SUMMARY

The people of the Eastern Himalayan regions of Nepal; the Darjeeling hills, Sikkim, Assam, Meghalaya and Manipur in India consume different types of traditionally processed smoked/sun-dried/fermented/salted fish products. Sukako maacha and gnuchi are typical smoked and dried fish products prepared and consumed in Nepal, the Darjeeling hills and Sikkim as curry or side-dish by the Nepalis and the Lepcha, respectively. Sidra and sukuti are sun-dried fish products common in the diet of the ethnic people of Nepal, the Darjeeling hills, Sikkim and Bhutan as side-dish or pickle. Ngari and hentak are unique fermented fish cuisine of Manipuri. Tungtap is a traditional fermented fish product consumed by the Khasia tribes in Meghalaya. Karati, bordia and lashim are sun-dried and salted fish products commonly sold in the local markets in Assam.

Some of these products are prepared using indigenous knowledge of the rural people for fish preservation. The traditional technique for fish preservation in the Eastern Himalayan regions involves dehydration (drying), smoking, fermentation and salting (low-salt) for preservation. No fish sauce and shrimp products are prepared and used as condiment in the local diet in the Eastern Himalayan regions.

Seventy-two samples of different fish products were collected from different places of Eastern Nepal (Maglung, Therathum and Aitabare), the Darjeeling hills (Gidhang in Kalimpong sub-division), Sikkim (Gangtok), Meghalaya (Shillong), Assam (Guwahati) and Manipur (Imphal) of the Eastern Himalayan regions and were analysed for microbial load. Microbial load of lactic acid bacteria was 10^4 cfu/g to 10^8 cfu/g, spore-forming rods was $<10^4$ cfu/g, yeasts was 10^3 cfu/g and total viable count was 10^4 cfu/g to 10^8 cfu/g.

Though the load of spore-formers was around 10^4 cfu/g, their presence shows the dominance in fish products next to lactic acid bacteria. Filamentous moulds were not recovered. Out of 527 isolates of microorganisms isolated, 369 strains were lactic acid bacteria, 77 were spore-formers, 27 were aerobic coccii and 54 were yeasts.

Out of 369 lactic acid bacteria strains isolated from seventy-two samples of fish products, 282 isolates were coccii and 87 isolates were non-sporeforming rods. Lactic acid bacteria were identified as *Lactococcus lactis* subsp. *cremoris, Lactococcus plantarum, Lactococcus lactis* subsp. *lactis, Leuconostoc mesenteroides, Enterococcus faecium, Enterococcus faecalis, Pediococcus pentosaceus, Lactobacillus confuses, Lactobacillus fructosus, Lactobacillus amylophilus, Lactobacillus corynifomis* subsp. *torquens* and *Lactobacillus plantarum.*

Endospore-forming rods were identified as *Bacillus subtilis* and *Bacillus pumilus*, aerobic coccii isolates were *Micrococcus*. Yeasts were identified as *Candida chiropterorum, Candida bombicola, Saccharomycopsis* spp.

Prevalence of lactic acid bacteria was 100 %, whereas that of *Bacillus* species, *Micrococcus* species and yeasts was only 83 %, 41 % and 53 % in seventy-two samples analysed, respectively. Lactic acid bacteria (70%) were the dominant microflora in 72 samples of fish products, followed by *Bacillus* spp. (15%), yeasts (10%) and *Micrococcus* (5%). Out of 369 lactics isolated from the different fish products, 77% of coccii dominant the lactic microflora, whereas only 27% rods were present in the fish products.

Pathogenic contaminant was detected in the selective media used. *Bacillus cereus, Staphylococcus aureus* and enterobacteriaceae were

detected in 66%, 54.7% and 68.3% of fish products, respectively. However, none of the sample was found to contain more than 10^2 cfu/g of *Bacillus cereus*, 10^3 cfu/g of *Staphylococcus aureus* and enterobacteriaceae population.

Only three strains, viz. *Enterococcus faecium* GG6, *Lactobacillus cornyformis* subsp. *torquens* T2:L1 and *Leuconostoc mesenteroides* BA4, isolated from gnuchi, tungtap, bordia, respectively showed proteolytic activity with low protease activity (1.0 U/ml). Seven strains of LAB showed amylolytic activity with 3.2 U/ml to 5.8 U/ml α-amylase activity. All *Bacillus subtilis* strains showed amylolytic activity. Six strains of LAB and four strains of *Bacillus* showed lipolytic activity on tributyrin agar plates.

Enzymatic profiles of randomly selected lactic acid bacteria strains of fish products were assayed using the API zym galleries. Each of the predominant LAB strains produced a wide spectrum of enzymes. These strains showed relatively weak esterase and no lipase (C14) activities. *Lactoctoccus lactis* subsp. *cremoris* SM:T1 and *Enterococcus faecium* SM:A1 showed strong phosphatase activities, while other strains showed moderate activities. This method is also of relevance for selection of strains as potential starter cultures on the basis of superior enzyme profiles, especially peptidases and esterase, for accelerated maturation and flavour development of fish products. The absence of proteinases (trypsin and chymotrypsin) and presence of peptidase (leucine-, valine- and cystine-arylamidase) and esterase-lipase (C4 and C8) activities produced by the predominant organisms isolated from fish products are possible traits of desirable flvaour in the products.

The antagonistic properties of the strains, isolated from fish products of the Eastern Himalayan regions were tested against the

indicator strains (*Listeria monocytogenes* DSM 20600, *Bacillus cereus* CCM 2010, *Enterococcus faecium* DSM 20477 and *Streptococcus mutans* DSM 6178). Some of the strains such as *Enterococcus faecium* SM:A1, *Pediococcus pentasaceus* GG2, *Lactococcus plantarum* CG1:B1, *Lactococcus plantarum* SG1:B3, *Leuconoctoc mensenteroides* BA4, *Lactobacillus coryniformis* subsp. *torquens* T2:L1, *Lactococcus lactis* subsp. *cremoris* KA1 showed the antagonistic properties against the indicator strains. This reveals that some of these LAB strains have antimicrobial activities, which can reduce the number of other undesired microorganism in the fish products as well as help in the preservation of fish. However, none of the strains were found to produce any bcteriocin with the methods applied.

Thirty three strains were tested for biogenic amine production with the surface plate method applied. None of the strains were produced tyramine, cadaverine, histidine and putrescine in the applied method. This result indicated that biogenic amine is not produced by the dominant microorganisms (LAB and *Bacillus* spp.) in fish products, which also correlated that these traditionally processed fish products are safe to eat.

Nine strains of LAB isolated from traditionally processed fish products showed high degrees of hydrophobicity (>75%), among which *Pediococcus pentosaceus* GG2 (isolated from gnuchi) showed the highest degree of hydrophobicity of 94%, showing strong hydrophobic properties. All strains of LAB had >30% hydrophobicity, indicating that the strains isolated from fish products of the Eastern Himalayas was not hydrophilic in nature. High degree of hydrophobicity by the lactic acid bacteria isolated from lesser-known traditional fish products of the

Eastern Himalayas indicates the potential of adhesion to gut epithelial cells of human intestine, advocating their 'probiotic' character.

Proximate composition of sukako maacha, gnuchi, sidra, sukuti, ngari, hentak, tungtap, karati, bordia and lashim was analysed. The pH of all these products was 6.2-6.5 with titratable acidity ranging from 0.5 to 1.1%. Moisture content was low upto 10 % in sukako maacha, however, hentak, the simi-paste product contained 40% of moisture. Drying in the sun or smoking during preservation, as a result of dehydration, most of the fish products have low moisture content. High content of protein was observed in all analysed fish products, indicating increasing protein intake in the local diet. Among the minerals of the fish products, calcium content was higher than other minerals estimated. Among the fish products analysed, gnuchi contained highest amount of calcium and magnesium. Due to low moisture content and slightly acidic in nature, the shelf-like of the product can be prolonged and can be kept for longer period at room temperature.

Traditional processing of perishable fish such as smoking, drying, salting and fermentation are principal methods of bio-preservation without refrigeration or addition of any synthetic preservative in the Eastern Himalayas. Though, the traditionally processed fish products are lesser-known, role of LAB in fermentation/process enhancing functional properties such as wide spectrum of enzymatic activities or profiles, antimicrobial activities, probiotic (adherence character showing high degree of hydrophobcity), and even non-producer of biogenic amine is remarkable observation in this study.

This study has demonstrated that microbial diversity ranging from species of lactic acid bacteria belonging to coccii-lactics (*Lactococcus, Enterococcus, Pediococcus, Leuconostoc*) to species of

homo- and hero-fermentative rods (*Lactobacillus*) belonging to lactic acid bacteria, *Bacillus subtilis, Bacillus pumilus., Micrococcus* spp. to species of yeasts (*Candida, Saccharomycopsis*) were present in the lesser-known traditionally processed fish products of the Eastern Himalayan regions.

Table C shows the schematic presentation of microbial diversity in the traditionally processed fish products of the Eastern Himalayan regions. The isolated, identified and preserved microorganisms from lesser-known fish products may contribute significant information on unknown microbial gene pool as genetic resources of the Himalayan regions.