SUMMARY

The major objective of this thesis was to document indigenous knowledge of ethnic people of Meghalaya and Mizoram on production of fermented soybean products; *turangbai* and *bekang* respectively and to isolate, characterise and identify the dominant microorganisms in fermented soybean products collected from different places. The objective of the thesis was also to analyse microbial load, molecular characterization of predominant bacilli testing for occurrence of pathogenic contaminants in the product as food safety, functional or technological properties of microorganisms such as acidifying capacity, enzymatic profiles, and their antimicrobial activities against pathogenic bacteria, degradation of antinutritive factors, probiotic properties, production of biogenic amines, poly-glutamic acid (PGA) production by *bacilli*, study of antioxidant activity. Firstly, an extensive survey was conducted in different regions of Meghalaya and Mizoram. Based on personal observation and interviews with the ethnic people (producers), *turangbai*, an indigenous fermented soybean product from Meghalaya and *bekang*, a similar ethnic fermented soybean product from Mizoram were documented.

Scientific knowledge on *turangbai* of Meghalaya and *bekang* of Mizoram is unknown outside this region. A total of 39 samples of *turangbai* and 43 samples of *bekang* were collected from different

villages and markets of Meghalaya and Mizoram respectively and were analysed for microbial load. In *turangbai*, microbial load of bacilli, lactic acid bacteria, yeasts and total viable counts were found at the level of 10^8 cfu/g, 10^4 cfu/g, 10^3 cfu/g and 10^9 cfu/g respectively. In *bekang*, the average microbial populations of bacilli, lactic acid bacteria, yeasts and total viable counts were found at the level of 10^8 cfu/g, 10^5 cfu/g, 10^3 cfu/g and 10^9 cfu/g respectively. In case of *turangbai* samples, a total of 211 isolates of bacilli, 58 isolates of LAB and 26 isolates of yeasts were selected for detailed identification. Similarly, for *bekang* a total of 217 isolates of bacilli, 60 isolates of LAB and 26 isolates of yeasts were selected for detailed identification. On the basis of a combination of phenotypic and genotypic characterization, strains of bacilli isolated from *turangbai* and *bekang* were identified as *Bacillus subtilis*, *B. pumilus*, *B. licheniformis*, *B. cereus*, *B. coagulans*, *B. circulans*, *B. brevis and Lysinibacillus fusiformis*. Similarly, based on phenotypic characterization profiles, strains of LAB were identified as *Lactobacillus brevis* (only in *turangbai* samples), *Enterococcus faecium*, *E. durans*, *E. hirae*, *E. raffinossus and E. cecorum*. Based on the detailed characterizations and identification profiles of yeasts, *Saccharomyces cerevisiae*, *Debaryomyces hansenii* and *Pichia burtonii* were identified. The most dominant microorganism in both

the products was *Bacillus subtilis* (Bacilli) followed by *Enterococcus faecium* (LAB) and *Saccharomyces cerevisiae* (Yeast). Pathogenic bacteria such as *Listeria* sp., *Salmonella* sp., *Shigella* sp. and *Staphylococcus aureus* were not detected in any of the samples analysed. *Bacillus cereus* and enterobacteriaceae were detected in few samples at the level of $\leq 10^2$ cfu/g and they might have introduced during handling of raw materials for preparation. About 45 % of LAB strains of *turangbai* and about 49 % of LAB strains of *bekang* caused coagulation of milk at 30° C and *Enterococcus faecium* showed the lowest acidification value of pH 4.3 in both the products followed by other species of enterococci.

The use of the API-zym technique has 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. Absence of proteinases and presence of strong peptidase and esterase-lipase activities produced by the predominant *Bacillus* strains are possible traits of desirable quality for their use in production of typical flavour and aroma. Most of the bacilli and LAB strains showed proteolytic and amylolytic activity.

B. subtilis TS1:B25 (*turangbai*) and *B. subtilis* BT:B9 (*bekang*) accounted for the highest production og PGA (2.8 mg/ml each)

amongst the other strains tested which suggests that *B. subtilis* is the most potent PGA producer than the other *Bacillus* sp.. Though, all strains of LAB isolated from *turangbai* and *bekang* showed antimicrobial activities against pathogenic bacteria, none of them produced bacteriocin under the applied condition. None of the strains were found to produce biogenic amines in the products analysed. Bacterial adherence to hydrocarbons such as hexadecane, proved to be a simple and rapid method to determine cell surface hydrophobicity. *E. faecium* TM2:L6 (*turangbai*) and BAV:E2 (*bekang*) showed the highest degree of hydrophobicity of 72.7 % and 71.6 % respectively. Percent of hydrophobicity greater than 70 % was arbitrarily classified as hydrophobic, which probably indicates the potential of adhesion to gut epithelial cells of human intestine, advocating their 'probiotic' character. The experimental data also showed that LAB strains were able to degrade anti-nutrients like phytic acid/phytates and oligosaccharides like raffinose and stachyose. Phytic acid has the strong ability to chelate multivalent metal ions, especially zinc, calcium, and iron. The binding can result in very insoluble salts that are poorly absorbed from the gastrointestinal tract, which results in poor bioavailability (BV) of minerals. Oligosaccharides such as raffinose, stachyose and verbascose cause flatulence, diarrhea and indigestion. The present

study also revealed that the extracts of *turangbai* and *bekang* possess antioxidant and free radical (DPPH and ABTS) scavenging activity as they were found to contain phenolic compounds. This thesis would explore the possibility of upgrading traditional fermented soybean products into national and global markets.

Starter cultures of bacilli, previously isolated from native *turangbai* and *bekang* were tested singly or in combination for their ability to ferment soybean to produce *turangbai* and *bekang*. Sensory evaluations were carried out in order to choose the best culture or their combination. It was found that *turangbai* produced using starter 'D'- a mixture of pure culture strains of *B. subtilis* TS2:B24, *B. licheniformis* TSB:B13 and *B. pumilus* TSA:B15 and *bekang* produced using starter 'H'- a mixture of pure culture strains of *B. subtilis* BT:B9, *B. licheniformis* BK1:B13 and *B. pumilus* BK2:B6 selected on the superior technological property as mentioned in the result section, at 40° C for 48 h, organoleptically scored the highest acceptability among the consumers. None of the strains of *Bacillus* used singly, as starters could produce organoleptically acceptable *turangbai* and *bekang*. It is also showed that *turangbai* and *bekang* prepared by cell suspension mixture (starter-'D' and starter-'H' respectively) were more acceptable than *turangbai* and *bekang* prepared by conventional method (market

samples). *Turangbai* and *bekang* prepared using starter cultures had 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. This study has provided a detailed information on the microbiology and functionality of lesser-known ethnic fermented soybean products of Meghalaya and Mizoram.