

SUMMARY

The major objective of this thesis was twofold: (a) the identification of the predominant lactic acid bacteria present in traditional fermented vegetables including bamboo shoot products of Sikkim, Arunachal Pradesh and Manipur in North East India, and (b) the technological properties of the functional lactic acid bacteria to know their role in fermentation and biopreservation of the perishable vegetables. During the first phase, a substantial documentation has been prepared on the twelve different types of traditional fermented vegetables and wild edible bamboo shoot products of Sikkim, Arunachal Pradesh and Manipur, such as goyang, gundruk and inziangsang (fermented leafy vegetables), khalpi (fermented cucumber), sinki (fermented radish tap root), ekung, eup, herring, mesu, soibum, soidon and soijim (all fermented bamboo shoot products). Indigenous knowledge of the ethnic people on preparation and biopreservation methods, and their mode of consumption were documented. All these fermented foods have important bearing in the food habits of the ethnic people of North East India.

A major emphasis in this thesis have been microbiological investigations on the products of vegetable fermentation with regard to both desirable and undesirable microorganisms and their contribution to quality, safety and to biopreservation of the products. For the products goyang, gundruk, inziangsang, khalpi, sinki, eukung, eup, herring, mesu, soibum, soidon and soijim, total microbial numbers of 10^5 to 10^8 cfu/g were detected. The lactic acid bacteria (LAB) dominated in all products whilst yeasts were detected only in low numbers in some samples. Filamentous fungi were not found in any samples. In total 547 bacterial

isolates were grouped as LAB on the basis of phenotypic features. Further phenotypic properties and also genotypic properties (in few strains) were used for the further characterisation of representative strains from each product. Taxonomically diverse species of LAB have been identified from fermented vegetable and bamboo shoots of North East India, with the genus *Lactobacillus* as a major group, representing about 65.4 % of the total isolates. A number of strains were also allocated to the genera *Pediococcus*, *Leuconostoc*, *Lactococcus*, *Enterococcus* and one strain as *Tetragenococcus*.

Identified species of LAB were *Lactobacillus* (*Lb.*) *plantarum*, *Lb. brevis*, *Lactococcus* (*Lc.*) *lactis*, *Enterococcus* (*E.*) *faecium*, *Pediococcus* (*P.*) *pentosaceus* (isolated from goyang); *Lb. plantarum*, *P. pentosaceus* (from gundruk); *Lb. plantarum*, *Lb. brevis*, *P. acidilactici* (from inziangsang); *Lb. plantarum*, *Lb. brevis*, *Leuconostoc* (*Leuc.*) *fallax* (from khalpi); *Lb. plantarum*, *Lb. brevis*, *Lb. casei*, *Leuc. fallax* (from sinki); *Lb. plantarum*, *Lb. brevis*, *Lb. casei*, *Tetragenococcus halophilus* (from ekung); *Lb. plantarum*, *Lb. fermentum* (from eup); *Lb. plantarum*, *Lc. lactis* (from herring); *Lb. plantarum*, *Lb. brevis*, *Lb. curvatus*, *Leuc. citreum*, *P. pentosaceus* (from mesu); *Lb. plantarum*, *Lb. brevis*, *Leuc. fallax*, *Leuc. lactis*, *Leuc. mesenteroides*, *E. durans* (from soibum); *Lb. brevis*, *Leuc. fallax*, *Leuc. lactis* (from soidon); *Lb. brevis*, *Leuc. fallax*, *Leuc. lactis* (from soijim).

Occurrence of *Staphylococcus aureus*, *Bacillus cereus* and enterobacteriaceae in few finish samples below 10^2 cfu/g was observed. *Listeria*, *Salmonella* and *Shigella* were not detected in analysed samples.

Yeasts mostly *Pichia*, *Candida*, *Saccharomyces* and *Rhodotorula* were also present in few samples of goyang, khalpi, sinki, ekung, herring and mesu, possibly as spoilage.

With the aim towards starter culture development, different technological properties of selected strains were investigated. LAB strains isolated from fermented vegetable and bamboo shoot products were screened for their acidifying capacity, and found that most of the strains acidified with lowering of pH. The strains though 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. LAB strains showed antimicrobial activities against a number of potentially pathogenic Gram-negative and Gram-positive bacteria, this can contribute towards the safeguarding of a product. *Lb. plantarum* IB2 (inziangsang) was the only strain found to produce a bacteriocin against *Staphylococcus aureus* S1. Absence of proteinases and presence of strong peptidase and esterase-lipase activities produced by the LAB strains are possible traits of desirable quality for their use in production of typical aroma. Most of the strains degraded phytic acid, and oligosaccharides in the applied methods, showing ability to degrade antinutritive factors. The inability of most strains of LAB to produce biogenic amines is a good indication of their acceptability and their potential for the possible development as starter cultures. High surface hydrophobicity was detected for only a few strains; this property serves as indication of adhesion to intestinal epithelial cells and thus of possible functional properties.

During natural fermentation of gundruk and khalpi, indigenous lactic acid bacterial flora changed spontaneously and at the end of the process *Lactobacillus* species, mainly *Lb. plantarum* was involved. As expected in a typical lactic fermentation, the pH decreased and the titratable acidity increased as the gundruk fermentation progressed. The population of yeasts and pathogenic contaminants disappeared during fermentation. An attempt was made to produce gundruk and also khalpi under optimised condition using selected strains of LAB. It was found that gundruk produced under optimised conditions using a mixture of pure culture strains of *Lb. plantarum* GLn:R1 and *P. pentosaceus* GLn:R1, selected on the superior technological property, at 20° C for 6 days, had highest score of general acceptability. 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. Khalpi produced at 20° C for 72 hour had organoleptically accepted by the consumers. Gundruk and khalpi 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. Proximate composition of fermented vegetable products of North East India was also determined. This dissertation has provided new and practical information on the microbiology and safety of lesser-known traditional fermented vegetable and bamboo shoots of North East India.