensory likings (Tamang and Samuel, 2010). Fermented foods are produced from raw materials of plant or animal sources either naturally or by adding starter culture(s) containing functional microorganisms which modify the substrates biochemically and organoleptically into edible products that are culturally and socially acceptable to the consumers (Tamang, 2010 a,b). Based on sensory and physico-chemical properties fermented foods bear $A^3$ connotation representing acidic, alkaline and alcoholic nature which are biologically engineered by complex microbial community (Tamang, 2010b). More than 5000 varieties of major and minor unlisted fermented foods and alcoholic beverages are consumed across the world by billions of people, representing about 5-40 % of the total daily meals (Tamang, 2010b). Several fermented foods have health-promoting benefits and are substituted as health-foods and functional foods (Tamang, 2007; 2010; Farhad *et al.*, 2010).

Soybean is a major leguminous crop in the world, and its utilization as foods are mostly confined to Asia. Soybean was probably introduced to India from China through the Himalayas

several centuries ago and some believe that soybeans were also brought via Myanmar by traders from Indonesia (Shurtleff and Aoyagi, 2010). Preparation and consumption of sticky, non-salty, flavorsome fermented soybean foods are the traditional wisdom of the people by several of South-East Asia and they remain a distinct food culture of the people (Tamang, 2010a). There are two types of fermented soybean products: one is solely fermented by *Bacillus* spp. (mostly *B. subtilis*) and has characteristic stickiness, and other is fermented by moulds. Again, fermented soybean is two types on the basis of salty taste: Non-salted fermented soybeans eg. *kinema, natto, tempe*, etc., and salted fermented soybeans eg. *miso*, soy-sauce, *douchi*, etc.

*Bacillus subtilis*, a rod-shaped, endospore-forming bacterium is an important starter culture for many Asian and African fermented soybean foods (Kiers *et al.*, 2000). *Bacillus*-fermented, non-salted and sticky soybean foods are concentrated in an imaginary triangle with three vertices on Japan (*natto*), east Nepal and North East India (*kinema* and its similar products) and northern Thailand (*thua-nao*) named as '*natto* triangle' (Nakao, 1972) and renamed as '*kinema-natto-thua nao* (KNT)-triangle' by Tamang, (2010a). Within the KNT-triangle-bound countries, many *Bacillus*-fermented sticky non-salty soybean foods are produced and consumed such as *natto* (Japan), *kinema* (India, Nepal and Bhutan), *tungrymbai, bekang, hawaijar, aakhuni* and *peruyaan*

(India), *thua nao* (Thailand), *chungkokjang* (Korea), *pepok* (Myanmar) and *sieng* (Cambodia) (Nagai and Tamang, 2010). Beyond this hypothetical KNT-triangle, there is no report of *Bacillus*-fermented sticky fermented soybean foods and the KNT triangle does not include non-bacilli and non-sticky fermented soybean products such as *tempe, miso, sufu, shoyu*, etc. (Tamang, 2010a). Although the method of production and culinary practices vary from product to product, plasmids and phylogenetic analysis of *B. subtilis* showed the similarity among strains of *B. subtilis* isolated from common sticky fermented soybean foods of Asia (Hara *et al.*, 1986, 1995; Tamang *et al.*, 2002, Meerak *et al.*, 2007) suggesting origination from the same stock. Moulds-fermented soybean products are *miso* and *shoyu* of Japan (Sugawara, 2010), *tempe* of Indonesia (Nout and Kiers, 2005), *douchi* of China (Zhang *et al.*, 2007), *sufu* of China (Han *et al.*, 2001), *doenjang* of Korea (Kim *et al.*, 2009). Among non-soybean common fermented legumes of the world are *dawadawa, iru, ugba, bikalga, soumbala* of Africa (Parkouda *et al.*, 2009, Ouoba *et al.*, 2010), *papad, dhokla,* and *wari* of India (Nagai and Tamang, 2010), *ontjom* of Indonesia (Nagai and Tamang, 2010), *maseura* of India and Nepal (Chettri and Tamang, 2008).

Geographically North East (NE) India is located within the Eastern Himalayas and Purvanchal Himalayas (Tamang, 2010a). The Purvanchal Himalayas lie between the latitudes 21° 5' - 28°

23' north and longitudes 91° 13'-97° 25' east, covering a total area of 108,229 km$^2$ comprising hills of Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura. Meghalaya (or *megh* meaning cloud; *alay* means home) with an area of 22,429 sq km, is located in the North East region of India (Fig 1) and extends latitude 20°1"N-26°5"N and longitude 85°49"E-92°52"E (Tamang, 2010 a; Census of India, 2011). It is bounded on the north and east by the Indian state of Assam and on the south and west by Bangladesh. Around 30% of total land in Meghalaya is under forest cover. Meghalaya currently has 7 districts, viz: East Garo Hills, East Khasi Hills, Jaintia Hills, Ri-Bhoi, South Garo Hills, West Garo Hills and the West Khasi Hills. The population of Meghalaya is 29,64,007 dominated mostly by Khasi, Garo, Jaintia, and Gorkha (Census of India, 2011). A common cultural tradition of all the tribes of Meghalaya is the matriarchal law of inheritance by which, custody to property and succession of family position runs through the female line, passing from the mother to the youngest daughter, instead of the male line as is common elsewhere in the country.

Mizoram or 'the Land of Hill People' (from *mi* 'people', *zo* 'hill', *ram* 'country') with an area of 21,087 sq. km is situated in the southern-most corner of NE India (Fig 1) and is bounded on the north by the States of Assam and Manipur, on the east and south by Chin Hills of Myanmar and on the west by Chittagong

Hill Tracts of Bangladesh and the State of Tripura. Mizoram lies between 21.58° to 24.35° north latitudes and 92.15° to 93.29° east longitudes. The major ethnic groups living in Mizoram are Mizo, Hmar, Gorkha, Lakher, Pawi etc. (Tamang, 2010 a; Census of India, 2011). Mizoram is divided into 8 districts. Viz: Aizawl District, Lunglei District, Kolasib District, Mamit District, Champhai District, Serchhip District, Saiha District and Lawngtlai District. The population of Mizoram is 1,091,014 with a literacy rate of 91.6 % which is the second highest in the country (Census of India, 2011).

Indigenous knowledge of ethnic people of NE regions on food fermentation, conservation biology, medicinal herbs, hill farming, livestock management, etc. are well known and one such example of innovative knowledge is production of ethnic fermented foods and alcoholic drinks (Jeyaram *et al.*, 2009). The ethnic people of NE has developed the ethnic foods to adapt to the harsh conditions and environment, the in-take of such foods has been in the systems for centuries and people have adapted such foods to protect and sustain them (Tamang *et al.*, 2010). More than 250 types of ethnic fermented foods and alcoholic beverages are produced and consumed in NE as staple, curry, side dish, fried, cooked, paste, condiment, pickle, confectionery, soup, drink, masticator, alcoholic and non-alcoholic beverages (Tamang *et al.*, 2012).

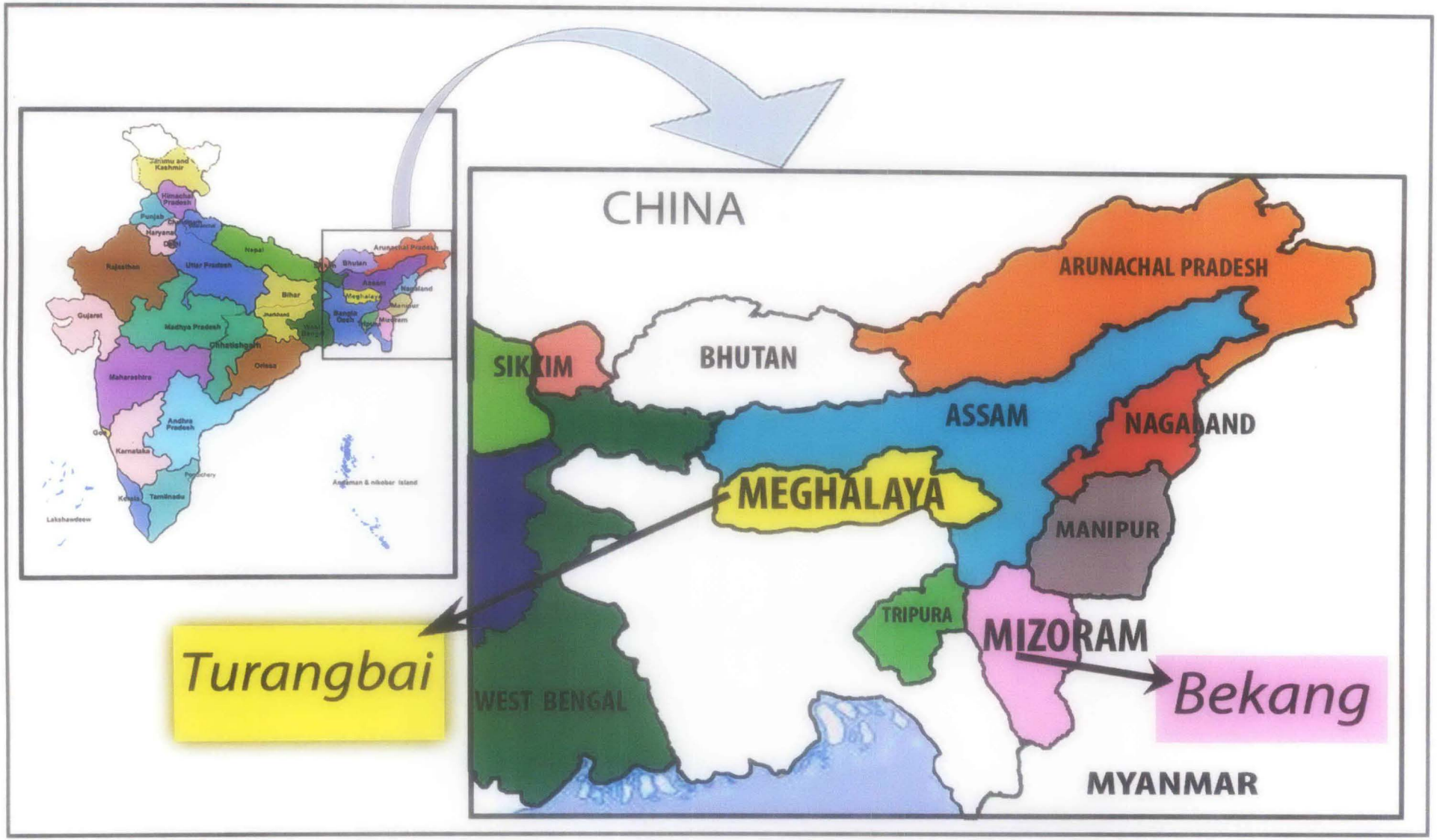


Fig 1. Collection sites of *turangbai* and *bekang* from NorthEast India

**Objectives**

The present Thesis was aimed to document the indigenous knowledge of ethnic people of Meghalaya and Mizoram for production of lesser-known fermented soybeans foods; and to isolate, characterize and identify the predominant *Bacillus* species, lactic acid bacteria and yeasts associated with *turangbai* and *bekang*, two ethnic fermented soybeans of Meghalaya and Mizoram, respectively using both conventional and molecular identification tools, and also their microbial population. The objective of the thesis was also to study the technological properties of the identified strains of *Bacillus* and lactic acid bacteria such as enzymatic activities and profiles, acidifying capacity, degradation of antinutritive factors such as phytic acid and oligosaccharides, antimicrobial and bacteriocin activities against potential strains, ability to produce biogenic amines, to determine the degree of hydrophobicity of strains, production and degradation of poly-γ-glutamic acid (PGA), antioxidant activity and total phenol contents of the products. Proven strains of *Bacillus* were selected on the basis of superior technological properties and tested for production of fermented soybean products under laboratory conditions.