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Role of Lactic Acid Bacteria and their Functional Properties in *Goyang*, a Fermented Leafy Vegetable Product of the Sherpas

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ABSTRACT

Goyang *is an ethnic fermented leafy vegetable prepared and consumed by the Sherpas of Sikkim, the Darjeeling hills and Nepal. Isolation, characterization and identification of predominant lactic acid bacteria (LAB) from samples of* goyang, *and their functional properties were studied. Total population of LAB was recorded at the level of* 10^7 *cfu/g, and was identified as* Lactobacillus plantarum, Lact. brevis, Lact. lactic, Enterococcus faecium, Pediococcus pentosaceus. *Prevalence of LAB showed that lactobacilli were reprenting 59.5 %, pediococci and lactococci 10.8 % , and enterococci 18.9 %, respectively. This study showed that strains of LAB played important role by their functional properties related to acidifying capacity, degradation of anti-nutritive factors, tolerance to bile-salt, and non-producers of biogenic amines.*

INTRODUCTION

Ethnic fermented vegetables constitute an important part of the local diet in the Himalayan food culture (Tamang *et al*., 2005). 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. The daily per capita consumption of fermented foods in Sikkim is 163.8 g representing 12.6 % of the total daily food consumed in the local diet (Tamang *et al*., 2007). A variety of fermented vegetable products are prepared and consumed in the Eastern Himalayan regions of India, Nepal and Bhutan (Tamang *et al*., 1988).

Goyang is an ethnic fermented non-salted, slightly acidic vegetable food in the local diet of the Sherpas of Sikkim and Nepal. *Goyang* is prepared during rainy season when the leaves of wild plant locally called 'magane-saag' (*Cardamine macrophylla* Willd.), belonging to the family Brassicaceae, are plenty. During preparation, 'magane-saag' are collected, washed and cut into pieces, then squeezed to drain off excess water, and are tightly pressed into the bamboo baskets lined with 2-3 layers of leaves of fig plants. Top of the baskets are then covered with fig plant leaves, and fermented at room temperature (~15-25° C) for a month (Fig 1). Now-a-days, poly-bags or glass jars are used instead of bamboo baskets which are easy for maintaining anaerobic condition during preparation of *goyang*.

After completion of desired fermentation, fresh *goyang* is transferred into an air tight container which can be stored for 2-3 months. However, shelf-life of *goyang* can be prolonged by making the freshly fermented *goyang* into balls and sun dry. Such sun-dried *goyang* can be kept for several months at the room temperature. *Goyang* is generally prepared at household only for consumption; there is no report of selling in the local markets. *Goyang* is boiled in a soup along with yak or beef meat and noodles to make a thick 'thukpa', a common staple food of the Sherpas (Tamang, 2006).

To the best of our knowledge, *goyang* has so far not been investigated scientifically. The present investigation aims at identifying and studying the role of lactic acid bacteria in *goyang* production.

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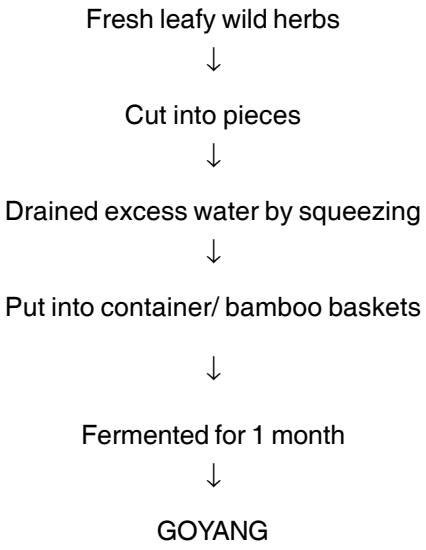

Fig 1. Flow sheet of traditional method of goyang preparation at Toong in Sikkim.

MATERIALS AND METHODS

Collection of Samples

Samples of *goyang* were collected from different regions of Sikkim aseptically in sterile bottles kept in an ice-box, and transported to the laboratory for analyses.

Microbiological analysis

Samples (10 g) of each product were mixed with 90 ml of 0.85 % (w/v) sterile physiological saline and homogenised in a Stomacher lab-blender (400, Seward, UK) for 1 min. A serial dilution in the same diluent was made. Lactic acid bacteria (LAB) were isolated on plates of MRS agar (M641, HiMedia) supplemented with 1 % $CaCO_3$ and incubated at 30° C in an anaerobic gas-jar (LE002, HiMedia) for 48-72 h. Total viable counts were determined on plate count agar (M091A, HiMedia) incubated at 30° C for 48-72 h. Colonies of moulds and yeasts were examined on potato dextrose agar (M096, HiMedia) and yeast-malt (YM) agar (M424, HiMedia), supplemented with 10 IU/ml benzylpenicillin and 12 mg/ ml streptomycin sulphate, respectively, which were incubated aerobically at 28° C for 72 h. Isolated colonies based on colony morphology were selected randomly from the highest diluted plates. Purity of the isolates was checked by streaking again and sub-culturing on fresh agar plates of the isolation media, followed by microscopic examinations. Purified isolates of LAB were preserved at -20° C in MRS broth (M369, HiMedia) with 15 % (v/v) glycerol added. Enumeration of pathogenic contaminants from the samples were done in selective media such as *Bacillus cereus* agar base (M833, HiMedia) for *Bacillus cereus*, Baird Parker agar base (M043, HiMedia) for *Staphylococcus aureus* and Violet Red Bile Glucose agar w/o lactose (M581, HiMedia) for enterobacteriaceae (Han *et al*., 2001). *Salmonella-Shigella* Agar (M108, HiMedia) was used for the detection of *Salmonella* and *Shigella* and *Listeria* identification agar base (M1064, HiMedia, Mumbai) with *Listeria* selective supplement (FD 061, HiMedia) for *Listeria* in the samples following the method of Metaxopolous *et al*. (2001).

Characterization and identification

Cell morphology of all bacterial isolates and their motility were determined using a phase contrast microscope (Olympus CH3-BH-PC, Japan). Bacterial isolates were Gram-stained and tested for catalase production by placing a drop of 10 % hydrogen peroxide solution on isolates, and were preliminarily identified on the basis of carbon dioxide production from glucose, ammonia production from arginine, growth at different temperatures (10°C, 15°C, 45°C), the ability to grow in different concentrations of sodium chloride (6.5 %, 10 %, 18 %) and pH (3.9, 9.6) in MRS broth (M369, HiMedia, India), following the methods of Schillinger and Lücke (1987), and Dykes *et al*. (1994). The configuration of lactic acid produced from glucose was determined enzymatically using D-lactate and L-lactate dehydrogenase test kits

(Tamang *et al*., 2005). The presence of *meso*-diaminopimelic acid (DAP) in the cell walls of LAB was determined on cellulose plates using a thin layer-chromatography (Tamang *et al*., 2000). Sugar fermentation of LAB isolates were determined by the API 50 CHL test strips (bioMérieux, France) and the identifications were interpreted using APILAB PLUS software (bioMérieux, France). Taxonomical keys of Simpson and Taguchi (1995), Wood and Holzapfel (1995) were followed for identification of LAB isolates.

pH and Acidification

The pH of the samples (10 g) was determined directly using a digital pH meter (Type 361, Systronics) calibrated with standard buffer solutions (Merck, Germany), after homogenization in 20 ml of carbondioxide free distilled water (AOAC, 1990). The acidification and coagulating abilities of the LAB isolates was assayed by inoculating 10 % skim milk with 24 h old cultures (RM1254, HiMedia) at 1 % level, and incubated at 30° C. Observation was made for commencement of clotting, and pH was measured after 72 h of incubation (Olasupo *et al*., 2001).

Bile salt tolerance

The ability of LAB strains to grow in presence of bile salt was performed by spotting about 10 µl of the overnight culture directly on the assay medium consisted of MRS agar supplemented with 0.5% bile salt (Merck) (Olasupo *et al*., 2001).

Phytic acid degradation

Ability of LAB isolates to degrade phytic acid was determined on a synthetic phytic acid screening medium (Holzapfel, 1997), containing calcium phytate (Sigma, USA) as sole phosphate source. Control was prepared without calcium phytate. In preparing the medium, phytate and salts were added separately. After adding glucose, Na-citrate, magnesium sulfate, manganese sulfate and ferrous sulfate to the phytate solution, the pH was adjusted to 6.0 and the medium was autoclaved. Vitamins, amino acids and nucleotides were filter sterialised and added to the medium before plating. The pH of the medium was finally adjusted between 5.8-6.0. The plates were streaked with 24 hour-old broth culture and incubated aerobically at 30° C for 5 days. Clear zone around the colony of the test organism indicated a positive reaction.

Degradation of oligosaccharides

Screening of LAB for degradation of oligosaccharides such as stachyose and raffinose were performed in MRS broth without beef extract (pH 6.4) containing 2% stachyose and 1% raffinose (instead of glucose), respectively, and 0.004% chlorophenol red as indicator. Inoculation was followed by incubation at 30° C for 3 days (Holzapfel, 1997).

Hydrophobicity assay

Bacterial adhesion to hydrocarbons was determined using n-hexadecane (RM 2238, HiMedia) as described by Dewan and Tamang (2007). Fresh cultures were grown in MRS broth at 30°C for 24 hour and centrifuged at 8,000 *g* for 5 min. The pellet was washed with 9 ml of Ringer solution (Merck, Germany), and 1 ml of the suspension was taken for measurement of absorbance at 580 nm. Then, 1.5 ml of the suspension was mixed with equal volume of n-hexadecane (RM 2238, HiMedia) and mixed thoroughly in a vortex. The phases were allowed to separate for 30 min at room temperature, after which aqueous phase was carefully removed and absorbance at 580 nm was measured. The percentage hydrophobicity was expressed as follows: hydrophobicity % = $[A_0\text{-}A/A] \times 100$, where A_0 and A are the absorbance values of the aqueous phase before and after contact with n-hexadecane**.** The percent hydrophobic index greater than 70% was arbitrarily classified as hydrophobic (Nostro *et al*., 2004).

Antimicrobial activity

The LAB isolates were screened for antimicrobial activity by the agar spot method (Schillinger and Lücke, 1989). The indicator strains used for antagonism were *Listeria innocua* DSM 20649, *L. monocytogenes* DSM 20600, *Bacillus cereus* CCM 2010, *Staphylococcus aureus* S1, *Pseudomonas aeruginosa* BFE 162, *Enterobacter agglomerans* BFE 154, *Ent. cloacae* BFE 282, and *Klebsiella pneumoniae* subsp. *pneumoniae* BFE 147. Cell-free neutralized supernatants of LAB isolates were also screened for bacteriocin activity by the agar spot test method (Uhlman *et al*., 1992).

Screening for biogenic amine production

Biogenic amine-forming capacity of LAB isolates was determined qualitatively in medium (Joosten and Northold, 1989) containing histidine, lysine, ornithine and tyrosine. Change of the bromocresol purple used as indicator to purple colour was considered as positive reactions of amino acid decarboxylase activity (Bover-Cid and Holzapfel, 1999).

RESULTS AND DISCUSSION

A total of 20 samples of goyang were analysed for microbial counts (Table 1). The population of LAB, yeasts and total viable counts was found at the level of 10^7 cfu/g, 10^5 cfu/g and 10^8 cfu/g, respectively. Filamentous mould

Table 1. Microbiological populations of *goyang*

Product	Region	Place of collection	Log cfu/g sample		
			LAB	Yeast	AMC
Goyang (pH 6.5 ± 0.1)	North Sikkim	Toong (n = 5)	6.8 ± 0.1	4.5 ± 1.0	8.3 ± 0.5
	East Sikkim	Tshangu (n = 7)	6.8 ± 0.1	5.0 ± 0.6	7.8 ± 0.2
	East Sikkim	Chipsu (n = 8)	7.0 ± 0.1	4.8 ± 0.1	8.0 ± 0.1

n, number of samples collected. Mould was not detected.

Data represents the means (± SD) of number of samples. Mean pH (± SD) of each sample is shown in parenthesis.

LAB, lactic acid bacteria; AMC, aerobic mesophilic count.

was not recovered in any sample. *Staphylococcus aureus* and enterobacteriaceae were detected in few samples at the level of less than 10^2 cfu/g. Food borne pathogens *Listeria* sp., *Salmonella* sp. and *Shigella* sp. were not detected in any sample of goyang.

All bacterial strains isolated from *goyang* were considered lactic acid bacteria because they grew well in anaerobic agar and formed clear halo in $CaCO_3$ supplemented MRS agar plates, were Gram-positive, catalase-negative bacteria, non-motile and did not form spores. A grouping of all LAB isolates was done based on cell morphology, gas production from glucose and ammonia production from arginine (Table 2). Representative strains of LAB were selected randomly from each grouped strains having similar morphology, ability to produce gas from glucose and hydrolyse arginine, isolated from the respective sample. Of the 26 representative strains of LAB isolated from goyang, 3 strains were heterofermentative rods, 12 were homofermentative rods, 5 were homofermentative cocci, 3 coccoid rod and 3 tetrad-forming cocci (Table 2). All cocci forming tetrads were presumptively grouped as pediococci. Further differentiation of all tetrad forming cocci was performed by using the key proposed by Simpson and Taguchi (1995) based on the ability to grow at pH 8.5, pH 4.2, at 50° C and in the presence of 10 % NaCl. On the basis of these tests, 3 tetrad strains Go1, Go17, Go29 from *goyang* (Table 3) were identified as *Pediococcus pentosaceus*. The sugar fermentation

Table 2. Grouping of representative strains of the LAB isolated from *goyang*

Product	Cell shape	Gas from glucose	Arginine hydrolysis	Grouped Strains	Representative strains	
					Total No.	Strain code
Goyang (55)	Rod	–	–	21	12	Go6, Go12, Go14, Go16, Go22, Go23, Go25, Go30, Go31, Go37, Go39, Go40
	Rod	+	+	7	3	Go11, Go24, Go41
	Coccoid rod	–	–	5	2	Go7, Go8
	Coccoid rod	–	+	5	1	Go5
	Coccus	–	+	10	5	Go9, Go20, Go34, Go43, Go45
	Coccus/ tetrad	–	+	7	3	Go1, Go17, Go29

Total number of isolates is given in parenthesis

Table 3. Phenotypic characteristics of the LAB isolated from *goyang*

Species	**Number of strains**	**CO_2 from glucose**	**NH_3 from arginine**	**Growth at/in**					**Isomer of lactate**
				45°C	**pH 3.9**	**pH 9.6**	**NaCl 6.5%**	**DAP**	
Lactobacillus brevis	3	+	+	-	+	-	1/2	-	DL
Lactobacillus plantarum	12	-	-	-	+	-	+	+	DL
Lactococcus lactis	3	-	1/2	-	-	+	+	-	L
Enterococcus faecium	5	-	+	+	-	+	+	-	L
Pediococcus pentosaceus	3	-	+	+	+	-	+	-	DL

+, all strains positive; -, all strains negative; (../..), number of positive/negative strains; all s

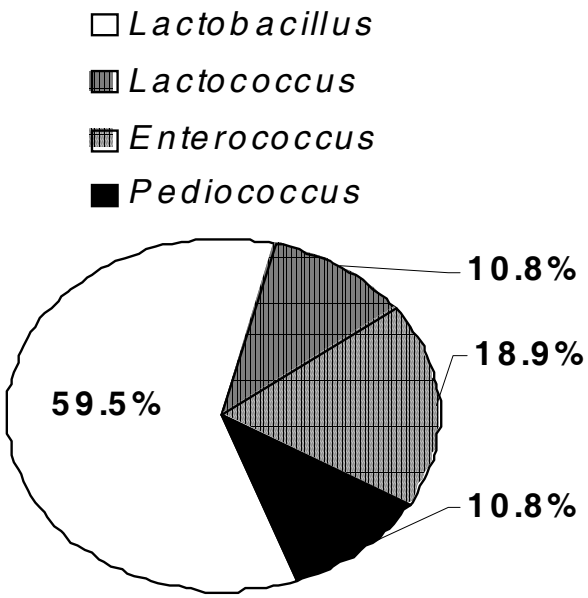

Fig 2. Percentage prevalence of LAB in *goyang*

profiles using API system also confirmed them as *Pediococcus pentosaceus.* All 12 representative homofermentative rods strains isolated from goyang (Table 2), shared the ability to grow at 15° C, contained DAP in the cell wall and produced DL lactate from glucose. They were able to ferment the majority of the 16 carbohydrates shown in Table 3 and were presumptively identified as *Lactobacillus plantarum*. The heterofermetative rod produced DL lactate from glucose and were able to grow at 15° C, but not at 45° C (Table 3). All the heterofermentative rods were identified as *Lactobacillus brevis* based on the sugar fermentation using API system.

Three coccoid rod strains (Go5, Go7, Go8) (Table 3) which were non-gas producer, unable to grow at pH 3.9, in 6.5 % NaCl and at 45° C but grew well at 10° C, 15° C and in pH 9.6 were identified as *Lactococcus lactis* based on sugar profiles (Table 3); while 5 cocci strains (Go9, Go20, Go34, Go43, Go45) growing at 6.5% NaCl, at 45° C and on the basis of sugar fermentation profiles (Table 3) were identified as *Enterococcus faecium*. The identity of the LAB seems to correspond with that of LAB typically reported for fermented vegetable products of different origin and in various regions (Steinkraus, 1996; Lee, 1997). The most dominant LAB in *goyang* were *Lactobacillus* represented by 59.5 % (homofermentative lactobacilli 51.4 % and heterofermentative lactobacilli 8.1 %) followed by *Enterococcu*s (18.9 %), *Lactococcus* (10.8 %) and *Pediococcus* (10.8 %) (Fig 2).

All yeast strains were phenotypically characterized including sugar fermentation and assimilation tests (data not shown). Based on the taxonomic keys of Kurtzman and Fell (1998) and Yarrow (1998), yeasts strains were identified as species of *Candida*. Identification to species level was not confirmed.

Technological properties

The pH of the samples of *goyang* was around 6.5 (Table 1). Acidification is an important technological property in relevance of selection for starter culture among the LAB (De Vuyst, 2000). The ability of some species of LAB particularly *Lb. plantarum* in lowering pH of the substrates is significant in food preservation (Brown and Booth, 1991). All the LAB strains acidified and coagulated skim milk (Table 4). The coagulation of skim milk occurred within 19-24 hour at 30° C.

The ability of the LAB strains to hydrolyse bile salts was tested. All strains showed positive reaction. Bile tolerance is one of the criteria for selection of strains for probiotic properties (Hyronimus *et al*., 2000).

LAB strains were screened for their ability to degrade antinutritive factors (Table 4). About 50 % of LAB strains degraded phytic acids, and 46 % degraded raffinose. None of the strains were able to degrade stachyose. Oligosaccharides such as raffinose, stachyose and verbascose cause flatulence, diarrhea and

Table 4. Technological properties of LAB isolated from *goyang*

Strain	Acidification (pH)	Phytic acid	Raffinose	% Hydrophobicity
Control	6.8			
P. pentosaceus Go1	5.1	+	–	1.9 ± 0.2
Lc. lactis Go5	4.6	+	–	3.2 ± 0.7
Lb. plantarum Go6	4.5	–	–	6.4 ± 2.3
Lc. lactis Go7	5.4	–	+	8.0 ± 0.9
Lc. lactis Go8	5.6	+	–	5.3 ± 0.4
E. faecium Go9	4.9	+	+	2.5 ± 0.9
Lb. brevis Go11	6.0	–	–	2.0 ± 0.8
Lb. plantarum Go12	5.0	+	–	63.4 ± 7.4
Lb. plantarum Go14	5.0	–	–	55.2 ± 5.2
Lb. plantarum Go16	4.5	+	+	30.4 ± 4.3
P. pentosaceus Go17	4.9	–	–	46.3 ± 3.7
E. faecium Go20	4.9	–	+	3.2 ± 1.0
Lb. plantarum Go22	4.8	–	–	1.1 ± 0.4
Lb. plantarum Go23	4.1	–	–	6.3 ± 3.4
Lb. brevis Go24	4.2	+	–	10.6 ± 3.5
Lb. plantarum Go25	4.0	+	+	8.8 ± 3.0
P. pentosaceus Go29	4.7	+	–	23.1 ± 4.2
Lb. plantarum Go30	4.2	+	+	6.5 ± 1.2
Lb. plantarum Go31	4.2	+	+	7.2 ± 1.3
E. faecium Go34	4.8	+	–	4.2 ± 1.7
Lb. plantarum Go37	4.6	+	+	20.2 ± 2.1
Lb. plantarum Go39	4.3	–	+	24.5 ± 2.5
Lb. plantarum Go40	4.3	–	+	4.7 ± 1.4
Lb. brevis Go41	5.0	–	+	1.1 ± 0.3
E. faecium Go43	4.8	–	+	3.1 ± 0.4
E. faecium Go45	4.9	–	–	1.9 ± 0.4

Data represents the means (± SD) of four sets.

All strains coagulated skim milk except *Lactococcus lactis* Go8

All strains hydrolysed bile salt. None of the strains fermented stachyose.

indigestion (Holzapfel, 1997). Due to these nutritional consequences, the degradation of antinutritive factors in food products by fermentation is desirable as reported for a number of foods of plant origin (Chavan and Kadam, 1989; Mbugua *et al*., 1992).

Table 4 shows the percentage hydrophobicity of the LAB strains. Out of 26 strains, 23 strains had less than 30 % hydrophobicity. Only 3 strains *Lb. plantarum* Go12 (63.4 %) *Lb. plantarum* Go14 (55.2 %), and *P. pentosaceus* Go17 (46.3 %) showed appreciable hydrophobicity property. Ability to adhere to the hydrocarbons, such as hexadecane is one of the criteria to determine the cell surface hydrophobicity (van Loosdrecht *et al*., 1987).

Antagonistic activities of LAB strains were tested against different indicator strains used such as *Listeria innocua* DSM 20649, *L. monocytogenes* DSM 20600, *Bacillus cereus* CCM 2010, *Staphylococcus aureus* S1, *Enterococcus faecium* DSM 20477*, Streptococcus mutans* DSM 6178*, Klebsiella pneumoniae* subsp. *pneumoniae* BFE 147, *Enterobacter cloacae* BFE 282, *E. agglomerans* BFE 154 and *Pseudomonas aeruginosa* BFE 162. The LAB strains showing the inhibition zones of than of >4 mm in agar-spot plates were *Lb. plantarum* Go12, Go14, Go22, Go40 and *P. pentosaceus* Go29 (data not shown) against *L. innocua* DSM 20649. This reveals that antimicrobial properties of functional LAB can reduce the number of other undesired microorganisms in vegetable products. However, none of the strain produced bacteriocin against any other indicator strains under the test conditions applied.

Strains of LAB isolated from goyang were tested for their ability to produce biogenic amines (Table 4). None of the strains produced biogenic amines with the method applied. Leafy vegetables usually contain low level of biogenic amines but it may increase during fermentation due to decarboxylase activity of microorganisms (Simon-Sarkadi *et al*., 1994; Silla-Santos, 2001). The inability of strains of LAB to produce biogenic amines is a good indication of their acceptability and their potential for the possible development as starter culture. The production of biogenic amines by LAB to be selected as starter cultures is not a desirable property (Buchenhüskes, 1993; Holzapfel, 1997).

Indigenous knowledge of the Sherpa on production of fermented vegetable products was worth documenting, both as low-cost functional foods, and for socio-cultural reasons. The strains of LAB play an important and partly complex role in the traditional fermentation processes by their functional properties in *goyang*.

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REFERENCES

AOAC (1990). *Official Methods of Analysis*, 15th edition. Association of Official Analytical Chemists, Virginia.

Bover-Cid, S. and Holzapfel, W.H. (1999). Improved screening procedure for biogenic amine production by lactic acid bacteria. *International Journal of Food Microbiology* **53**: 33-41.

Brown, M.H. and Booth, J.R. (1991). Acidulants and low pH. In: *Food Preservatives*, (Eds. Russel, N.J. and Gould, G.W.), pp. 22-43, Blackie, Glasgow.

Buchenhüskes, H.J. (1993). Selection criteria for lactic acid bacteria to be used as starter cultures in various food commodities. *FEMS Microbiology Reviews* **12**: 253-272.

Chavan, J.K. and Kadam, S.S. (1989). Nutritional improvement of cereals by fermentation. *Critical Review on Food Science and Nutrition* **28**: 349-400.

De Vuyst, L. (2000). Technology aspects related to the application of functional starter culture. *Food Technology and Biotechnology* **38 (2)**: 105-112.

Dewan, S. and Tamang, J.P. (2007). Dominant lactic acid bacteria and their technological properties isolated from the Himalayan ethnic fermented milk products. *Antonie van Leeuwenhoek* **92**: 343-352.

Dykes, G.A., Britz, T. J. and von Holy, A. (1994). Numerical taxonomy and identification of lactic acid bacteria from spoiled, vacuum packaged Vienna sausages. *Journal of Applied Bacteriology* **76**: 246-52.

Han, B.Z., Beumer, R.R., Rombouts, F.M. and Nout, M.J.R. (2001). Microbiological safety and quality of commercial sufu- a Chinese fermented soybean food. *Food Control* **12**: 541-547.

Holzapfel, W.H. (1997). Use of starter cultures in fermentation on a household scale. *Food Control* **8 (5&6)**: 241-258.

Hyronimus, B., Le Marrec, C., Hadj Sassi, A. and Deschamps, A. (2000). Acid and bile tolerance of spore–forming lactic acid bacteria. *International Journal of Food Microbiology* **1**: 193-197

Joosten, H.M.L.J. and Northolt, M.D. (1989). Detection, growth and amine-producing capacity of lactobacilli in cheese. *Applied Environmental Microbiology* **55**: 2356-2359.

Kurtzman, C.P. and Fell, J.W. (1998). *The Yeast, A Taxonomic Study*, 4th edition. Elsevier Science, Amsterdam.

Lee, C.-H. (1997). Lactic acid fermented foods and their benefits in Asia. *Food Control* **8**: 259-269.

Mbugua, S.K., Ahrens, R.H., Kigutha, H.N., Subramanian, V. (1992). Effect of fermentation, malted flour treatment and drum drying on nutritional quality of uji. *Ecology of Food Nutrition* **28**: 271-277.

Metaxopoulos, J., Samelis, J. and Papadelli, M. (2001). Technological and microbiological evaluation of traditional process as modified for the industrial manufacturing of dry fermented sausages in Greece. *Italian Journal of Food Science* **13**: 3-18.

Nostro, A., Cannatelli, M.A., Crisafi, G., Musolino, A.D., Procopio, F. and Alonzon, V. (2004). Modifications of hydrophobicity, *in vitro* adherence and cellular aggregation of *Streptococcus mutans* by *Helichrysum italicum* extract. *Letters in Applied Microbiology* **38**: 423-427.

Olasupo, N.A., Schillinger, U. and Holzpafel, W.H. (2001). Studies on some technological properties of predominant lactic acid bacteria isolated from Nigerian fermented foods. *Food Biotechnology* **15 (3)**: 157-167.

Schillinger, U. and Lücke, F.K. (1987). Identification of lactobacilli from meat and meat products. *Food Microbiology* **4**: 199-208.

Schillinger, U. and Lücke, F.K. (1989). Antibacterial activity of *Lactobacillus sake* isolated from meat. *Applied and Environmental Microbiology* **55 (8)**: 1901-1906.

Silla-Santos, M.H. (2001). Toxic nitrogen compounds produced during processing: biogenic amines, ethyl carbamides, nitrosamines. In: *Fermentation and Food Safety*, (Eds: Adams, M.R. and Nout, M.J.R.), pp. 119-140. Aspen Publishers, Inc. Gaithersburg, Maryland.

Simon-Sarkadi, L., Holzapfel, W.H. and Halasz, A. (1994). Biogenic amines content and microbial contamination of leafy vegetables during storage at 5 C. *Journal of Food Biochemistry* **17**: 407-418.

Simpson, W.J. and Taguchi, H. (1995). The genus *Pediococcus*, with notes on the genera *Tetragenococcus* and *Aerococcus*. In: *The Genera of Lactic Acid Bacteria*, (Eds. Wood, B.J. and Holzapfel, W.H.), pp.125-172. Blackie Academic and Professional, London.

Steinkraus, K.H. (1996). *Handbook of Indigenous Fermented Food*, 2nd edition. Marcel Dekker, Inc., New York.

Tamang, B. (2006). *Role of lactic acid bacteria in fermentation and biopreservation of traditional vegetable products*. Ph. D. Thesis, Sikkim Government College (under North Bengal University), Gangtok. pp. 274.

Tamang, J.P., Dewan, S., Thapa, S., Olasupo, N. A., Schillinger, U. and Holzapfel, W. H. (2000). Identification and enzymatic profiles of predominant lactic acid bacteria isolated from soft-variety *chhurpi*, a traditional cheese typical of the Sikkim Himalayas. *Food Biotechnology* **14** (1&2): 99-112.

Tamang, J.P., Sarkar. P.K. and Hesseltine, C.W. (1988). Traditional fermented foods and beverages of Darjeeling and Sikkim - a review. *Journal of Science of Food and Agriculture* **44**: 375-385.

Tamang, J.P., Tamang, B. Schillinger, U., Franz, C.M.A.P., Gores, M., and Holapfel, W. (2005). Identification of predominant lactic acid bacteria isolated from traditionally fermented vegetable products of the Eastern Himalayas. International Journal of Food Microbiology **105**: 347-356.

Tamang, J.P., Thapa, N., Rai, B., Thapa, S., Yonson, Y., Dewan, S., Tamang, B., Sharma, R.M., Rai, A. and Chettri, R. (2007). Food consumption in Sikkim with special reference to traditional fermented foods and beverages: A micro-level survey. *Journal of Hill Research* **20 (1)**, 2007 (Supplementary Issue).

Uhlman, L., Schillinger, U., Rupnow, J.R. and Holzapfel, W.H. (1992). Identification and characterization of two bacteriocin-producing strains of *Lactococcus lactis* isolated from vegetables. *International Journal of Food Microbiology* **16**:141-151.

van Loosdrecht, M.C.M., Lyklema, J., Norde, W., Schraa, G. and Zehnder, A.J.B. (1987). The role of bacterial cell wall hydrophobicity in adhesion. *Applied and Environmental Microbiology* **53 (8)**:1893-1897.

Wood, B.J.B. and Holzapfel, W.H. (1995). *The Lactic Acid Bacteria,* vol. 2: *The Genera of Lactic Acid Bacteria*. Blackie Academic & Professional, London.

Yarrow, D. (1998). Methods for the isolation, maintenance and identification of yeasts. In: *The Yeast, a Taxonomic Study*, 4th edition, (Eds: Kurtzman, C.P. and Fell, J.W.), pp. 77-105. Elsevier Science, Amsterdam.