Role of Lactic Acid Bacteria in Fermentation and Biopreservation of Traditional Vegetable Products

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This is to certify that the work presented in the thesis entitled: **Role of Lactic Acid Bacteria in Fermentation and Biopreservation of Traditional Vegetable Products** has been carried out by **Shri Buddhiman Tamang** under my guidance and supervision at Food Microbiology Laboratory of Department of Botany, Sikkim Government College, Gangtok. The results incorporated in this thesis have not been submitted for any degree elsewhere.

I also certify that Shri Tamang has followed the rules and regulation of this college in carrying out the work, and also fulfilled the conditions for submission of thesis to North Bengal University.

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INTRODUCTION

Biopreservation refers to extended storage life and enhanced safety of foods using the natural microflora and their antibacterial products (Stiles, 1996). Food fermentation has been used for centuries as a method to preserve perishable food products (Geisen and Holzapfel, 1996; Hansen, 2002). Lactic acid fermentation serves as affordable and manageable techniques for food preservation in absence of facilities for home refrigeration and freezing (Steinkraus, 1983; Nout and Sarkar, 1999). It represents a distillation of knowledge and wisdom gained by experience and based on trial and error. People might not be able to explain what is going on during fermentation, but they certainly know what do to get the desired products. They knew how to provide favourable conditions for fermentation and thus to promote the beneficial microorganisms for fermentative production of foods (Lee, 2001). However, scientific knowledge on the biochemical changes and on the identity of these microorganisms were unknown, partly unto the recent time. Numerous types of traditional fermented foods all over the world have already been microbiologically explored.

Bacteria, mostly lactic acid bacteria (LAB), yeasts and filamentous moulds constitute the microbiota of fermented foods and beverages, which are present in or on the ingredients, utensils, environment, and are selected through adaptation to the substrates (Hesseltine, 1983, Steinkraus, 1997). In the Indian sub-continent, mostly due to wide variation in agro-climatic conditions and diverse form of food culture of different ethnic people, a diversity of microorganisms is associated with traditional fermented foods and beverages (Soni and Sandhu, 1990;

Tamang, 1998). Microorganisms change the chemical composition of raw materials during food fermentation enhancing the nutritive value, enriching the blend diet with improved flavour and texture, preserving the perishable foods, fortifying the products with essential amino acids, vitamins, minerals, degrading the undesirable components and antinutritive factors, improving the digestibility, and stimulating the probiotic functions (Steinkraus, 1994; Stiles and Holzapfel, 1997; Adams and Nout, 2001). Among the various microorganisms present in food fermentation, LAB are the dominant microfloras in many traditional fermented foods such as vegetable products (Kim and Chun, 2005), fermented milk products (Shah, 2005), cereal products (Nout, 2001), meat products (Rantsiou and Cocolin, 2006), and fish products (Adams, 1998). Lactic acid bacteria are Gram-positive, nonsporing, catalase-negative, devoid of cytochromes, of nonaerobic habit but aerotolerant, fastidious, acid-tolerant, and strictly fermentative with lactic acid as the major end-product during sugar fermentation (Axelsson, 1998; Klein *et al.*, 1998). LAB produce lactic acid during traditional fermentation, the characteristic fermentative product, which reduce pH of the substrate to a level where growth of putrefactive, pathogenic and toxinogenic bacteria are inhibited (Holzapfel *et al.*, 1995). The LAB which are usually designated as "GRAS" (generally recognised as safe) status in foods (Donohue and Salminen, 1996), can also exert as 'biopreservative' (Holzapfel *et al.*, 2003). The genera of LAB mostly present in foods are *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus*, *Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus*, *Vagococcus* and *Weissella* (Stiles and Holzapfel, 1997;

Carr *et al.*, 2002). The advantages of lactic acid food fermentations include resistant to spoilage and food toxins; they make the foods less likely to transfer pathogenic microorganisms; they generally preserve the foods between the time of harvest and consumption, and they modify the flavour of the original ingredients and often improve the nutritional value (Nout and Ngoddy, 1997; Salminen and Wright, 1998).

North East India has eight states; most of them are situated in hills and foothills. Samples of fermented vegetables and bamboo shoots were collected from three states viz. Sikkim, Arunachal Pradesh and Manipur, respectively, for the study materials in this thesis. Sikkim is a mountainous state of India in the Eastern Himalayas with an area of 7096 sq. km and altitudes ranging from 300 m to 8,500 m. The state comprises four districts. A total population of Sikkim is 5,40,493 (Census of India, 2001) and is populated by three major ethnic groups, Nepali, Bhutia and Lepcha. Arunachal Pradesh is also in the Eastern Himalayan range with an area of 83,743 sq. km. The state comprises thirteen districts with a total population of 10,96,702 (Census of India, 2001) and is populated by many tribes including Nyishing, Adi, Abors, Khampti, Apatami, etc. Manipur has an area of 22,327 sq. km with a population of 23,88,634 (Census of India, 2001). It has nine districts and is populated by many ethnic communities mostly Meiteis, Kuki, etc.

Traditional fermented foods constitute an important part of the local diet in the Sikkimese culture (Tamang, 2005a). 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 of time. The daily per capita consumption of fermented foods in the Sikkim Himalayas is 87.6 g representing 10 % of the total daily food consumed in the local diet (Yonzan and Tamang, 1998). A variety of fermented vegetable products are prepared and consumed in the Eastern Himalayan regions of India (the Darjeeling hills, Sikkim, Manipur, Nagaland, Meghalaya), Nepal and Bhutan (Tamang *et al.*, 1988; Tamang, 2001). These traditional products are usually prepared by rural women using their indigenous knowledge of fermentation for the biopreservation of perishable vegetables for storage and consumption.

Food culture in North East India has been reflected in the pattern of food production in a mixed farming system. Depending on the altitudinal variation, seasonal vegetables such as cabbage, cauliflower, leafy mustard 'rayo-saag', young tendrils and fruits of squash 'iskus', brinjal, chili, cucumber, young tendrils and fruits of pumpkin, sponge gourd, tomato, tree tomato, radish, carrot, etc. are cultivated and eaten in Sikkim (Annual Report, 2005), and also in other hill regions of North East India. Preparation of wild edible plants including young bamboo shoots, ferns, stinging nettles, and their parts such as seeds, fruits, roots, leaves, flowers in local diet are important components of food culture (Rai *et al.*, 2005).

About 78 indigenous and exotic species of bamboo belonging to 19 genera are reported in the biodiversity-rich regions of North East India (Hore, 1998). Bhatt *et al.* (2003) reported that about 266.4 quintal, 4420.3 quintal and 4336.3 quintal of bamboo shoots are harvested annually in

Sikkim, Meghalaya and Mizoram, respectively. Young succulent shoots of these bamboo species are mostly used as an edible delicacy by different tribes of North East India (Sharma, 1989), and some as fermented products (Tamang, 2001). Some popular fermented bamboo tender shoots of North East India are mesu of Sikkim, soidon, soibum and soijim of Manipur, ekung, eup and hiring of Arunachal Pradesh, kharisa and bah gazar of Assam, syrwa of Meghalaya, zusem and zutsuk of Nagaland and rep of Mizoram (Singh, 2002; Bhatt *et al.*, 2005; Tamang, 2005b).

Varieties of ethnic fermented foods and beverages are traditionally prepared and consumed, and even marketed within the region in North East India (Tamang, 2001). Some of the traditional food products of North East India were extensively studied (Tamang, 2005b), however, majority of them are yet to be investigated scientifically. Though, there have been no published papers or reports on many traditional fermented vegetable and bamboo shoot products of North East India except few vegetable-based fermented products which were reported earlier. Tamang and Sarkar (1993) reported the microbiology of sinki, a traditional fermented radish tap-root product of the Darjeeling hills and Sikkim. Tamang and Sarkar (1996) also reported the microbiology and biochemistry of mesu, a traditional fermented bamboo shoot product of the Darjeeling hills and Sikkim. Karki *et al.* (1983a, 1983b, 1983c, 1983d) studied the microbiology and biochemistry of gundruk from Nepal. Pravabati and Singh (1986), Giri and Janmejay (1987, 1994, 2000) studied some parameters of soibum, fermented bamboo shoot of Manipur. To the best of our knowledge, no microbial systematic of predominant lactic acid

bacteria isolated from most of the traditional fermented perishable vegetables and wild bamboo shoot products of North East India, and their technological properties were studied.

Objectives

The present thesis was aimed to document the indigenous knowledge of biopreservation methods of perishable vegetables including edible bamboo shoots of North East India; and to isolate, characterise and identify the predominant lactic acid bacteria associated with the various traditional fermented vegetable and bamboo shoot products. The objective of the thesis was also to study the technological properties of the identified strains of functional lactic acid bacteria such as acidifying capacity, enzymatic profiles, their antimicrobial and bacteriocin activities against potential strains, ability to produce biogenic amines, degradation of antinutritive factors such as phytic acid and oligosaccharides and to determine the degree of hydrophobicity of such strains. The LAB Strains were selected on the basis of technological properties and tested for production of fermented vegetable products under laboratory conditions, and subjected to sensory evaluation. Microbial population dynamics of LAB and other microbial groups during fermentation were also examined. Proximate composition of samples of fermented vegetables and bamboo shoots was also calculated.

REVIEW OF LITERATURE

Canned or frozen foods are too expensive or not easily available for majority of people living in underdeveloped and developing countries, acid fermentation combined with salting remains one of the most practical methods of preserving and often enhancing the organoleptic and nutritional quality of fresh vegetables (Steinkraus, 1996). The knowledge of the art of pickling vegetables (fermentation) is a process of preservation of foods that is lost in antiquity (Battcock and Azam-Ali, 1998). It may have been developed in Asia as suggested by Pederson (1979) or in Mediterranean (Hulse, 2004) but until more evidence is available its origin will remain obscure (Steinkraus, 1996). In any event, this method of food preservation has been used for many centuries and is one of the important methods of food preservation still in use for vegetables and fruits where production by canning, drying or freezing is not the method of choice (Vaughn, 1985).

Record of acid-fermented vegetables has been well documented by several authors (Fleming, 1982; Steinkraus, 1996; Battcock and Azam-Ali, 1998). The Chinese labourers working on the Great Wall were eating acid-fermented mixed vegetables in the 3rd century B.C. (Pederson, 1979). Centuries ago, the Koreans developed kimchi made from acid-fermented Chinese cabbage, radish, and other ingredients (Lee, 1994a). Similarly, in the Western world, cabbage was fermented to sauerkraut and cucumbers to pickles (Pederson and Albury, 1969).

Table A shows a comprehensive list of fermented vegetable products of the world. Review of available literature on some common LAB-based fermented vegetables was illustrated.

Table A. LAB-based fermented vegetable products of the world

Product	Raw material	Functional LAB	Country	Reference
Cucumber pickle	Cucumber	*Leuc. mesenteroides, P. cerevisiae, P. acidilactici, Lb. plantarum, Lb. brevis*	European countries, USA, Canada	Vaughn (1985)
Gundruk	Leafy vegetables	*Lb. plantarum, Lb. casei, Lb. fermentum, P. pentosaceus.*	India, Nepal	Karki *et al.* (1983d)
Jeruk	Fruits and vegetables	*Leuconostoc, Lactobacillus, Pediococcus, Streptococcus*	Malaysia	Merican (1996)
Kimchi	Chinese cabbage, radish	*Leuc. mesenteroides, E. faecalis, P. cerevisiae, Lb. plantarum, Lb. brevis*	Korea and China	Cheigh and Park (1994)
Mesu	Bamboo shoots	*P. pentosaceus, Lb. brevis, Lb. plantarum*	India, Nepal	Tamang and Sarkar (1996)
Naw-mai-dong	Bamboo shoots	*Leuc. mesenteroides, P. pentosaceus, Lb. fermentum, Lb. buchneri, Lb. brevis*	Thailand	Dhavises (1972)
Olives	Olive	*Lb. plantarum, Lb. brevis, Lb. casei, E. faecium*	USA, Spain, Portugal, Peru, Chile	Randazzo *et al.* (2004)
Pak-gard-dong	Leafy vegetable	*Lb. plantarum, Lb brevis, P. cerevisiae*	Thailand	Mingmuang (1974)

Product	Raw material	Functional LAB	Country	Reference
Pak-sian-dong	Leaves of *Gynandropis pentaphylla*	*Leuc. mesenteroides, Lb. fermentum, Lb. buchneri, P. pentosaceus Lb. plantarum, Lb. brevis*	Thailand	Dhavises (1972)
Sauerkraut	Cabbage	*Leuc. mesenteroides, P. cerevisiae, Lb. plantarum, Lb. brevis, Leuc. fallax*	European countries, USA, Canada	Pederson and Albury (1969), Breidt *et al.* (1995)
Sayur asin	Mustard cabbage and similar vegetable	*Leuc. mesenteroides, P. pentosaceus, Lb. confusus, Lb. plantarum*	Indonesia	Puspito and Fleet (1985)
Sinki	Radish tap-root	*Lb. fermentum, Lb. brevis, Lb. plantarum*	India, Nepal, Bhutan	Tamang and Sarkar (1993)
Soibum	Bamboo shoot	*Leuc. mesenteroides, Lb. coryniformis, S. lactis, Lb. brevis, Lb. delbruckii*	India	Giri and Janmejay (1987)
Suan-cai	Vegetables	*Lb. harbinensis*	China	Miyamoto *et al.* (2005)
Sunki	Turnip	*Lb. plantarum, Lb. brevis, P. pentosaceus*	Japan	Itabashi (1986)

Cucumber pickle

Cucumber pickle is produced and consumed in North America and Europe (Fleming, 1984). Pickling cucumbers are harvested while still immature, washed and are either dry salted or brined (Etchells *et al*, 1973). Most of the companies in USA do not brush-wash the fruit before brining although washing has been suggested for use in controlled fermentation procedures (Fleming *et al.*, 1988). Maintenance of structural integrity of cucumbers during fermentation is greatly dependent on chemical and physical properties of the fresh fruit, including size, maturity, cultivar, and physiology (Fleming, 1984). Microbial fermentation is not limited to the cover brine, as once thought (Etchells *et al.*, 1968). Lactic acid bacteria have been shown to enter and proliferate within brined cucumber (Daeschel and Fleming, 1981). Sugar content and buffering capacity of cucumbers are important in determining the extent to which LAB can ferment before inhibited by low pH (Fleming, 1984). Cucumbers contain about 2 % fermentable sugars (Handley *et al.*, 1983) consisting essentially of glucose and fructose (Fleming *et al*, 1988) and traces of sucrose (Handley *et al.*, 1983). Glucose and fructose are readily fermented by LAB during cucumber fermentation (Phar *et al.*, 1977), however, differential utilization of glucose and fructose by the LAB have also been reported (Lu *et al.*, 2001).

Three kinds of cucumber pickles are produced viz. fresh pack (also known as fresh cure, home style and by other names) which at most, are held in salt brine for up to 2 days, then packed in tin cans or glass jars and pasteurised; salt stock pickles from which a variety of

processed products are produced; and fermented dill pickles (Fuller and Dull, 1983). The fresh pack pickles undergo a marginal fermentation, whereas the other two undergo a complete fermentation (Vaughn, 1985). The initiation of the fermentation by *Leuconostoc mesenteroides* at lower temperature and salt concentration was verified by Pederson and Ward (1949), Pederson and Albury (1950). The other species of LAB, *Pediococcus cerevisiae*, *P. acidilactici*, *Lb. brevis* and *Lb. plantarum* predominate in cucumber fermentation at 5-8 % brines (Etchells *et al*, 1975). *P. cerevisiae* and *Lb. brevis* are less resistant to salt than *Lb. plantarum* so sometimes they are absent in the higher salt concentrations (30° Saltometer) brine (Pederson and Albury, 1961). *Lb. plantarum* is the most salt resistant species and it terminates the fermentation (Pederson and Albury, 1961). But it is severely retarded at salt concentration of 40° Saltometer and above and little if any growth occurs above 45° Saltometer (Vaughn, 1985).

Vigorous activity in the cover brine by coliform bacteria, obligate halophiles, heterofermentative LAB, and fermenting species of yeast is associated with gaseous fermentation and resulting bloater spoilage (Fleming *et al.*, 1992). Even homofermentative LAB such as *Lb. plantarum* and *P. cerevisiae* produce sufficient CO_2 when combined with CO_2 from cucumber tissue, to form bloater formation in brined cucumbers (Fleming *et al.*, 1973). *Lb. plantarum* and *P. pentosaceus* decarboxylate malic acid to yield CO_2 and lactic acid (Daeschel *et al.* 1984; McFeeters *et al.*, 1984). Malic acid is a natural constituent of pickling cucumbers (McFeeters *et al.*, 1982). Procedures have been developed to produce

and isolate non-malate decarboxylating mutants of *Lb. plantarum* (McFeeters *et al.*, 1984; Daeschel and Fleming, 1987). Purging fermenting cucumber brines with nitrogen has been shown to be effective in preventing bloater formation (Etchells *et al.*, 1973; Fleming *et al.*, 1973, 1975). Butyric acid spoilage of brined cucumbers was found to occur after an apparently narrow primary fermentation by LAB (Fleming *et al.*, 1989). *Clostridium tertium* was identified as contributing to spoilage (Fleming *et al.*, 1992). At completion, the total acidity of fermented cucumber may be as high as 0.9 % (as lactic acid) and have a pH value as low as 3.3 if oxidative yeasts have been kept inactive (Vaughn, 1985).

Gundruk

Gundruk is a non-salted and fermented leafy vegetable product which has been one of the major appetizers for the people of Nepal (Dietz, 1984) and also of India particularly in the Darjeeling hills and Sikkim (Tamang *et al.*, 1988).

In the traditional method of gundruk preparation practiced in Nepal (Karki, 1986), fresh leaves of mustard, radish and cauliflower are wilted for 2-3 days. The leaves are shredded, pressed into an earthen jar and covered with lukewarm (30-35° C) water. After fermentation at 16-20° C for 5-7 days, the leaves are removed from the jar and sun-dried (Karki *et al.*, 1983d, Karki, 1986). Karki *et al.* (1983d) studied the microorganisms of gundruk prepared in Nepal. They found that gundruk fermentation was initiated by *Lb. cellobiosus* (= *Lb. fermentum*) instead of *Leuconostoc mesenteroides* as in other fermented vegetable products

and was followed by *Pediococcus pentosaceus* and finally by *Lb. plantarum. Lb. casei*, *Lb. casei* subsp. *pseudoplantarum* were also isolated from gundruk of Nepal (Karki *et al.,* 1983d).

The pH and acidity (as lactic) in gundruk were 4.0-4.3 and 0.8-1.0 % respectively (Karki *et al.*, 1983d). In gundruk 90 % of the organic acids consisted of lactic and acetic acids, besides, citric and malic acids were found in lower concentrations (Karki, 1986). The level of palmitic, oleic, linoleic and linolenic acids was much higher in mustard leaf gundruk compared to those in the unfermented vegetables (Karki *et al.*, 1983c). In mustard gundruk, free amino acids, particularly glutamic acid, alanine, leucine, lysine and threonine remarkably increased with the corresponding decrease in asparagine, glutamine, histidine and arginine, indicating the influence of fermentation (Karki *et al.*, 1983b).

Mustard vegetable gundruk comprised of cyanides (15.7 %), isothiocyanates (8.5 %), as the main flavour components, followed by alcohols (12.3 %) and esters (4.1 %). Phenyl acetaldehyde (6.4 %) was the only aldehyde identified in mustard leaf gundruk. Cauliflower gundruk consisted of alcohols (50 %) as the major component followed by cyanides (6.5 %), isothiocyanates (6.1 %) and esters (3.2 %) (Karki *et al.*, 1983a). The levels of iron and calcium are high while carotenoids are reduced by more than 90 % probably during sun drying (Diez, 1984).

Jeruk

Jeruk are homemade fermented pickles indigenous to many races in Malaysia, which are prepared from common fruits and vegetables (Merican, 1996). Among the common vegetable used are gherkins and cucumbers, ginger, onion, chili, bamboo shoot, mustard leaves, etc. Fruits such are mango, papaya, nutmeg, etc. are used for pickling.

Low-income rural people consume large quantities of jeruk because pickling is an inexpensive way to preserve surplus food. Pickled vegetables are prepared like fresh vegetables; pickled fruit is eaten as a relish especially by children and expectant mothers because of the sweet-sour flavour. *Leuconostoc*, *Lactobacillus*, *Pediococcus* and *Streptococcus* have been isolated from fermenting cucumber and mustard leaf pickles (Kiam chye) produced in Malaysia (Merican, 1996).

Kimchi

Kimchi is the generic name given to a group of lactic acid fermented vegetable foods of Korea (Cheigh and Park, 1994). It is a unique vegetable product of long tradition in Korea considered as a main side-dish served along with cooked rice and other dishes (Lee, 1994a). Kimchi is closely related to sauerkraut but differs in having less acid and being carbonated (Lee, 1986; Mheen *et al.*, 1996). More specific names are used for these pickled vegetables depending on the raw material, processing methods, seasons and localities. For example, Tongbaechu-kimchi and Bossam kimchi are based on Korean cabbage; Kakduggi, Dongchimi, chonggyak-kimchi are based on Korean radish; and oisobaegi

and oiji are based on cucumber (Mheen *et al.*, 1996). Korean cabbage and radish are the major substrates while garlic, green onion, zinger, leaf of mustard, hot pepper, parsley pear, chestnut and carrot are minor ingredients. Additional minor ingredients are pickled shrimp, frozen pollack, oyster, small octopus and seasonings, table salt, monosodium glutamate, chengyak (a type of seaweed), pear, etc. The ratio of major to minor ingredients varies depending on the households and makers, the range being 70 to 90:30 to 10. A recipe for typical Tongbaechu-kimchi includes 100 g Korean cabbage, 2 g garlic, 2 g green onion, 2 g powdered hot pepper, and 0.5 g ginger. Although the proper combination of minor ingredients is reported to be the key to good-tasting kimchi, the most important factor seems to be the salt concentration. Salting of cabbage can be done at 5 to 7 % salt concentration for 12 hour or in 15 % saline solution for 3 to 7 hour, followed by rinsing and draining. Optimum salt concentration during kimchi fermentation is approximately 3 % and is adjusted by experience at the household level (Yu and Chung, 1974).

Production methods of kimchi differ depending on the variety of kimchi and the ingredients used, as well as whether production is home-based or industrial-scale (Yu and Chung, 1974; Koo and Choi, 1990; Han *et al.*, 1993; Mheen *et al.*, 1996). However, the principal process described by Cheigh and Park, (1994) consists of pretreatment, brining, blending of ingredients, and fermentation. Chinese cabbage or Oriental radish may be treated in various ways before brining (Lee, 1986). Pretreatment include grading, washing and cutting. Other materials also are graded, washed and cut or chopped for the blending and fermentation

steps. Pretreated cabbage or radish is brined at various salt concentrations by either dry salt or brine solution. Brine-treated and rinsed ingredients (cabbage, radish, cucumber, etc.) are blended together with dry salt and a premixture of chopped or sliced minor ingredients (spices, seasonings, salt-pickled fishes, and other vegetables) according to the recipes (Lee, 1986). The blend is then fermented under the appropriate conditions. Accordingly, the preparation methods, as well as the ingredients, significantly affect the biochemical, microbiological, and nutritional characteristics (Cheigh and Park, 1994).

Fermentation of kimchi in the home is usually done at ambient temperature (Mheen *et al.*, 1996) Using a 3 % salt concentration, the optimum fermentation period is one day at 30° C, 2-3 days at 20° C, 12-15 days at 10° C and 30-60 days at 5° C. The initial pH of 5.5 to 5.8 falls to an optimum of 4.5 to 4.2 and drops further to pH 4.0 if fermentation is too prolonged (Song *et al.*, 1966). Optimum total acidity (as lactic acid) is 0.4 to 0.75 %, rising to 1.0 % if over-ripening occurs and becoming 1.5 to 2.0 % at the spoilage stage (Lee and Yang, 1970). Studies on the microbial composition of kimchi using conventional methods of isolation and phenotypic identification has been done by Lim *et al.* (1989), Park *et al.* (1990) and Shin *et al.* (1996). Some important species of LAB thought to be responsible for kimchi fermentation are *Leuc. mesenteroides, Leuc. pseudomesenteroides, Leuc. lactis, Lb. plantarum, Lb. brevis, E. faecalis, P. cerevisiae,* (Kim and Whang, 1959; Kim and Chun, 1966; Mheen and Kwon, 1984). LAB isolates from kimchi were re-examined using a batterey of polyphasic methods including 16S rRNA sequencing and DNA-DNA

hybridization leading to the discovery of several novel species: *Leuconostoc kimchii* (Kim *et al.*, 2000), *Lb. kimchii* (Yoon *et al.*, 2000), *Weissella kimchii* (Choi *et al.*, 2002), *Weissella koreensis* (Lee *et al.*, 2002) and *Leuc. inhae* (Kim *et al.*, 2003). *Leuc. geladium*, *Leuc. gasicomitatum* and *Lb. sakei*, *Weissella koreensis* were elucidated by using 16S RNA gene analysis (Kim and Chun, 2005). The main microorganisms responsible for ripening are *Leuc. mesenteroides* but *Lb. plantarum* is responsible for excessive acidification in the later stages (Whang *et al.*, 1960; Kim and Chun, 1966). Han and Yang (1994) found that *Leuc. mesenteroides* subsp. *mesenteroides* and *Leuc. paramesenteroides* were dominant at lower temperature (5°-10° C) while at higher temperature (15°-25° C) *Lb. plantarum* was dominant. Addition of pepper and ginger stimulated the fermentation while onion, garlic and green onion inhibited the fermentation of kimchi (Han and Yang, 1994). Fermentation at lower temperatures (5 to 15° C) is recommended for obtaining acceptable kimchi (Mheen and Kwon, 1984; Lim *et al.*, 1989; Lee *et al.*, 1992). As the pH falls to 4.0, *Lb. plantarum* become predominant (Mheen and Kwon, 1984). The mixed strains are more effective than a single strain to produce better organoleptic quality (Lee and Kim, 1988). Optimum pH and acidity for the best taste of kimchi is 4.2 and 0.6 % (as lactic acid), respectively (Lee, 1994b). Reducing sugars decreases as fermentation progresses (Lee and Lee, 1965).

Kimchi blended at low temperature (15° C) with a 3 % salt concentration and fermented slowly at –1° C can be kept for more than 3 months at this same low temperature. Kimchi is packed in polyethylene

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film bags and heat treated at low temperature (60° C for 20 min). Kimchi prepared this way can be stored at 4° C for more than 3 months with a constant acidity (near 0.57 %) and good eating quality (Lee and Yang, 1970). Growth inhibition of pathogenic bacteria in kimchi has been reported by Chung *et al.* (1967), Choi and Beuchat (1994).

Kimchi contains 75-95 % moisture, and per 100 g dry matter: 10-30 g protein, 3-10 g fat, 30-50 g carbohydrate, 5-10 g fibre, 10-20 g ash, 250-330 kcal energy, 20-300 mg Ca, 250-600 mg P, 2-11 mg Fe, 0.15-0.7 mg thiamine, 0.2-1.0 mg riboflavin, 3-40 mg niacin, 100-300 µg cyanocobalamine and 75-450 mg vitamin C (Campbell-Platt, 1987). Kimchi is an important source of B group vitamins, carotene, and ascorbic acid. These are already present in the raw materials; however, some vitamins particularly those of the B group (Lee *et al.*, 1960) and ascorbic acid (Lee *et al.*, 1984), may be synthesized during the fermentation process. The total free amino acids increase from about 300-600 mg/100g in kimchi, thereby glutamic acid, alanine, valine, leucine, lysine and arginine being the most important ones and their concentrations being closely related to the contents of non-volatile organic acids, free amino acids and pH (Hawer *et al.*, 1988). The amount of nitrite and secondary amines in kimchi is very low compared to those in sausage and fish (Yim *et al.*, 1973).

Kimchi is known to improve digestion, prevent constipation, control intestinal flora, and have antimutagenic, anticarcinogenic, and other pharmaceutical functions (Park and Cheigh, 1992; Lee, 1997).

Mesu

Mesu, a non-salted fermented bamboo shoot product, has traditionally been consumed by the people in the bamboo-growing regions of the Darjeeling hill and Sikkim in India (Tamang *et al.*, 1988). Tamang and Sarkar (1996) reported *Pediococcus pentosaceus*, *Lb. brevis* and *Lb. plantarum* from samples of mesu collected from markets of Kalimpong, Darjeeling and Sikkim.

Mesu is commonly used as a pickle by mixing it with salt, mustard oil and green chilies. While fresh mesu has a shelf-life of only about a week, its pickle can be stored for a year or more (Tamang and Sarkar, 1996). Proximate composition of mesu was: moisture 94.1 %, pH 4.2, acidity 0.75 %, ash 10.9 % DM (dry matter basis), protein 17.1 % DM, fat 2.4 % DM (Tamang, 1992).

Naw-mai-dong

Naw-mai-dong is a traditional fermented bamboo shoot (*Bambusa arundinacea* Willd.) product of Thailand (Dhavises, 1972). The sweeter species of bamboo such as *Bambusa burmanica* Gamble and *Dendrocalamus asper* Back. are also used as raw materials (Boon-Long, 1986).

During preparation, young bamboo shoots are harvested, woody and defective portions are removed from the shoots by trimming. After washing, shoots are sliced and mixed with salt at 2 % level. The bamboo shoots are boiled in water and the bitter liquor is discarded. These are then packed into earthen jar, covered with plastic sheet and weight kept at

the top. Fermentation is complete in 3–4 weeks at room temperature. Functional microorganisms identified from naw-mai-dong are *Leuc. mesenteroides, Lb. fermentum, Lb. buchneri, Lb. brevis* and *P. pentosaceus* (Dhavises, 1972). Titratable acidity reaches to 1.0-1.2 % as lactic acid (Dhavises, 1972).

Olives

Olive fermentation is practiced in southern parts of Europe, the Middle East, North Africa and California in USA (Campbell-Platt, 1987). Olive pickling for table use, practiced as an art in the missions, on the ranches, and in the homes from many years, was not of much commercial importance until about 1900 (Vaughn, 1985). The response of three Sardinian olives cultivars to processing as table olives was evaluated and characterized from the marketing, technological and chemical-physical point of view and brined with 8 % NaCl (Piga *et al.*, 2001). Cruess (1958) reported around 1900 it was independently discovered by Professor Bioletti and Mrs. Freda Ehmann that ripe olives, after preliminary treatment with lye (sodium hydroxide solution) to destroy the bitter glucoside oleuropein, could be canned and preserved by heat in much the same way as other foods. Total world production has increased linearly in the last 35 years at an annual rate of 1.5-2 % reaching over 9,00,000 tonnes in 1994-95 (Garrido Fernandez *et al.*, 1997). The disposition of California olives for products in approximate order of importance include black-ripe and green-ripe canned olives (whole and pitted), Spanish-style, Sicilian-type, Greek-type including brined and salted-cured fruits and oil

(Vaughn, 1985). Fresh or direct cure black olives also are processed and canned throughout the harvest period of up to 2-5 months (Vaughn, 1985).

Because of lack of processing space, the remainder of the olives destined for processing has to be stored in salt brine prior to making them into canned ripe olives, which undergo lactic acid fermentation (Vaughn, 1985). Thus, there are four brine fermentations including 'storage', Sicilian-type, Spanish-type and Greek-type brined olives. The 'storage' and Sicilian-type fermentations may be considered to be identical, for the fruits are placed directly in brine with no prior lye treatment whereas the Spanish-type olives are lye treated to destroy most of the bitterness, washed to remove some of the alkali, and then brined in which they undergo lactic fermentation (Fernández-Diéz, 1983). Physiochemical characteristics, i.e. NaCl concentration, external temperature, etc. are responsible for changes in the microbial flora during fermentation (Randazzo *et al.*, 2004). The use of NaCl and the progressive pH decrease select lactic acid microflora that increase from 1 % of the total microbial population in the fresh brine to 80 % after few days (Robinson, 1988). Indigenous LAB change spontaneously during natural fermentation and at the end of the process *Lactobacillus* species are involved, mainly *Lb. plantarum* (Fernandez Gonzalez *et al.*, 1993; Harris, 1998). Using PCR/RFLP and by partial sequence analysis Randazzo *et al.*, (2004) found *Lb. casei* to be the most dominant species in naturally fermented Sicilian green olives. The brined Greek-type olives are placed in high salt brine, which, on equilibration, may be too strong for the lactic acid

bacteria to ferment the olives. Attempts have been made to use lactic starter culture in olives by Fleming and McFeeters (1981). *P. cerevisiae* and *Lb. plantarum* have been used in pure culture or controlled fermentation of olives (Etchells *et al.*, 1966).

Leuc. mesenteroides and streptococci were found in low salt fermentations of Sevillano variety (Vaughn *et al.*, 1943). While *Lb. plantarum* dominated the final stage of fermentation, *P. cerevisiae* was isolated from Spanish-type brines in the last part of the initial stage of fermentation and the first phases of the intermediate stage of fermentation, then it declined rapidly (Vaughn, 1985). Preparation of starter culture for fermentation of Spanish-type olive has been described by Vaughn *et al.* (1943). The total acidity of the final product is 0.7-1.0 % as lactic acid, and pH of 3.8-4.2 (Campbell-Platt, 1987). The most important control of protecting olives in fermentation, whether storage, Sicilian-type or Spanish-type brines, is the maintenance of anaerobic conditions (Vaughn, 1985). Control of the oxidative moulds and yeasts is mandatory to prevent destruction of desired acidity, but only recently has become recognized as a problem by the industry as a whole (Mrak *et al.*, 1956; Balatsouras and Vaughn, 1958; Vaughn *et al.*, 1969).

The coliform bacteria are responsible for most of the blister and gassy spoilage of fermenting olives (Foda and Vaughn, 1950). The species of *Bacillus polymyxa* and *B. macerans* (Gililland and Vaughn, 1943; Vaughn, 1954) and yeasts *Saccharomyces* and *Hansenula* (Vaughn *et al.*, 1972) also cause gassy spoilage in olives.

Olives contain 60-70 % moisture and per 100 g dry matter: 2-4 g protein, 25-45 g fat, trace amount of carbohydrate, 12-18 g fibre, 15-35 g ash, 15-20 g NaCl, 250-400 kcal energy, 5000-9000 mg Na, 300 mg K, 200 Ca, 80 mg Mg, 50 mg P, 3 mg Fe, 0.8 mg Cu, 100 mg S, 2000-7000 mg Cl, trace amount of thiamine, riboflavin, 0.5 mg niacin, 0.07 mg vitamin B_6, 0.07 mg pantothenic acid, 150 µg (black olives)-700 µg (green olives) carotene, vitamin C and D nil (Campbell-Platt, 1987).

In a study by Ruiz-Barba *et al.* (1994) on bacteriocin-producing *Lb. plantarum* LPCO10 and its non-bacteriocin-producing, bacteriocin-immune derivative, *Lb. plantarum* 55-1, were evaluated separately for growth and persistence in natural Spanish-style green olive fermentations. Their results indicated that *Lb. plantarum* LPCO10 may be useful as a starter culture to control the lactic acid fermentation of Spanish-style green olives (Ruiz-Barba *et al.*, 1994).

Antimicrobial substances that inhibited other strains of the genera *Lactobacillus*, and some foodborne pathogens including *Listeria monocytogenes*, *Staphylococcus viridans*, *Escherichia coli* and *Salmonella* was produced by *Lb. plantarum* Zn 42, *Lb. plantarum* Zn 50 and *Lb. plantarum* Zn 52 isolated from Portuguese olive brines (Cordeiro *et al.*, 2002), and also reported the antimicrobial activities of LAB isolated from fermented table olives (Rubia-Soria *et al.*, 2006).

Pak-gard-dong

Pak-gard-dong is the fermented vegetable product of Thailand prepared from leaf of mustard (Boon-Long, 1986). During its preparation,

leaves of black mustard are collected, defective leaves are removed, washed and wilted in the sun, 2.5 % salt are added into the wilted leaves and packed into a container and left for 12 hours. After removing salt water, 3 % sugar is added and fermented at room temperature. Fermentation is completed in 3-5 days (Boon-Long, 1986).

Mingmuang (1974) reported *Pediococcus pentosaceus*, *Lb. brevis*, *and Lb. plantarum* as the major microorganisms involved in the fermentation of pak-gard-dong.

Pak-sian-dong

Pak-sian-dong is a very common pickle leafy vegetable of Thailand (Dhavises, 1972). It is prepared from leaves of pak-sian (*Gynandropis pentaphylla* DC.). The fresh vegetable is thoroughly cleaned with water and then spread out in the air or under the sun to lose water until the sample is distinctly flacid. It is then mixed with water, salt, and sugar and kept in a tightly covered container. To reduce bitter flavour, the leaves are sometimes soaked in water and salt overnight. The liquor is thrown away and fresh water and sugar are added. Usually raw cane sugar or palm sugar is preferred to refined sugar because it enhances the flavour of the finished product. Pak-sian-dong is ready in 72 hours. At this stage the pH of the liquor is 3.9 and acidity between 0.7 to 0.8 %. Dominant functional microorganisms in pak-sian-dong are *Leuc. mesenteroides*, *Lb. fermentum*, *Lb. buchneri*, *Lb. plantarum*, *Lb. brevis* and *P. pentosaceus* (Dhavises, 1972).

Sauerkraut

Sauerkraut or sauerkohl is a German term for 'sour cabbage' (Pederson, 1979). It is the salted and fermented cabbage product produced and consumed with main meals in Germany, Switzerland, Central Europe, USA, Canada and former USSR (Vaughn, 1985; Campbell-Platt, 1987).

Sauerkraut is prepared from shredded white cabbage (Vaughn, 1985). Mature, sound heads of cabbage are trimmed to remove the outer green, broken, or dirty leaves, then the cores are cut by reversing cores that leaves the cut core in the head. The head of cabbage is then sliced into long, finely cut shreds of 3-5 mm x 5-7 cm size. The cut cabbage is then brined (containing 2.25-2.5 % salt) in the tank. Once the tank has been filled to proper level it is closed and the fermentation has started. At the time of filling to the desired level the shreds are covered with a sheet of plastic large enough to cover much more than the area in the top of the tank. The plastic sheeting is placed against the top of the slaw with the edges draped over the sides of the vat to form an open bag. Then water or preferably salt brine is placed in the bag so that the weight of the added solution forces the shredded cabbage down into the brine until the uppermost shreds are covered. This method of weighting the cover provides nearly anaerobic conditions once carbon dioxide begins to be formed by fermentation (Vaughn, 1985).

The fermentation of sauerkraut by a sequence of flora (Pederson, 1930a, 1930b) has been confirmed by Holtman (1939, 1941), Murray (1940), and Stamer *et al.* (1971). Pederson (1930a) found that the

fermentation was initiated by the heterofermentative species *Leuc. mesenteroides* and was followed by heterofermentative rods and finally by homofermentative rods and cocci. Additional studies by Pederson and Albury (1954, 1969) firmly established that the *Leuc. mesenteroides* initiated the lactic fermentation of sauerkraut. It is now accepted that the sauerkraut fermentation is initiated by *Leuc. mesenteroides*, a gas-forming species whose initiation of growth is more rapid than other lactic bacteria and is active over a wide range of temperatures and salt concentrations. The CO_2 replaces air and creates an anaerobic atmosphere conducive to prevention of oxidation of ascorbic acid and darkening the natural colour of the cut cabbage and also stimulates the growth of many lactic acid bacteria (Vaughn, 1985).

At 7.5° C, fermentation is very slow, only *Leuc. mesenteroides* grow slowly attaining an acidity of about 0.4 % in about 10 days, and an acidity of 0.8 % to 0.9 % acid in a month (Pederson and Albury, 1969). At 18° C with 2.25 % salt, a total acidity of 1.7 to 2.3 % will be attained in about 20 days. At higher temperatures, i.e. 23° C, the rate of fermentation will be greater so that a brine acidity of 1.0 to 1.5 %, may be attained in 8 to 10 days. *Lb. plantarum* and *Lb. brevis* grows actively and completes fermentation in approximately in one month. At 32° C, a similar acidity will be reached in about 8-10 days with most of the acid being lactic acid produced by the homofermentative bacteria *P. cerevisiae* and *Lb. plantarum* (Pederson and Albury, 1969).

Lb. brevis produces a red pigment under certain conditions which may result in discoloration or darkening of sauerkraut (Stamer *et al.*,

1973). The growth of pigmented yeast may be the cause of kraut defect or 'pink kraut' (Brunkow *et al.*, 1925). In fact, anaerobiosis helps to eliminate aerobic growth of moulds and yeasts in sauerkraut (Pederson and Albury, 1969).

The potential of sauerkraut production in India was studied by Mukherjee *et al.* (1996), and observed the loss of total nitrogen from 0.23 % (30 days) to 0.12 % (120 days), loss of total ash, and gradual increase of crude fibre during sauerkraut production. The major amount of volatiles in sauerkraut are acetyl, isoamyl alcohol, n-hexanol, ethyl lactate, cis-hex-3-ene-1-ol and allyl isothiocyanate (Lee *et al.*, 1976).

Sauerkraut contains 35-45 % moisture, and per 100 g dry matter: 3-5 g protein, trace amount of fat, 15-20 g carbohydrate, 25-30 g fibre, 35-45 g ash, 15-25 g NaCl, 70-100 kcal energy, 150 mg Ca, 2 mg Fe, 0.1 mg thiamine, 0.15 mg riboflavin, 0.7 mg niacin, 50 µg carotene and 50-70 mg ascorbic acid (Campbell-Platt, 1987). Pederson *et al.* (1939) found that ascorbic acid value of kraut during active fermentation equaled to those of original fresh cabbage. Kraut showed a slow loss of vitamin C during vat storage after completion of fermentation (Pederson and Albury, 1969). Marked changes were observed in some of the minor lipid components of cabbage during sauerkraut fermentation (Vorbeck *et al.*, 1963). Dawson and Herrington (1967) found that purified phospholipase of cabbage slowly attach a lecithin suspension at pH 5.4 and some of the hydrolysis products were utilized by the bacterial cells during growth. The unsaponifiable fractions remain relatively stable during fermentation of sauerkraut (Hill and Mattick, 1966). The characteristic

flavour of cabbage and closely related vegetables is usually associated with the sulfur-containing constituents, particularly the glucosides (Steinkraus, 1996). Edmond and Lewis (1926) found that the sulfur content of different varieties varied from 0.075-0.341 %. Mild flavoured sauerkraut blends well with meats, fruits, other vegetables, spices and other foods without imparting a strongly distinctive flavour (Pederson and Albury, 1969).

The 16S rRNA sequence of an unknown *Leuconostoc* originally isolated from sauerkraut was investigated by reverse transcription revealed a new species, *Leuc. fallax*, which represents a new albeit peripheral line within the genus *Leuconostoc* sensu stricto (Schillinger *et al.*, 1989; Martinez-Murcia, 1991).

Breidt *et al.* (1995) proposed a paired starter culture system for sauerkraut fermentation consisting of a nisin-producing strain of *Lactococcus lactis* and a nisin-resistant strain of *Leuc. mesenteroides*. They concluded that nisin produced *in situ* or added to brined cabbage can direct the progression of the species in the resultant fermentations, by preventing the growth of naturally present LAB.

Yoon *et al.* (2006) determined the suitability of cabbage as a raw material for production of probiotic cabbage juice by lactic acid bacteria (*Lb. plantarum* c3, *Lb. casei* A4, and *Lb. delbrueckii* D7). They reported that fermented cabbage juice could serve as a healthy beverage for vegetarians and lactose-allergic consumers.

Sayur asin

Sayur asin is an indigenous fermented mustard cabbage leaves of Indonesia (Puspito and Fleet, 1985). Mustard cabbage leaves are wilted, rubbed or squeezed with 2.5–5 % salt. As in Hum choy preparation, the liquid from boiled rice is added to provide fermentable carbohydrate to assure sufficient acid is produced during the fermentation. The fermentation is initiated by *Leuc. mesenteroides* and *Lb. confusus* and later dominated by *Lb. plantarum* and *P. pentosaceus*. The pH falls from 6.5 to 4.2 in 8 days of fermentation (Puspito and Fleet, 1985).

Sinki

Sinki, a non-salted fermented radish tap root product, is traditionally consumed as a base for soup and as a pickle in some north-eastern states of India, in Nepal and a few places of Bhutan (Tamang *et al.*, 1988).

Tamang and Sarkar (1993) studied the microbiology of sinki samples collected from the Darjeeling hills and some parts of Sikkim and reported the presence of *Lb. fermentum*, *Lb. brevis*, and *Lb. plantarum*. Fresh sinki had 93 % moisture content while dried sinki had 21 % moisture. This reduced moisture level increases the shelf-life of the product, for which it can be stored for several months (Tamang and Sarkar, 1993). The proximate composition of sinki was determined by Tamang (1992): pH 4.4, acidity 0.8 %, protein 14.6 % (dry matter) DM, fat

2.5 % DM, ash 11.5 % DM, Ca 120.5 mg/100 g DM, K 443.1 mg/100 g DM, Fe 18 mg/100 g DM.

Soibum

Soibum is an indigenous fermented bamboo shoot product of Manipur (Pravabati and Singh, 1986). It is prepared from the succulent bamboo shoots of *Bambusa tulda, Dendrocalamus giganteus* and *Melocana bambusoides* (Bhatt *et al., 2005*). Giri and Janmey (1987) had reported the presence of *Leuc. mesenteroides*, *Lb. brevis*, *Lb. coryniformis*, *Lb. delbrueckii* and *Streptococcus lactis* and yeasts *Candida, Saccharomyces* and *Torulopsis* in soibum. Microorganisms from 'soibum exudates' involved in microbial bioconversion of phytosterol were isolated and identified as *Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans* and *Micrococcus luteus* (Sarangthem and Singh, 2003). During fermentation of soibum, degradation of protein and gain of free amino acids was reported (Giri and Janmejay, 1994; 2000).

Suan cai

Suan cai is a traditional Chinese fermented vegetables (Miyamoto *et al.*, 2005). Taxonomical analysis of two genetically distinguished *Lactobacillus* strains isolated from suan cai, which formed L-lactate from glucose, were facultative heterofermentative, and had a DNA G+C content of 53-54 mol %. Based on DNA-DNA hybridization analysis, Miyamoto *et al.* (2005) proposed the new species be named *Lb. harbinensis* sp. Nov., isolated from suan cai of China.

Sunki

Sunki is a non-salted and fermented vegetable product prepared from the leaves of turnip in Japan (Battcock and Azam-Ali, 1998). Sunki is eaten with rice and miso (fermented soybean) soup. During its preparation, turnip is boiled, inoculated with 'zumi' (a wild small apple), dried sunki from the previous year and allowed to ferment for 1-2 months. Sunki is produced under low temperature (in winter season). Microorganisms involved include *Lb. plantarum*, *Lb. brevis*, *Lb. buchneri*, *E. faecalis*, *B. coagulans* and *P. pentosaceus* (Itabashi, 1986).

MATERIALS AND METHODS

CULTURE MEDIA USED

(1). Arginine Hydrolysis Medium (Thornley, 1960)

Peptone	10.0 g
Yeast extract	5.0 g
D (+) glucose	0.5 g
$K_2HPO_4.3H_2O$	2.0 g
Magnesium sulphate	0.1 g
Manganese sulphate	0.05 g
Sodium acetate	5.0 g
Tri-sodium citrate	20.0 g
Tween 80	1.0 ml
Arginine	0.3 %
Phenol red	0.01 g
Distilled water	1000 ml
pH	5.0

(2). Ascospore Agar (M804, HiMedia, Mumbai)

(3). *Bacillus cereus* Agar Base (M833, HiMedia, Mumbai)

(4). Bacteriocin Screening Medium (Tichaczek *et al.*, 1992)

Peptone	10 g
Beef extract	5.0 g
Yeast extract	5.0 g
Glucose	2.0 g
K_2HPO_4	2.0 g
Tween 80	1.0 g
Diammonium citrate	2.0 g
Sodium acetate	5.0 g
$MgSO_4$	0.1 g
$MnSO_4$	0.05 g
Distilled water	1000 ml
pH	6.5
Agar	12 g

(5). Baird Parker Agar Base (M043, HiMedia, Mumbai)

(6). Biogenic Amine Sub-culturing Medium (Bover-Cid and Holzapfel, 1999)

MRS Broth (M369, HiMedia, Mumbai)	52.2 g
D-Tyrosine (RM 1520, HiMedia, Mumbai)	1.0 g
L-Histidine monohydrochloride (Merck)	1.0 g
L-Lysine monohydrochloride (Merck)	1.0 g
L-Ornithine monohydrochloride (Merck)	1.0 g
Pyridoxal-5-Phosphate (RM 1554, HiMedia)	0.001 g
Distilled water	1000 ml
pH	6.00

(7). Biogenic Amine Screening Medium (Joosten and Northold, 1989; modified by Bover-Cid and Holzapfel, 1999)

Tryptone	5.0 g
Yeast extract	5.0 g
Meat extract	5.0 g
Sodium chloride	2.5 g
Glucose	0.5 g
Tween 80	1.0 g
K_2HPO_4	2.0 g
Ammonium citrate	2.0 g
Calcium carbonate	0.1 g
$MgSO_4.7H_2O$	0.2 g
$MnSO_4.4H_2O$	0.05 g
$FeSO_4.7H_2O$	0.04 g
Thiamine	0.001 g
Pyridoxal-5-phosphate	0.005 g
Bromocresol purple	0.05 g
Agar	22.0 g
Amino acid	5.0 g
Distilled water	1000 ml

Amino acids used were D-Tyrosine (pH 5.3) (RM 1520, HiMedia, Mumbai); L-Histidine monohydrochloride (pH 5.0) (Merck, Germany); L-Lysine monohydrochloride (pH 5.15) (Merck, Germany); L-Ornithine monohydrochloride (pH 5.0) (Merck, Germany).

(8). Egg Yolk Emulsion (FD045, HiMedia, Mumbai)

(9). Egg Yolk Tellurite Emulsion (FD046, HiMedia, Mumbai)

(10). Fermentation Basal Medium for yeasts (Wickerham, 1951)

Yeast extract	4.5 g
Peptone	7.5 g
Distilled water	1000 ml

Bromothymol blue (Till sufficiently dense green colour appears)

(11). Lactate Configuration Medium (Tamang and Holzapfel, unpublished)

Peptone from casein	10.0 g
Yeast extract	4.0 g
Glucose	20.0 g
Di-potassium hydrogen phosphate	2.0 g
Tween 80	1.0 g
Di-ammonium hydrogen phosphate	2.0 g
Magnesium sulphate	0.2 g
Manganese sulphate	0.04 g
Distilled water	1000 ml

(12). *Listeria* Identification Agar Base (PALCAM) (M 1064, HiMedia, Mumbai)

(13). *Listeria* Selective Supplement (FD 061, HiMedia, Mumbai)

(14). Malt Extract Agar (M137, HiMedia, Mumbai)

(15). MRS Agar (M641, HiMedia, Mumbai)

(16). MRS Broth (M369, HiMedia, Mumbai)

(17). Nitrate Broth (Gordon *et al.*, 1973)

Peptone	5.0 g
Beef extract	3.0 g
Potassium nitrate	1.0 g
Distilled water	1000 ml
pH	7.0

(18). Nutrient Agar (MM012, HiMedia, Mumbai)

(19). Nutrient Broth (M002, HiMedia, Mumbai)

(20). Phytase Degrading Screening Medium (Holzapfel, 1997)

Ca-Phytate	5.0 g
Glucose	2.0 g
Na-Citrate	0.1 g
$MgSO_4$	0.075 g
$MnSO_4$	0.010 g
$FeSO_4$	0.005 g
L-Glutamate	0.150 g
DL-Alanine	0.20 g
L-Arginine-HCl	0.05 g
L-Asparagine	0.20 g
L-Cysteine-HCl	0.20 g
L-Phenylalanine	0.04 g
L-Histidine	0.05 g
L-Isoleucine	0.06 g
L-Leucine	0.06 g
L-Lysine-HCl	0.05 g
L-Methionine	0.05 g
L-Proline	0.04 g
L-Tyrosine	0.002 g
L-Threonine	0.025 g
Thiamine	0.005 g

L-Tryptophan	0.025 g
L-Valine	0.015 g
Adenine	0.025 g
Guanosine	0.025 g
Thymidine	0.025 g
Uracil	0.025 g
Vitamin B12	0.0001 g
Biotin	0.0001 g
Ca-Pantothenate	0.01 g
Folic acid	0.001 g
Niacin	0.01 g
Riboflavine	0.005 g
Agar Agar	18 g
Distilled water	1000 ml
pH	6.0

(21). Plate Count Agar (M091, HiMedia, Mumbai)

(22). Potato Dextrose Agar (M096, HiMedia, Mumbai)

(23). *Salmonella–Shigella* Agar (M108, HiMedia, Mumbai)

(24). Skim Milk Powder (RM1254, HiMedia, Mumbai)

(25). Sucrose Broth (Garvie, 1960)

Tryptone	10 g
Yeast extract	5.0 g
K_2HPO_4	5.0 g
Trisodium citrate	5.0 g
Sucrose	50 g
Distilled water	1000 ml

Ingredients were dissolved in distilled water by heating in a steamer and sterialised by autoclaving.

(26). Tryptone Soya Agar (M290, HiMedia, Mumbai)

(27). Violet Red Bile Glucose Agar w/o Lactose (M581, HiMedia, Mumbai)

(28). Yeast-Malt Extract (YM) Agar (M424, HiMedia, Mumbai)

(29). Yeast Malt Extract (YM) Broth (M425, HiMedia, Mumbai)

(30). Yeast Morphology Agar (M138, HiMedia, Mumbai)

(31). Yeast Nitrogen Base (M139, HiMedia, Mumbai)

REAGENTS

(1). Acidic Ninhydrin

1-Butanol/water saturated	465 ml
Acetic acid	35 ml
Ninhydrin	2.5 ml

(2). Burke's Iodine Solution (Bartholomew, 1962)

Iodine	1.0 g
Potassium iodide	2.0 g
Distilled water	100 ml

(3). Gram's Crystal Violet (S012, HiMedia, Mumbai)

(4). Malachite Green (S020, HiMedia, Mumbai)

(5). Nessler's Reagent

Potassium iodide	50.0 g
Mercuric chloride (saturated)	35.0 ml
Distilled water (ammonia free)	25.0 ml
Potassium hydroxide (50 %)	400.0 ml

Potassium iodide was dissolved in 35 ml of distilled water followed by addition of saturated aqueous solution of mercuric chloride till the appearance of precipitate. Then, 400 ml of potassium hydroxide was added and made the final volume to 1000 ml by adding distilled water. The solution was left for a week, the supernatant was decanted and stored in capped amber bottle at 4° C.

(6). Nitrate Reduction Test Reagent

Solution A

Sulphanilic acid	0.8 g
5 N acetic acid	100 ml
(Glacial acetic acid: water, 1:2.5)	

Solution B

∝-Napthylamine	0.5 g
5 N acetic acid	100 ml

The solutions A and B were mixed in equal quantities just before use.

(7). Phenolphthalein (I009, HiMedia, Mumbai)

(8). Ringer solution (Merck, Germany)

(9). Safranin (S027, HiMedia, Mumbai)

Reference Strains

Reference Strains	Origin	Purpose in this experiment
Bacillus cereus CCM 2010	CCM	Indicator strain for antimicrobial activity
Enterobacter agglomerans BFE 154	BFE	Indicator strain for antimicrobial activity
Enterobacter cloacae BFE 282	BFE	Indicator strain for antimicrobial activity
Enterococcus faecium DSM 20477	DSM	Indicator strain for antimicrobial activity
Klebsiella pneumoniae subsp. *pneumoniae* BFE 147	BFE	Indicator strain for antimicrobial activity
Lactobacillus brevis DSM 20054	DSM	Molecular identification
Lactobacillus buchneri LMG 8692	LMG	Molecular identification
Lactobacillus collinoides DSM 20595	DSM	Molecular identification
Lactobacillus diolivorans DSM 14421	DSM	Molecular identification
Lactobacillus hilgardii DSM 20176	DSM	Molecular identification
Listeria innocua DSM 20649	DSM	Indicator strain for antimicrobial activity
Listeria monocytogenes DSM 20600	DSM	Indicator strain for antimicrobial activity
Lactobacillus paraplantarum LTH 5200	LTH	Molecular identification
Lactobacillus pentosus DSM 20314	DSM	Molecular identification
Lactobacillus plantarum DSM 20174	DSM	Standard strain for *meso*-diaminopimelic acid determination (DAP); molecular identification
Lactobacillus sakei DSM 20017	DSM	Ammonia from arginine
Pediococcus acidilactici DSM 20333	DSM	Molecular identification
Pseudomonas aeruginosa BFE 162	BFE	Indicator strain for antimicrobial activity
Pediococcus pentosaceus MTCC 3817	MTCC	Molecular identification
Staphylococcus aureus S1	FMR	Indicator strain for antimicrobial activity
Streptococcus mutans DSM 6178	DSM	Indicator strain for antimicrobial activity

Originally, these reference strains were obtained from DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany), CCM (Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia), BFE (Instutute of Hygiene and Toxicology, Karlsruhe, Germany), MTCC (Microbial Type Culture Collection, Chandigarh, India), LTH (Universität Hohenheim, Germany) and FMR (Food Microbiology Laboratory, Sikkim Government College, Gangtok, India).

Listeria monocytogenes DSM 20600, *Listeria innocua* DSM 20649, *Staphylococcus aureus* S1, *Bacillus cereus* CCM 2010, *Enterobacter agglomerans* BFE 154, *Enterobacter cloacae* BFE 282, *Klebsiella pneumoniae* subsp. *pneumoniae* BFE 147, *Pseudomonas aeruginosa* BFE 162 were propagated in standard nutrient agar. *Enterococcus faecium* DSM 20477, *Streptococcus mutans* DSM 6178, *Lactobacillus plantarum* DSM 20174, *Lactobacillus sakei* DSM 20017, *Lb. brevis* DSM 20054 were cultivated in MRS broth. The cultures were maintained as frozen stocks at –20° C in 15% glycerol.

METHODS

Traditional knowledge

Detailed information on various types of traditional fermented vegetable products including wild edible bamboo shoot products was documented from different villages located in Sikkim, Arunachal Pradesh and Manipur of North East India. Indigenous knowledge on methods of preparation including biopreservation methods of perishable and seasonal vegetables practised by the ethnic and tribal people of North East India, the nature and use of the traditional fermented products were documented based on the information sought from the local people of the respective places.

Collection of samples

Twenty five samples of gundruk and twenty one samples of sinki were collected from local markets of Sikkim. Twenty samples of goyang and twenty five samples of khalpi were directly obtained from the producers in different rural areas in Sikkim. A total of forty six samples of mesu were collected from different places of Sikkim during rainy seasons when bamboo shoots were plenty. Six samples of inziangsang were obtained from Bishnupur market of Manipur. Sixteen samples of ekung, thirteen samples of eup and fifteen samples of hirring were collected from different places of Arunachal Pradesh. Twelve samples of soibum, eleven samples of soidon and eight samples of soijim were obtained from Bishnupur and Imphal of Manipur. All samples were collected aseptically

in sterile bottles and poly-bags, which were kept in an ice-box container, and transported to the laboratory for analyses.

Microbiological analysis

Ten g of sample were homogenised with 90 ml of 0.85 % (w/v) sterile physiological saline in a stomacher lab-blender (400, Seward, UK) for 1 min. A serial dilution (10^{-1} to 10^{-8}) in the same diluent was made. Lactic acid bacteria (LAB) were isolated on MRS agar (M641, HiMedia, Mumbai) supplemented with 1 % $CaCO_3$, and incubated under anaerobic condition in an Anaerobic Gas-Pack system (LE002, HiMedia, Mumbai) and incubated at 30° C for 48-72 hour. Aerobic mesophilic counts were determined using plate count agar (M091A, HiMedia, Mumbai) incubated at 30° C for 48-72 hour. Moulds and yeasts were isolated on potato dextrose agar (M096, HiMedia, Mumbai) and yeast-malt extract (YM) agar (M424, HiMedia, Mumbai), supplemented with 10 IU/ml benzylpenicillin and 12 µg/ml streptomycin sulphate, respectively, and incubated aerobically at 28° C for 72 hour. Colonies were selected randomly or all sampled if the plate contained less than 10 colonies, according to Leisner *et al.* (1997). Purity of the isolates was checked by streaking again on fresh agar plates of the isolation media and sub-culturing on corresponding broths/agar, followed by microscopic examinations. Microbiological data obtained were transformed into logarithms of the numbers of colony forming unit (cfu) per g of sample. Identified strains of LAB were preserved in MRS broth using 15 % (v/v) glycerol at –20° C.

Characterisation of Bacterial Isolates

Cell morphology

Smear of a 24 hour-old bacterial culture was made in a grease free slide, air-dried (not heated-fixed), stained for 30 sec with safranin (S027, HiMedia, Mumbai), washed in water, air-dried (Harrigan, 1998) and observed under oil-immersion objective. Cell dimensions were measured with a standardized ocular micrometer.

Gram staining

All isolates were Gram-stained following the method of Bartholomew (1962). A suspension of a 24 hour-old bacterial culture on slant was prepared, and smear was made in a grease-free slide. Then heated-fixed, flooded by crystal violet stain for 1 min, and washed for 5 sec with water. The smear was flooded with Burke's iodine solution, allowed to react for 1 min, and washed again for 5 sec with water. Holding the slide against a white surface, 95% ethanol was poured drop-wise from the top edge of the slide until no more colour came out from the lower edge of the slide. After washing with water, the smear was stained with safranin for 1 min and washed again with water. The slide was air-dried and observed under oil-immersion objective.

Motility

A hanging drop of a 24 hour-old culture in MRS broth was prepared in a cavity slide following the method of Harrigan (1998). The prepared

culture was observed in a phase contrast microscope (Olympus CH3-BH-PC, Japan) for motility of the strains

Production of catalase

The production of gas bubbles by the isolates were observed by adding 0.5 ml of 10 % hydrogen peroxide solution (Merck) to the cultures indicating the presence of catalase (Schillinger and Lücke, 1987).

Ammonia from arginine

Tubes of 5 ml arginine hydrolysis medium (Thornley, 1960) were inoculated with 24 hour-old culture. The tubes were incubated at 30° C for 3 days and formation of ammonia from arginine was detected by spotting 100µl culture onto a white porcelain tile and adding equal volume of Nessler's reagent. Appearance of dark orange colour indicated presence of ammonia. *Lactobacillus sakei* DSM 20017 was used as ammonia positive reference strain (Schillinger and Lücke, 1987).

Gas ($C0_2$) production from glucose

Tubes of 10 ml MRS broth without citrate and containing inverted Durham tubes was inoculated with 24 hour-old cultures and incubated at 30° C (Schillinger and Lücke, 1987). Accumulation of gas in the inverts indicated positive result.

Growth at different pH

The pH of MRS broth was adjusted to 3.9 and 9.6 using 1 N HCl or 10% w/v NaOH. The medium was distributed into tubes containing 5 ml in each. They were autoclaved, cooled to room temperature and inoculated with 24 hour-old MRS broth culture. The tubes were incubated at 30° C for 24-72 hour and observed for growth (Dykes *et al.*, 1994).

Growth at different temperatures

MRS broth were inoculated with 24 hour-old cultures and incubated at 10° C and 15° C for 7 days, and 45° C for 3 days, respectively and observed for growth (Dykes *et al.*, 1994).

Growth in different NaCl concentrations

Salt tolerance was tested by inoculating a loop-full of culture in MRS broth supplemented with 6.5 %, 10.0 % and 18.0 % NaCl, respectively, and incubated for 3 days at 30° C in a slanting position to improve aeration (Schillinger and Lücke, 1987). Cultures were observed for growth after incubation.

Acid from carbohydrates

The method was based on Schillinger and Lücke (1987). Tubes of 5 ml MRS broth without beef extract, containing 0.5% w/v of different carbohydrates instead of glucose and 0.004% phenol red indicator were inoculated and incubated at 30° C for 2-5 days. Colour change from red to yellow indicated acid production.

Production of dextran from sucrose

Production of dextran in sucrose broth (Garvie, 1960) was tested exclusively for leuconostoc isolates following the method described by Kelly *et al.* (1995).

Lactic acid configuration

The configuration of lactic acid produced was determined enzymatically using D-lactate and L-lactate dehydrogenase kits (Boehringer-Mannheim GmbH, Cat. No. 1112821, Germany). The LAB strains were grown in lactate configuration medium at 30° C overnight. One ml culture was centrifuged in a microcentrifuge (Heraeus, Germany) at 8,000 *g* for 5 min. The 20 µl of the supernatant was mixed with 980 µl of redistilled water to obtain 1:50 sample dilution. The 1 ml of Solution (1), 0.2 ml of Solution (2), 0.02 ml of Suspension (3), 0.1 ml sample solution and 0.9 ml of redistilled water was pipetted into a cuvette, followed by gentle swirling to mix the contents of the cuvette after closing it with parafilm. Similarly, a blank was prepared by adding all the reagents except the sample solution being replaced with 1.0 ml of redistilled water. After 5 minutes the absorbance of the solutions (A_1) was measured in UV-VIS Spectrophotometer (Analytik Jena, Germany) at 340 nm. The absorbance differences (A_2-A_1) for both, blank and sample was determined and the difference of the absorbance difference of the blank from that of the sample ($\Delta A_{D\text{-lactic acid}}$) was calculated. The reaction was started by adding 0.02 ml of Solution (4) to the sample as well as to the

blank. The cuvettes were swirled gently to mix the contents by closing it with parafilm. After 30 minutes the absorbance (A_2) of the sample and the blank were measured immediately one after another at 340 nm. The 0.02 ml of Solution (5) was added to both the sample and the blank followed by mixing. These were allowed to stand for 30 minutes. The absorbance (A_3) was measured immediately one after another for the sample as well as for the blank at 340 nm. The absorbance differences (A_3-A_2) for both, blank and sample was determined and the difference of the absorbance difference of the blank from that of the sample ($\Delta A_{L\text{-lactic acid}}$) was calculated. The lactic acid isomer concentration was calculated as: $c = (V \times MW \times \Delta A)/(\varepsilon \times d \times v \times 1000)$ (g/l), where, V = final volume (ml), v = sample volume (ml), MW = molecular weight of lactic acid = 90.1 (g/mol), d = light path = 1 cm, ε = extinction coefficient of NADH at 340 nm = 6.3 (l/m mol × cm). The result was multiplied by the dilution factor.

meso-Diaminopimelic acid (DAP)

The presence of *meso*-diaminopimelic acid in the cell walls of LAB was determined using thin-chromatography on cellulose plate (Tamang *et al.*, 2000). Cells were harvested from 48 hour old MRS broth culture by centrifuging 5 ml culture at 8,000 *g* for 5 min, and washed with 3 ml of distilled water. The sediment was resuspended in 1 ml of 6 N HCl and transferred to screw-capped tubes. The cells were hydrolysed overnight at 100° C in a water-bath. The contents of the tubes were blow-dried while immersed in boiling water. The sediment was resuspended in 1 ml of

distilled water and blow dried again and oven dried for 1 hour. Finally, the sediment was suspended in 0.1 ml of distilled water and each sample (5 µl) was spotted on thin-layer chromatography plates on cellulose plates (Merck, Germany). Descending one-dimensional chromatography was done by keeping the plates in a TLC chamber in a solvent solution containing methanol: pyridine: 10 N HCl: water (32:4:1:7). The solvent solution was prepared 1 hour before use. After keeping for 4-5 hour the plates were dried with a hair drier and the chromatograms were developed by spraying acidic ninhydrin and when almost dried, placed for 5 min in 100° C oven. Spots representing *meso*-diaminopimelic acid appeared dark green to grey and turned yellow within 24 hour. *Lactobacillus plantarum* DSM 20174 was used as standard (*meso*-DAP positive).

API Tests

Carbohydrate fermentation patterns of LAB were determined using API 50 CHL and API 20 STREP test strips (bioMérieux, France) according to manufacturer's instructions as well as the method described by Tamang and Holzapfel (1999). Cultures were grown on MRS agar at 30° C for 48 hour. The growth was harvested in 2 ml sterile normal NaCl solution which was used to prepare suspensions, corresponding to 10^7 cells/ml. The incubation box was prepared by distributing about 10 ml of sterile water into the honeycombed base of the 50 CHL trays. The strips were unpacked, placed them in the trays and the tubes were filled with the

bacterial suspensions. The inoculated strips were kept slightly tilted and incubated at 30° C for 48 h. The results were read by referring to the manufacturer's interpretation table at 24 hour and 48 hour, respectively. All spontaneous reactions were recorded. The APILAB PLUS database identification software (bioMérieux, France) was used to interpret the results.

Biolog system

The Biolog microplate bacterial identification system (Biolog Inc., USA; Oxoid GmbH, Wesel, Germany) based on the utilisation of 95 single carbon sources was used for the identification of *Leuconostoc* species. The metabolism of the substrates in the wells of the microplates results in a reduction of tetrazolium dye producing a colour change, and a specific "metabolic fingerprint" was obtained for each strain and compared with the data of the Biolog MicroLog database software (Biolog Inc.). Before inoculation of the Biolog AN microplates, strains were grown anaerobically on BUA (Biolog Universal Anaerobe) agar (Oxoid GmbH, Germany) with 5 % sheep blood at 30° C for 48 hour. The bacterial cells were swabbed from the surface of the agar and suspended in AN Inoculating fluid (Biolog Inc.) at the recommended cell density. 100 µl of the bacterial suspension was pipetted in each well of the Biolog AN microplate. The microplates were incubated in an anerobic jar with a hydrogen-free anaerobic atmosphere (Oxoid AnaeroGen; Oxoid GmbH) at 30° C for 48 hour and then read with the Biolog Microstation Reader (Biolog Inc.).

Phenotypic identification

Bacterial species were identified following the taxonomic keys of Bergey's Manual (Sneath *et al.*, 1986), Simpson and Taguchi (1995), Wood and Holzapfel (1995), and by APILAB PLUS Database software of API tests (bioMérieux, France).

Genotypic Characterization of LAB Strains

DNA extraction

Total genomic DNA of some selected strains of LAB was extracted from 2-ml samples of overnight cultures grown in MRS broth at 30° C according to the methods of Pitcher *et al.* (1989). Reference strains were obtained from DSMZ, LTH and LMG, as mentioned earlier in this chapter.

RAPD-PCR analysis

The primer M13 (5'-GAG GGT GGC GGT TCT-3') (Huey and Hall, 1989) was used for randomly amplified polymorphic DNA (RAPD)-PCR. Conditions of PCR reactions and amplification were performed as described by Schillinger *et al.* (2003). The PCR amplification was conducted with a Primus 96 Plus thermal cycler (MWG Biotech, Ebersberg, Germany).

Repetitive element (rep)-PCR analysis

Repetitive element (rep)-PCR analysis of LAB strains was carried out using reaction and amplification conditions as previously described by Gevers *et al.* (2001). For rep-PCR, the primer $(GTG)_5$ (5'-GTG GTG GTG

GTG GTG-3') recommended for LAB identification by Gevers *et al.* (2001) was used.

Species-specific PCR

For identification of *Lactobacillus brevis*, a species-specific PCR was applied. The oligonucleotide primer 5'-CTTGCACTGATTTTAACA-3' and 5'-GGGCGGTGTGTACAAGGC-3' were used as forward and reverse primers, respectively (Guarneri *et al.*, 2001). The PCR conditions were as described by Guarneri *et al.* (2001). To verify the identity of the PCR product, amplified fragments were digested with P*st*I (New England Biolabs, Frankfurt, Germany) in a 15 µl reaction mixture containing 11.5 µl of the PCR product, 1.5 µl incubation buffer and 10 U P*st*I (1 h, 37° C).

Gel electrophoresis

Amplification products from RAPD-PCR and rep-PCR were subjected to electrophoresis in 1.8 % agarose gels in 1 x TBE buffer for 15-17 hour at 48 and 55 V, respectively. The products resulting from the *Lactobacillus brevis* specific PCR were separated on 1.2 % agarose for 2 hour at 100 V. A DNA molecular mass marker (100 and 500 bp molecular ladders from Biorad, München, Germany) was used as a standard. After electrophoresis, the gels were stained in ethidium bromide and after washing visualised with a UV transilluminator. Gels were photographed using the Fluorchem Imager 5500 system (Alpha Innotech, USA) and the digitised images were analysed and processed using the Bionumerics software (Applied Maths, Kortrijk, Belgium). Groupings of the fingerprints

were performed by means of the Pearson product-moment correlation coefficient and the unweighted pair-group method using arithmetric averages clustering algorithm (UPGMA).

Characterisation of yeast isolates

Cell morphology

Cell morphology and mode of vegetative reproduction of yeast was observed following the method of Yarrow (1998). Sterile yeast morphology agar (M138, HiMedia, Mumbai) slants were inoculated with an actively growing (24 hour-old) yeast culture and incubated at 28° C for 3 days. Dimensions of cells were measured with a standardized ocular micrometer.

Pseudo- and True-mycelium

For observation of pseudo-mycelium and true-mycelium of yeast isolates, the slide culture method described by Kreger-van Rij (1984) was followed. A petri-dish, containing U-shaped glass rod supporting two glass slides, was autoclaved at 121° C for 20 min. The glass slides were quickly removed from the glass rod with a flame sterialized pair of tweezers, and were dipped into the molten potato dextrose agar (M096, HiMedia, Mumbai) after which they were replaced on the glass rod support. The solidified agar on the slides was inoculated very lightly with yeast isolates in two lines along each slide. Four sterile coverslips were placed over part of the lines. Some sterile water was poured into the petri-dish to prevent the agar from drying out. The culture was then incubated at 28° C for 4

days. The slides were taken out of the petri-dish and the agar was wiped off from the back of the slide. The edges of the streak under and around the coverslips were examined microscopically for the formation of pseudo-mycelium or true-mycelium.

Characteristics of asci and ascospore

Sterile ascospore agar (M804, HiMedia, Mumbai) slants were streaked with actively grown yeast cultures, incubated at 28° C for 3 days and examined at weekly intervals up to 4 weeks for observation of asci and ascospores. A heat fixed smear was flooded with 5 % w/v aqueous malachite green (S020, HiMedia, Mumbai) for 30 to 60 sec, heated to steaming 3 to 4 times over the flame of a spirit lamp and counterstained with safranin (S027, HiMedia, Mumbai) for 30 sec and observed under the microscope (Yarrow, 1998).

Reduction of nitrate

Yeast cultures were grown in 5 ml nitrate broth incubated at 28° C. After 3, 7 and 14 days, 1 ml of the culture was mixed with 3 drops of the reagent for nitrate reduction test and observed for the development of a red or yellow colour, indicating the presence of nitrate. A small amount of zinc dust was added to the tube that was negative even after 14 days and observed for the development of red colour, indicating the presence of nitrate, i.e. absence of reduction (Yarrow, 1998).

Growth at 37° C

Slants of malt-extract agar (M137, HiMedia, Mumbai) were inoculated with cells of actively grown yeast isolates and incubated at 37° C for 4 days and observed for growth (Yarrow, 1998).

Sugar fermentation

Yeasts isolates were grown at 28° C on yeast-malt extract (YM) agar (M242, HiMedia, Mumbai) slants for 3 days. Tubes of 10 ml of fermentation basal medium (Wickerham, 1951) supplemented with 2 % w/v sterile sugars containing inverted Durham tubes, were inoculated with the above yeast culture and incubated at 28° C and were shaken regularly to observe gas accumulation in the inverts (Yarrow,1998).

Sugar assimilation

Yeast isolates were grown at 28° C on yeast-malt extract (YM) agar (M242, HiMedia, Mumbai) slants for 3 days. Tubes containing 5 ml mixture of yeast nitrogen base (M139, HiMedia, Mumbai) and carbon source were inoculated with cultures and incubated at 28° C for 3 to 7 days. Control test tube was made by adding 0.5 ml of yeast nitrogen base in 4.5 ml of sterilized distilled water (devoid of any carbon source). Assimilation of carbon sources was observed by comparing with the control (Yarrow, 1998).

Identification of Yeast

Yeast isolates were identified to the genus level according to the criteria laid down by Kreger-van Rij (1984), Kurtzman and Fell (1998) and Yarrow (1998).

Pathogenic contaminants

Enumeration of pathogenic contaminants from the samples were done in selective media such as *Bacillus cereus* agar base (M833, HiMedia, Mumbai) for *Bacillus cereus*, Baird Parker agar base (M043, HiMedia, Mumbai) for *Staphylococcus aureus* and Violet Red Bile Glucose agar w/o lactose (M581, HiMedia, Mumbai) for enterobacteriaceae (Han *et al.*, 2001). *Salmonella-Shigella* Agar (M108, HiMedia, Mumbai) was used for the detection of *Salmonella* and *Shigella* and *Listeria* identification agar base (M1064, HiMedia, Mumbai) with *Listeria* selective supplement (FD 061, HiMedia, Mumbai) for *Listeria* in the samples following the standard method of Metaxopolous *et al.* (2001). Ten g of sample were blended with 90 ml of peptone-physiological saline (0.1% neutral peptone, 0.85% NaCl) and homogenized in a stomacher lab-blender 400 (Seward, UK) for 1 min. Serial decimal dilution series was prepared in the same diluent in duplicates.

***Bacillus cereus*:** Selective enumeration was carried out on spread plates of *Bacillus cereus* agar base (M833, HiMedia, Mumbai) with appropriate additions of Polymyxin B Selective Supplement (FD003, HiMedia, Mumbai) and Egg yolk emulsion (FD045, HiMedia, Mumbai). The

inoculated plates were incubated at 30° C for 24-48 hour. Characteristic turquoise to peacock blue colonies surrounded by zone of precipitate of the same colour were regarded as presumptive *Bacillus cereus*.

***Staphylococcus aureus*:** Spread plates of Baird Parker agar base (M043, HiMedia, Mumbai) with appropriate additions of Egg yolk tellurite emulsion (FD046, HiMedia, Mumbai) was used for selective enumeration of *Staphylococcus aureus*. After serial dilution plates were overlaid with the medium and incubated at 30° C for 24-48 hours. The black colonies surrounded by clear zone extending 2-5 mm into the opaque medium appeared were regarded as presumptive *Staphylococcus aureus*.

Enterobacteriaceae: Sample dilutions in tryptone soya broth (M011, HiMedia, Mumbai) were allowed to resuscitate on thinly plated tryptone soya agar (M290, HiMedia, Mumbai) plates for 1-2 hour at 27° C, followed by a thick overlay of selective Violet Red Bile Glucose agar (without lactose) (M581, Himedia, Mumbai) medium and incubated at 30° C for 20 hour. Pink colonies appeared were regarded as presumptive enterobacteriaceae.

***Listeria* sp:** *Listeria* identification agar base (M1064, HiMedia, Mumbai) with *Listeria* selective supplement (FD061, HiMedia, Mumbai) media were used for detection of *Listeria* in the samples following the standard method of Metaxopolous *et al.* (2001). Inoculated plates were incubated

at 30° C for 48 hour and observed in dark background for smooth glistening colonies indicating presence of *Listeria*.

***Salmonella* and *Shigella*:** *Salmonella-Shigella* (SS) agar (M108, HiMedia, Mumbai) was used for the detection of *Salmonella* and *Shigella* in samples following the method of Metaxopolous *et al.* (2001). After serial dilution plates were inoculated, followed by an overlay of the SS agar and incubated at 37° C for 48 hour and observed in dark background for presumptive colonies. *Salmonella* colonies appear dark-centred while colourless colonies are regarded as presumptive *Shigella*.

Technological Properties of LAB Isolates

Acidification and coagulation

Effect of acidification and coagulation of the LAB isolates was assayed by inoculating 10 % skim milk with 24 hour old cultures (RM1254, HiMedia, Mumbai) (centrifuged at 8,000 *g* for 20 min and sterialized at 110^0 C for 10 min) at 1 % level, and incubated at 30^0 C. Observation was made for commencement of clotting, and pH was measured after 72 hour of incubation (Olasupo *et al.*, 2001).

Antimicrobial and bacteriocin activity

The LAB isolates were screened for antimicrobial activity by agar spot method of Schillinger and Lücke (1989). The indicator strains used for antagonisms included: *Listeria innocua* DSM 20649, *Listeria 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, *Enterobacter agglomerans* BFE 154 and *Pseudomonas aeruginosa* BFE 162. Cell-free neutralized supernatants fluids of LAB isolates were screened for bacteriocin production by agar spot test method described by Uhlman *et al.* (1992), using the bacteriocin screening medium of Tichaczek *et al.* (1992).

Bacteriocin activity was quantified based on an agar diffusion assay as described by Cabo *et al.* (1999). The quotient between the inhibition zone area and the sensitivity of the indicator bacterium used to compare different bacteriocins. The twofold serial dilutions of the neutralized supernatants of bacteriocin are spotted (10 µl) onto the surface of lawns containing the required indicators bacteria. Bacteriocins titres were expressed as the reciprocal of the highest dilution exhibiting a zone of inhibition and were reported in activity units (AU) per ml.

Enzymatic profile by API-zym

The enzymatic profile of LAB were assayed following the method of Arora *et al.* (1990) using API-zym (bioMérieux, France) galleries by testing for the activity of the following 19 enzymes: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine-, valine- and cystine- arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-

glucosaminidase, α-mannosidase and α-fucosidase. Cultures were grown on MRS agar and cells were harvested in 2 ml sterile normal saline which was used to prepare suspension of 10^7 cells/ml. The strip was unpacked and 2 drops of cell suspensions was inoculated in each cupule of the strip containing ready-made enzyme substrates and incubated at 30° C for 6 hour. After incubation, 1 drop of ready-made zym-A and zym-B reagents was added and observed for colour development based on the manufacturer's colour chart.

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 are 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).

Biogenic amine

The ability of LAB isolates to produce biogenic amines was determined qualitatively on an improved screening medium as described by Bover-Cid and Holzapfel (1999) using a 'cocktail' of four precursor amino acids (histidine, lysine, ornithine and tyrosine). Cultures previously grown and subcultured twice in biogenic amine sub-culturing medium were spotted onto the plates containing screening medium. Change of the bromocresol purple indicator to purple was considered as index of significant amino acid decarboxylase activity, corresponding to >350 mg of a particular amino acid/litre (Olasupo *et al.*, 2001).

Hydrophobicity assay

Bacterial adhesion to hydrocarbons was determined and results were expressed according to Rosenberg (1984) and Perez *et al.* (1998), modified as follows. 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 three times with 9 ml of Ringer solution (Merck, Germany), and thoroughly

mixed in a vortex. The 1 ml of the suspension was taken and the absorbance at 580 nm was measured. Then, 1.5 ml of the suspension was mixed with equal volume of n-hexadecane (RM 2238, HiMedia, Mumbai) in duplicates 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 (Martin *et al.*, 1989; Nostro *et al.*, 2004).

Fermentation Dynamics *in situ*

Two types of gundruk were prepared following the traditional method, 'rayo-saag' gundruk, prepared from leaves of 'rayo-saag' [*Brassica rapa* L. sub-sp. *campestris* (L.) Clapham variety *cumifolia* Roxb.], and 'mula-saag' gundruk, prepared from leaves of radish. Leaves were wilted in the sun for 1 day, then crushed and soaked in hot water. About 400 g of crushed leaves were put into sterile 250 ml-bottle, pressed with sterile pestle to remove excess water, and loosely capped. Then, bottles were tightly capped and fermented at room temperature (20-25° C) for 16 days. Samples were taken at every one day interval till 16th day for analyses.

Ripened cucumber was collected from Gangtok market (Sikkim) and khalpi was prepared following the traditional method. Cucumber was

washed and cut into pieces and sun dried for 1 day. About 400 g of pieced cucumbers were filled into sterile 250 ml bottles and fermented at room temperature (20-25°C) for 3 days. Samples were taken at every 6 hour interval till 72 hours for analyses.

The pH and titratable acidity of the fermenting substrates were also determined. Changes in microbial population of major microbial groups were analysed as described in microbial analysis section. Temperature of each sample was recorded before taking sample for analysis.

Preparation of Gundruk and Khalpi using Selected Strains of LAB

'Rayo-saag' leaves of *Brassica rapa* L. sub-sp. *campestris* (L.) Clapham variety *cumifolia* Roxb. were purchased from Gangtok market. Leaves were washed thoroughly in sterile distilled water and wilted in oven (~30° C) for 6 hour. Leaves were crushed and put into sterile warm water (about 90° C) for 5 min. Leaves were transferred into another sterile glass container. Excess water in the leaves was removed by squeezing and then, about 400 g of crushed leaves of 'rayo-saag' were distributed aseptically into each sterile 250 ml capped-bottles, totalling 6 bottles for samplings. Each bottle was inoculated by a mixture (1 ml each) of actively grown culture strains of *Lactobacillus plantarum* GLn: R1 and *Pediococcus pentosaceus* GLn:R2, previously isolated from market samples of gundruk. Bottles were tightly capped and incubated at 20°, 25° and 30° C, respectively for 6 days. Samplings were done on 3rd and 6th days for organoleptic test followed by determination of pH and acidity.

Ripened cucumber (*Cucumis sativus* L.) was collected from Sadam village in Sikkim. Cucumber was cleaned, washed and cut into pieces and wilted at ~30° C in an oven for 6 hour. About 400 g of wilted pieced cucumbers were transferred into each sterile 250 ml bottles, totaling 9 bottles for samplings. Each bottle was inoculated by a mixture (1 ml each) of pure culture strains of actively grown *Lactobacillus plantarum* KG:B1, *Lactobacillus brevis* KG:B2 and *Leuconostoc fallax* KB:C1, previously isolated from traditionally prepared khalpi samples. Bottles were tightly capped and incubated at 20°, 25° and 30° C, respectively for 72 hours. Samples were taken on 24, 48 and 72 hours, respectively for sensory evaluation and also for determination of pH and acidity of the fermenting cucumbers.

Sensory evaluation

Sensory evaluation of gundruk and khalpi, prepared by selected starter cultures was evaluated in terms of aroma, taste, texture, colour and general acceptability as described by Meilgaard *et al.* (1990). Gundruk was organoleptically evaluated by a panel of 7 judges with score rate of 1, bad and 5, good considering market gundruk as control with scoring rate of 3, moderate (Table B). Similarly, khalpi was also evaluated organoleptically by a panel of 7 judges with score rate of 1, bad and 5, good considering market khalpi as control with scoring rate of 3, moderate (Table B).

Table B. Format for sensory evaluation of gundruk/khalpi produced by selected strains of LAB

Please use market gundruk/khalpi as a control with scoring rate of 3 (moderate)

Sample code:..... Name:.....

Attribute	Score					Comment
Aroma	Weak				Strong	
	1	2	3	4	5	
Taste: Mild acidic	Weak				Strong	
	1	2	3	4	5	
Acidic (sour)	Weak				Strong	
	1	2	3	4	5	
Texture:	Hard				Soft	
	1	2	3	4	5	
Colour:	Bad				Good	
	1	2	3	4	5	
General Acceptability:	Bad				Good	
	1	2	3	4	5	

Proximate Composition

pH

Ten g of sample were mixed with 20 ml carbon dioxide-free distilled water in a blender for 1 min and the pH of the slurry was determined directly (AOAC, 1990) using a digital pH meter (Model 361, Systronics, India) calibrated with standard buffer solutions (Merck).

Titratable acidity

Titratable acidity of sample was calculated by titrating the filtrates of a well blended 10 g sample in 90 ml carbon-dioxide free distilled water with 0.1 N sodium hydroxide to end point of phenolphthalein (0.1 % w/v in 95 % ethanol) (AOAC, 1990).

Moisture

Moisture content of sample was calculated by drying 2.5–3.0 g of well-mixed sample at 135 ± 1° C for 2 hour to constant weight (AOAC, 1990).

Ash

A sample (~ 2 g) was accurately weighed into a previously dried and weighed porcelain crucible and placed in a muffle furnace, preheated to 550° C for 3 hour. The crucible was transferred directly to a desiccator, allowed to cool to room temperature and weighed immediately (AOAC, 1990). The process of heating for 30 min, cooling and weighing was

repeated until the difference between two successive weighing was ≤ 1 mg.

Fat

Fat content was determined by ether extraction using glass soxhlet (AOAC, 1990). Flat-bottomed flask was oven dried and kept in a desiccator for cooling. The weight (W_1) of the round-bottomed flask was taken. A cellulose thimble (dry and fat free) was taken and in which ~ 2 g of sample was placed and put in the soxhlet. Fat was extracted by using petroleum ether with boiling range 40-60° C, on a heating mantle at 60° C for 5 hour. The flat bottomed flask was dried for 1 h at 100° C to evaporate ether and moisture, cooled in desiccator and weighed (W_2). Fat was calculated: Fat (%) = $W_2 - W_1$/Sample weight × 100

Protein

Total nitrogen of sample was determined following the method described in AOAC (1990). Approximately 1 g of sample was taken in a digestion flask, 0.7 g catalyst ($CuSO_4$: K_2SO_4, 1:9) and 25 ml of concentrated H_2SO_4 were added to it. The flask was heated gently until frothing ceased, boiled briskly until the solution became clear and then continued the boiling for about 1 hour. The solution was transferred quantitatively to a round-bottomed flask, and mixed with approximately 100 ml of distilled water and 25 ml 4 % w/v aqueous Na_2S to precipitate mercury. A pinch of zinc granules to prevent bumping and a layer of 40 %

w/v NaOH were added carefully. The flask was immediately connected to a distillation apparatus and the tip of the condenser was immersed in standard 0.1 N H_2SO_4 containing about 5 drops of methyl red indicator (I007, HiMedia, Mumbai). The flask was rotated to mix the contents thoroughly and heated until all the ammonia had distilled. The receiver was removed and the tip of the condenser was washed with distilled water. The remaining acid in the receiver was titrated with standard 0.1 N NaOH solution. The blank determination on reagents was considered for correction. Nitrogen was calculated in percentage. Total nitrogen (%) = [(ml of standard acid × N of standard acid) – (ml of standard NaOH – C.F.) × N of standard NaOH] ×1.4007/weight of sample (g). Correction factor (C.F.) = (titre of standard NaOH against blank – ml of standard acid).

Protein content was determined by multiplying total nitrogen value with 6.25 (for plants) (AOAC, 1990). Protein (%) = Total Nitrogen (%) × 6.25

Carbohydrate

The carbohydrate content of the samples was calculated by difference: 100 – (% protein + % fat + % ash) (Standal, 1963).

Nutritive Value

Nutritive value of each sample was determined by multiplying the protein, fat and carbohydrate contents by the factors 4, 9 and 4,

respectively, and adding all the multiplication values to get kcal per 100 g (Indrayan *et al.*, 2005).

Minerals

The method of AOAC (1990) for determination of mineral was followed. The ash after heating the sample at 550° C for 3 hour was dissolved in 5 ml of 20 % HCl. The solution was evaporated to dryness on a hot plate at a temperature of 100-110° C and in an oven at 110° C for 1 hour. The minerals in the dried residue were dissolved in about 10 ml of 100 % HCl and the solution was heated on a hot plate at a temperature of 100-110° C for 3-4 times. The solution was made up to 100 ml with 1 % HCl. Calcium, sodium and potassium were estimated in flamephotometer (Model CL 361, Elico, India) at 623, 589 and 766 nm, respectively following the method of Ranganna (1986).

RESULTS

Many perishable and seasonal leafy vegetables, radish, cucumbers including young edible bamboo tender shoots are traditionally fermented into edible products in North East India using indigenous knowledge of biopreservation and fermentation by the ethnic people (Table 1).

Table 1. Diversity of traditional fermented vegetable and bamboo shoot products of North East India

Sl. No.	Product	Substrate	Nature and Use	Region
1	*Goyang*	Leafy wild plant	Solid, sour; soup	Sikkim
2	*Gundruk*	Leafy vegetable	Solid, sour; pickle, soup	Sikkim
3	*Inziang-sang*	Mustard leaves	Solid, sour; soup	Nagaland and Manipur
4	*Khalpi*	Cucumber	Solid, sour; pickle	Sikkim
5	*Sinki*	Radish taproot	Solid, sour; pickle, soup	Sikkim
6	*Ekung*	Bamboo shoot	Solid, sour; curry	Arunachal Pradesh
7	*Eup*	Bamboo shoot	Solid, dry, sour; curry	Arunachal Pradesh
8	*Hirring*	Bamboo shoot	Solid, sour; curry	Arunachal Pradesh
9	*Mesu*	Bamboo shoot	Solid, sour; pickle, soup	Sikkim
10	*Soibum*	Bamboo shoot	Solid, sour; curry, pickle	Manipur
11	*Soidon*	Whole tips of bamboo shoot	Solid, sour; curry, pickle	Manipur
12	*Soijim*	Bamboo shoot juice	Liquid, sour; condiment	Manipur

Traditional methods of biopreservation of perishable vegetables including bamboo shoots and their mode of consumption were documented in this thesis.

1. GOYANG

Goyang is a traditional, fermented and slightly acidic vegetable product prepared and consumed by the Sherpas of Sikkim. Goyang is mainly prepared from the leaves of wild plant locally called 'magane-saag' (*Cardamine macrophylla* Willd.), belongs to the family Brassicaceae which are available in high altitude >6000 ft. in Sikkim.

***Traditional method of preparation*:** During preparation of goyang, 'magane-saag' are collected, washed and cut into pieces. Leaves are 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 *(Photo 1)*.

Fresh leafy wild herbs
↓
Cut into pieces
↓
Drained excess water by squeezing
↓
Put into container/ bamboo baskets
↓
Fermented for 1 month
↓
GOYANG

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

After completion of desired fermentation, fresh goyang is transferred into an air tight container which can be stored for 2-3 months.

Photo 1. Goyang preparation in North Sikkim

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 in Sikkim.

Mode of consumption: Goyang is one of the important components of diet for the Sherpas who live in high altitudes. 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.

2. GUNDRUK

Gundruk is a non-salted, fermented and acidic vegetable product commonly prepared by the Nepalis of Sikkim during winter when perishable leafy vegetables are plenty. Gundruk is also prepared in Nepal and Bhutan.

***Traditional method of preparation*:** During fermentation of gundruk, fresh leaves of local vegetable known as 'rayo-saag' [*Brassica rapa* L. ssp. *campestris* (L.) Clapham variety *cumifolia* Roxb.] (*Photo 2*), leaves of mustard [*Brassica juncea* (L.) Czern], leaves of radish (*Raphanus sativus* L.), leaves of cauliflower (*Brassica oleracea* L. variety *botrytis* L.) and leaves of cabbages (*Brassica oleracea* L. variety *capitata*) are wilted for 1-2 days. Wilted leaves are crushed mildly and pressed into a container or earthenware pot, made air tight and fermented naturally for about 15-22

days (Fig 2). Gundruk is similar to fermented vegetable products such as kimchi of Korea, sauerkraut of Germany and sunki of Japan.

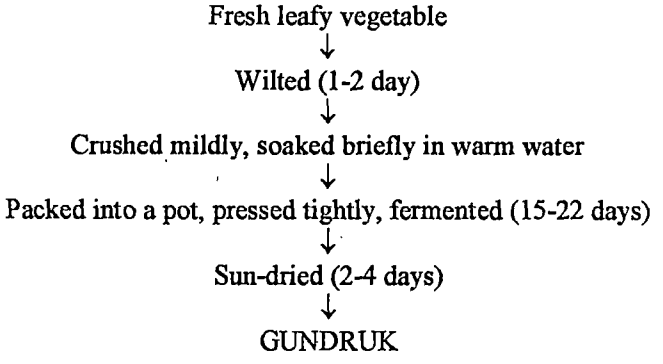


Fig 2. Flow sheets of traditional method of gundruk preparation in Sikkim.

Unlike Korean kimchi and German sauerkraut, freshly prepared wet gundruk is never eaten in Sikkim. After desirable fermentation, freshly prepared gundruk is removed from the container, and always sun dried for 2-4 days (*Photo 3a*) for consumption. Sun-dried gundruk (*Photo 3b)* can be kept for a year or more at room temperature.

Mode of consumption: It is usually eaten as soup with tomato and chilli. Pickle is also made by wetting the gundruk in hot water for 2-3 minute, squeezed out to remove water and mixed with sliced onion, chilly, unboiled mustard oil and table salt. It is usually prepared by women in rural area. It costs around Rs. 100 per kg in the local markets of Sikkim (*Photo 4*). Soup of gundruk is considered as an appetizer.

Photo 2. 'Rayo saag' (*Brasicca rapa* ssp. *camestris* var. cumifolia)

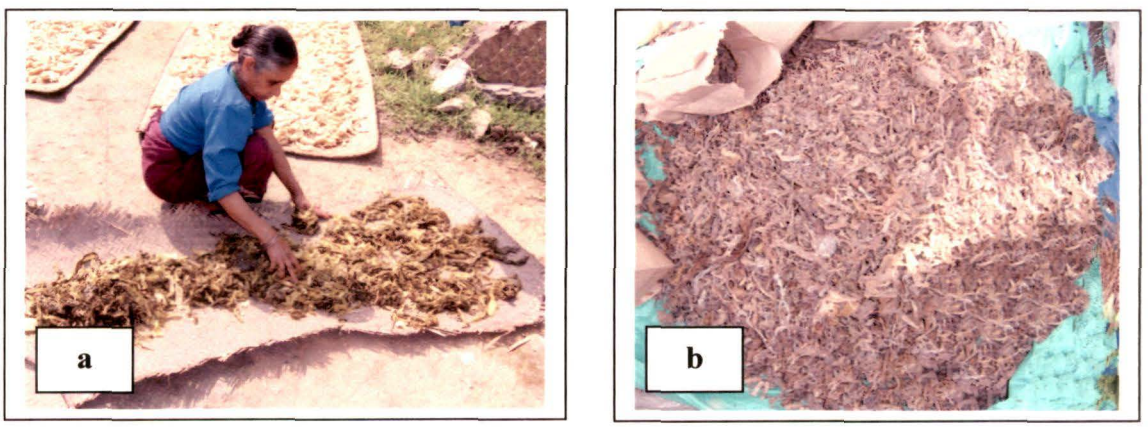


Photo 3. (a) Sun-drying of freshly prepared gundruk in East Sikkim and **(b)** Sun-dried gundruk

Photo 4. Gundruk being sold at Gangtok

Photo 5. Inziangsang

3. INZIANGSANG

Inziangsang (*Photo 5*) is a traditional fermented leafy vegetable product of Nagaland and Manipur mostly consumed by the Naga. It is prepared from mustard leaves and is very similar to gundruk.

***Traditional method of preparation*:** Freshly collected mustard leaves *Brassica juncea* (L.) Czern], are wilted, crushed and soaked in warm water. Leaves are then squeezed to remove excess water and put into air tight container, and fermented naturally at ambient temperature (~20-30° C) for 15-20 days (Fig 3).

Fresh leafy vegetable
↓
Wilted (1-2 day)
↓
Crushed mildly, soaked briefly in warm water
↓
Packed into a pot, pressed tightly, fermented (15-20 days)
↓
Sun-dried (4-5 days)
↓
INZIANGSANG

Fig 3. Flow sheets of traditional method of inziangsang preparation in Manipur.

Like gundruk, freshly prepared inziangsang is sun dried for 4-5 days and stored in a closed container for a year or more at room temperature for future consumption. Inziangsang is sold in the local market in Manipur and Nagaland.

Mode of consumption: It is consumed as soup by the Nagas with boiled rice.

4. KHALPI

Khalpi or khaipi is a non-salted fermented cucumber (*Cucumis sativus* L.) product, consumed by the Brahmin Nepalis in Sikkim.

Traditional method of preparation: During khalpi preparation, matured and ripened cucumber (*Photo 6a*) is cut into suitable pieces, sun dried for 2 days and then put into a bamboo vessel, locally called 'dhungroo' and made air-tight. It is fermented at room temperature for 4-7 days (Fig 4). Khalpi is prepared in the month of September–October (*Photo 6b-d*).

Ripened cucumber
↓
Cut into pieces
↓
Sun dried (1-2 days)
↓
Put into bamboo vessel, made air-tight
↓
Kept for 4-7 days
↓
KHALPI

Fig 4. Flow sheets of traditional method of khalpi preparation in Sikkim.

Khalpi (*Photo 6e*) is kept in air tight container for a week at room temperature. It is prepared to be consumed at the household only and has rarely been sold in few places of Sikkim.

Mode of consumption: Usually 3-5 days old khalpi is preferred by the consumers. When kept for more than 5 days it becomes sourer in taste which is not preferred. It is consumed as pickle by adding mustard oil, salt and powdered chillies, in meal with the boiled rice.

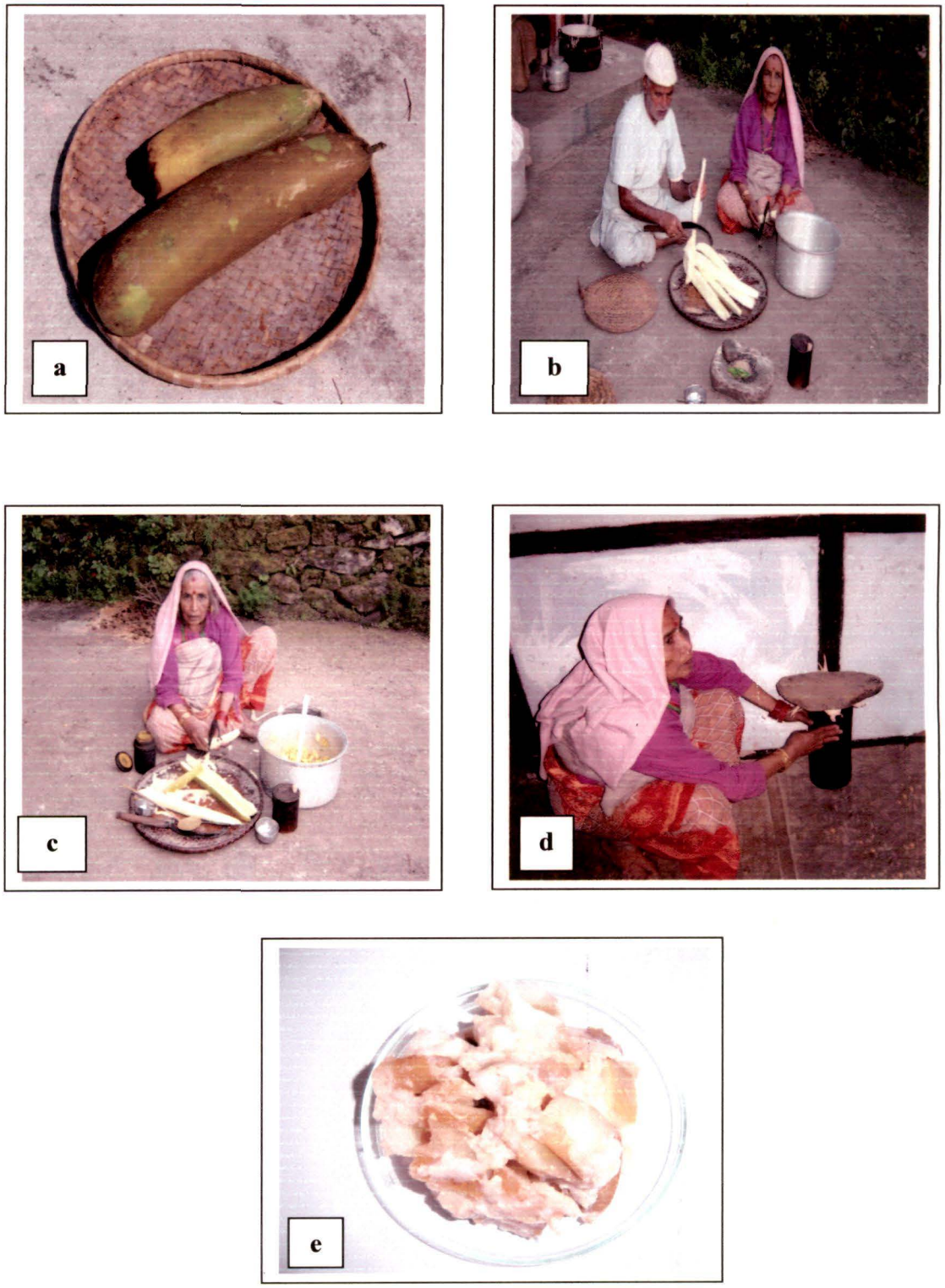


Photo 6. (a) Ripened cucumber; **(b)-(d)** Khalpi preparation; **(e)** Khalpi

5. SINKI

Sinki, a fermented radish tap-root food, is traditionally prepared by "pit fermentation", which is a unique type of biopreservation of perishable vegetables by lactic acid fermentation in the Sikkim Himalayas. Though sinki is consumed by all castes of Nepalis, it is traditionally prepared by the Nepali Brahmins in Sikkim. Sinki is prepared during December to February.

***Traditional method of preparation*:** During sinki production, a 2-3 ft pit of same diameter is dug in a dry place. The pit is cleaned, plastered with mud and warmed by burning. After removing the ashes, the pit is lined with bamboo sheaths and paddy straw. Radish (*Raphanus sativus* L.) tap-roots are wilted for 2-3 days, crushed, dipped in lukewarm water, squeezed and pressed tightly into the pit, then covered with dry leaves and weighted down by heavy planks or stones. The top of the pit is plastered with mud and left to ferment naturally for 22-30 days (Fig 5).

After completion of fermentation, fresh sinki is removed, cut into small pieces, sun-dried for 3-5 days (*Photo 7a-g*), and stored at room temperature for future consumption. Dry sinki can be kept for 2 years or more at room temperature. It is stored in an air tight container to avoid loss of typical sinki smell and to avoid absorption of moisture. Sinki is sold in local markets, mostly by rural women for their livelihood. A kg of sinki costs about Rs. 140 in the local market in Sikkim.

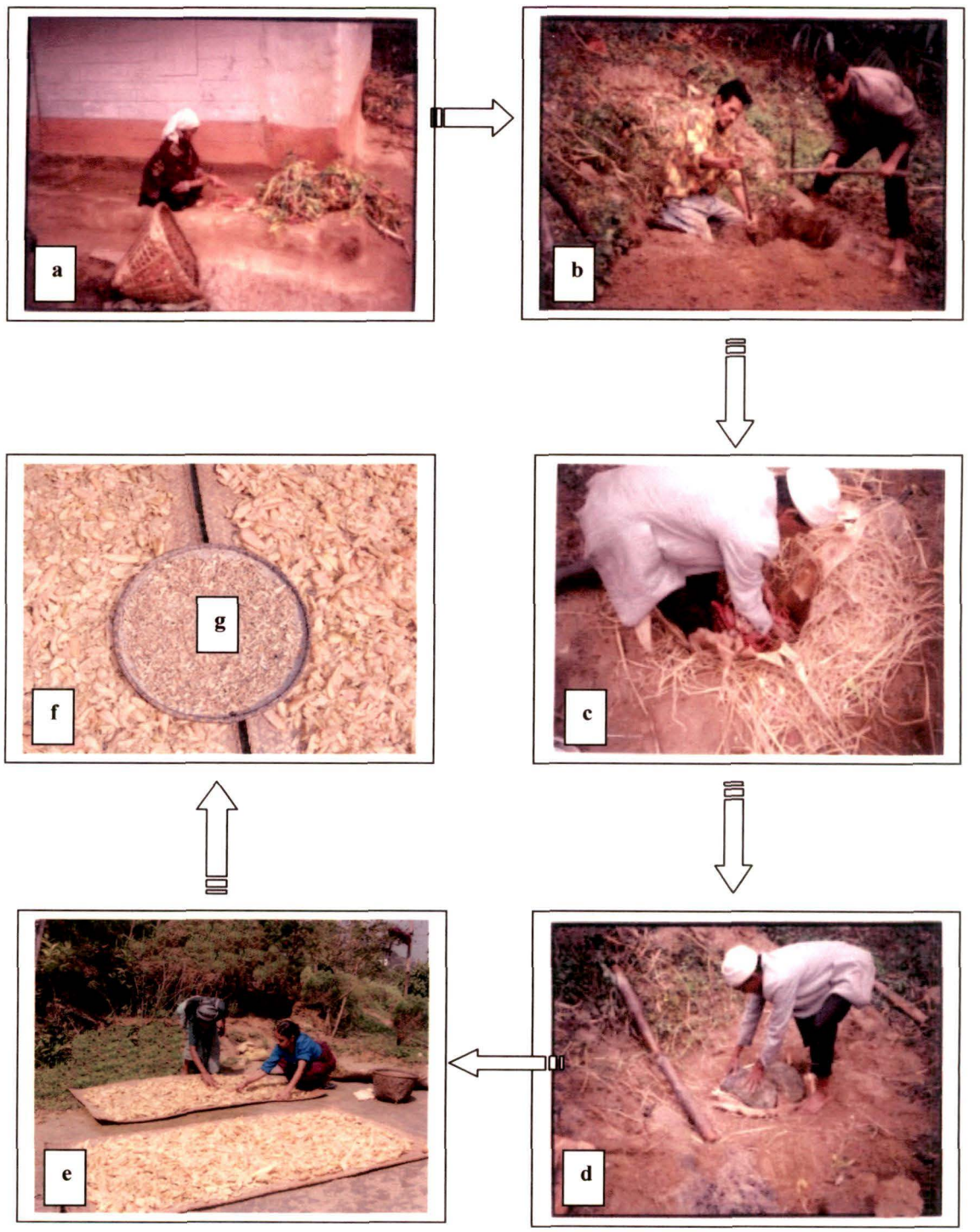


Photo 7. **(a)** Radish tap-roots are shredded; **(b)** Pit is dug; **(c)** shredded radish tap roots are pressed tightly into the pit; **(d)** heavy stones are placed to compress the substrates; **(e)** sun drying of freshly prepared sinki after fermentation; **(f)** freshly fermented sinki and sun dried sinki **(g).**

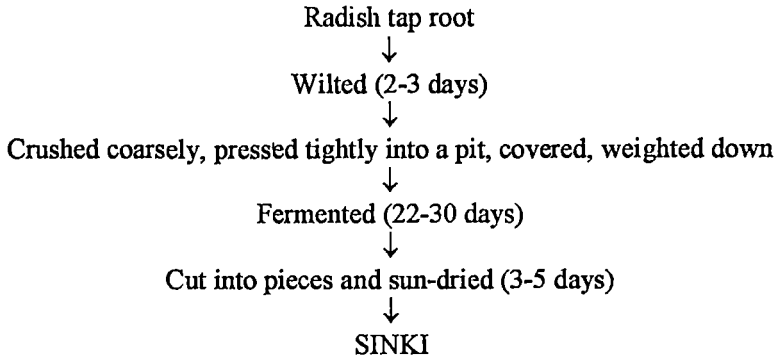


Fig 5. Flow sheets of traditional method of sinki preparation in Sikkim.

Mode of consumption: Sinki is consumed as soup and pickle in a meal. Soup of sinki is considered as an appetizer.

6. EKUNG

Ekung is an indigenous fermented bamboo tender shoot product of Arunachal Pradesh. The word ekung is called by the Nyishing tribes, Adi tribes call it iku and Apatani calls it hikku in Arunachal Pradesh (Basar and Bist, 2002). It is prepared during mid April to early September when the young bamboo shoots are plenty.

***Traditional method of preparation*:** Young bamboo tender shoot of *Dendrocalamus hamiltonii* Nees. et Arn. ex Munro, *Bambusa balcooa* Roxb. *Dendrocalamus giganteus* Munro, *Phyllostachys assamica* Gamble ex Brandis, *Bambusa tulda* Roxb. of about 8–10 ft height are collected and outer leaf sheaths are removed. The edible portions are chopped into very small pieces. Pit is dug in the forest usually in and around water source to facilitate washing of bamboo shoot pieces. The bamboo baskets

are laid into the pit and lined with leaves. Chopped bamboo shoot pieces are put into the basket. When the basket is full, it is covered with leaves and then sealed. Heavy stones are kept to give weight to drain excess water from the bamboo shoots and fermented for 1-3 months (Fig 6) (*Photo 8a-d*).

Bamboo tender shoot
↓
Remove outer casings
↓
Chopped into small pieces
↓
Laid down bamboo basket in the pit
↓
Leaves are spread over the basket
↓
Filled with bamboo shoot pieces
↓
Basket sealed and pit covered with soil and pile of stones to give weight
↓
Fermented for 1-3 months
↓
EKUNG

Fig 6. Flow sheet of traditional method of preparation of ekung in Arunachal Pradesh by Nyishing tribe

Ekung can be kept for a year in an air tight container at ambient temperature. It is sold in almost all the local markets of Arunachal Pradesh. Depending on the season, a kg of ekung costs Rs. 30 to Rs. 50.

Mode of consumption: It is consumed raw or are cooked with meat, fish and vegetables by the Nyishing tribes.

7. EUP

Eup is a fermented bamboo tender shoot product commonly prepared and consumed by different tribes of Arunachal Pradesh. It is a dry product and is known by different names in Arunachal Pradesh such as 'hi' by the Apatani, nogom by the Khampti, ipe by the Adi, while eup is a Nyishing dialect (Singh, 2002).

***Traditional method of preparation*:** Young bamboo shoots of *Dendrocalamus hamiltonii* Nees. et Arn. ex Munro, *Bambusa balcooa* Roxb., *Dendrocalamus giganteus* Munro, *Phyllostachys assamica* Gamble ex Brandis, *Bambusa tulda* Roxb. are collected and outer casings are peeled off and washed. Bamboo shoots are chopped into small pieces and fermented in similar manner as in ekung. Fermentation is completed in about 1-3 months (Fig 7). Unlike ekung, eup is a dry product. Eup is made by cutting the fermented bamboo shoot pieces into still smaller pieces and then drying in the sun for 5-10 days until its colour changes from whitish to chocolate brown.

Eup (*Photo 9*) can be kept up to 2 year at ambient temperature. Usually it is kept above the fireplace to avoid fungal attack. A kg of eup cost about Rs. 80-100. Eup is sold in the market in Arunachal Pradesh almost throughout the year.

Tender bamboo shoot

↓

Remove outer casings and tender shoots are chopped into small pieces

↓

Laid down bamboo basket that fits in the pit

↓

The baskets are covered with leaves

↓

Bamboo shoot pieces are pressed gradually

↓

Basket sealed and pit covered with mud and pile of stones to give weight

↓

Fermented for 1-3 months

↓

Removed from pits, cut into smaller pieces, and sun dried (5-10 days)

↓

EUP

Fig 7. Flow sheet of traditional method of preparation of eup in Arunachal Pradesh.

Mode of consumption: It is consumed as a curry along with meat, fish or vegetables. Curry of eup with meat is considered highly delicious by the people of Arunachal Pradesh.

8. HIRRING

Hirring (*Photo 10*) is a traditional fermented topmost whole bamboo shoot products, commonly prepared by the Apatani tribes of Arunachal Pradesh. Nyishing tribes call it hitch or hitak. It is usually prepared along with ekung. It is prepared during June to early September.

***Traditional method of preparation*:** Young tender bamboo shoot of *Dendrocalamus giganteus* Munro, *Phyllostachys assamica* Gamble ex Brandis, *Bambusa tulda* Roxb. are collected and outer leaf sheaths are removed. The topmost tender edible portions of the shoot are either cut

longitudinally into 2–3 pieces of size 1 1/2 inch to 2 inch x 9 to 15 inches or whole shoots are flattened by crushing, and are put into bamboo baskets lined with leaves. The baskets are placed into the pit, covered with leaves, sealed and weighted down with heavy stones, and fermented for 1-3 months. After 1-3 months, baskets are taken out from pits, hirring is ready for consumption which is sour and has acidic taste (Fig 8).

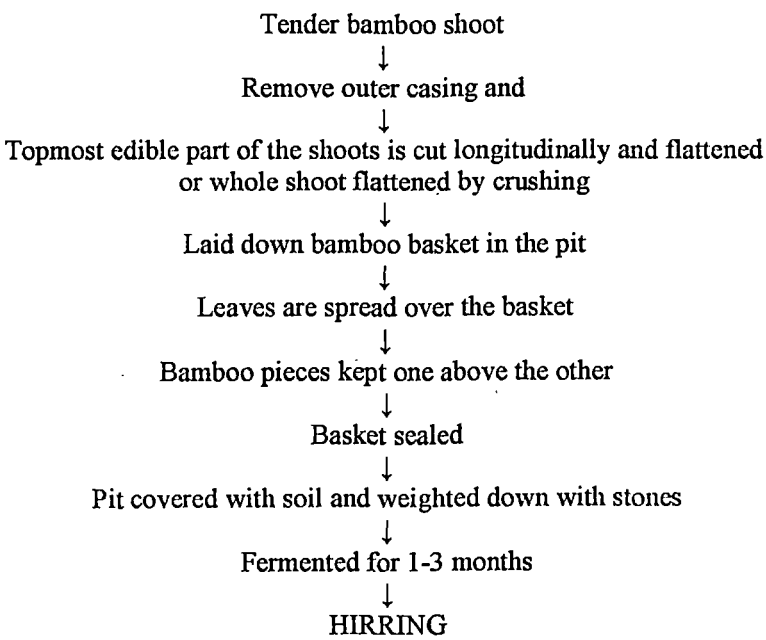
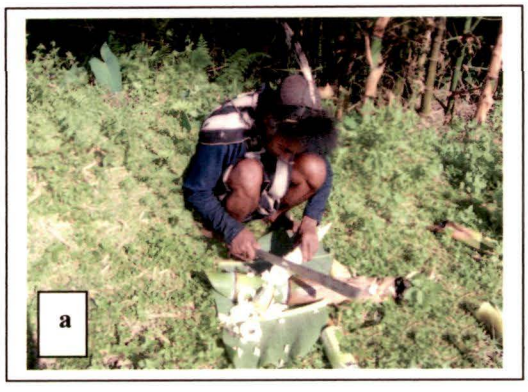


Fig 8. Flow sheet of traditional method of preparation of hirring in Arunachal Pradesh

Hirring can be kept for 2-3 months at ambient temperature. Hirring is sold in the local market in Arunachal Pradesh which costs about Rs. 150 - 200 depending on the season of availability of bamboo shoot.

Mode of consumption: Hirring is consumed as curry often mixed with vegetables, meat and fish.



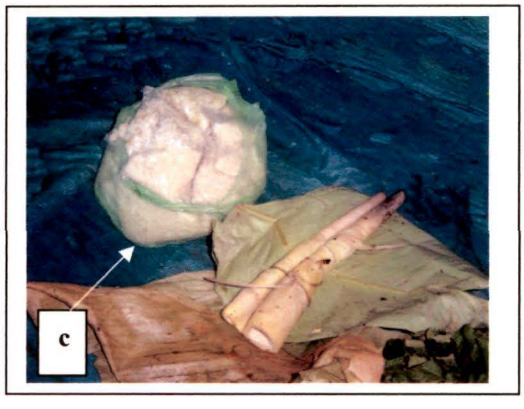


Photo 8. (a)-(b) Ekung preparation in Arunachal Pradesh by Nyishing tribe: **(c)** Ekung; **(d)** Ekung being sold at Itanagar

Photo 9. Eup

Photo 10. Hirring

9. MESU

Mesu is a traditional fermented bamboo tender shoot consumed by the Nepalis, Bhutias and Lepchas of Sikkim. It has sour-acidic taste. The word mesu is derived from the Limboo dialect (Tamang, 2000). In the Limboo (one of the castes of the Nepalis) dialect, 'me' means young bamboo shoot and 'su' means sour. Bhutia calls it 'rakyu' and the Lepcha calls it 'sitit'.

***Traditional method of preparation*:** During preparation of mesu, tender shoots of bamboo (*Dendrocalamus sikkimensis* Gamble., *Dendrocalamus hamiltonii* Nees. et Arn. ex Munro and *Bambusa tulda* Roxb.) are collected, their outer hard casings are removed and inner portion is then chopped into small pieces with a knife. The chopped pieces are washed thoroughly with clean water, drained well and pressed tightly in cylindrical vessels made of bamboo, (locally called as 'dhungro') or in big glass bottles. The vessel is made air tight with a lid, placed in an upside-down position to drain out any liquid, and fermented under natural anaerobic condition for 7-15 days (Fig 9).

Mesu (*Photo 11*) is kept in an air-tight container for a year at ambient temperature. It is also preserved mixing with mustard oil and mustard powder.

Young bamboo tender shoot
↓
Peel off outer inedible casings
↓
Chopped the inner part, washed with water, cut into pieces
↓
Pressed tightly into a fresh bamboo-vessel, made air-tight
↓
Kept the container in an upside down position
↓
Fermented for 7-12 days
↓
MESU

Fig 9. Flow sheet of mesu preparation in Sikkim.

Mesu is sold in the local markets during the months of July and September, when young bamboo tender shoots are plenty. It is sold along with the fermenting bamboo vessel which contains about 250 g of mesu and costs Rs. 15 per vessel. Some rural women are dependent on this product for their livelihood in Sikkim.

Mode of consumption: Mesu is consumed as a curry, pickle or soup. Mesu pickle is kept in a closed jar for several months.

10. SOIBUM

Soibum is an indigenous fermented young bamboo shoot product of Manipur similar to mesu.

Traditional method of preparation: It is prepared from succulent bamboo shoots (*Bambusa tulda* Roxb., *Dendrocalamus giganteus* Munro, *Dendrocalamus strictus* Nees. *and Melocana bambusoides* Trin.) of Manipur. It is whitish in colour and with faint aroma and sour taste. During preparation, outer casings of young shoots are removed, the inner part is sliced into pieces, washed and placed in a covered earthen pot and fermented for 3-12 months (Fig 10). It can be prepared from the single species of bamboo or from intermixed of more than one species.

Young bamboo shoot
↓
Peel off outer inedible casings; sliced into pieces, wash with water
↓
Put inside an earthen pot, covered
↓
Fermented for 3 months-1 year
↓
SOIBUM

Fig 10. Flow sheet of soibum preparation in Manipur

Soibum (*Photo 12a*) can be kept in air tight container for a year at ambient temperature. It is commonly sold in local vegetable markets in Manipur. It costs about Rs. 60 per kg.

Mode of consumption: Soibum is consumed as pickle and curry. It is one of the most delicious in the Manipuri dish.

11. SOIDON

Soidon is a fermented tip of matured bamboo shoot product of Manipur.

***Traditional method of preparation*:** Tips of matured bamboo shoot (*Bambusa tulda* Roxb., *Dendrocalamus giganteus* Munro *and Melocana bambusoides* Trin.) are collected; outer casings and lower portions are removed. Whole tips are submerged in water in an earthen pot, sour liquid (soijim) of previous batch is added as starter in 1:1 dilution, covered and fermented for 3-7 days at room temperature (Fig 11). Leaves of *Garcinia pedunculata* Roxb., (family: guttiferae), locally called heibung in Manipuri language, may be added in the fermenting vessel during fermentation to enhance the flavour of soidon.

Tips of matured bamboo shoot
↓
Peel off outer inedible casings
↓
Removed unwanted lower portions, washed with water
↓← Sour liquid (soijim) of previous batch added
Put inside an air-tight container/earthen pot
↓← Leaves of *Garcinia pedunculata* added
Fermented for 3-7 days
↓
Removed from water filled container after 3-7 days
↓
SOIDON

Fig 11. Flow sheet of soidon preparation in Manipur

After 3-7 days, fermented products, now soidon are removed. Soidon (*Photo 12b*) can be kept in closed container at room temperature for a year. Soidon is sold in the local market in Manipur. It costs about Rs 40 per kg (*Photo 12d*).

Mode of consumption: Soidon is consumed as a curry as well as pickle.

12. SOIJIM

Soijim is the liquid formed during fermentation of soidon (*Photo 12c*). It is acidic and sour in taste.

***Traditional method of preparation*:** During fermentation of soidon pieces of bamboo shoot tips are merged in water in an earthen pot, covered and fermented for 3-7 days at room temperature. After fermentation, pieces of bamboo shoots tips called soidon are taken out, and the acidic liquid portion called soijim is kept in a bottle. Soijim can be kept in closed container at room temperature for a year or more.

Mode of consumption: Soijim is used as a starter for fermentation of soidon and as a condiment to supplement the sour-taste in curry in Manipuri dishes.

Photo 11. Mesu

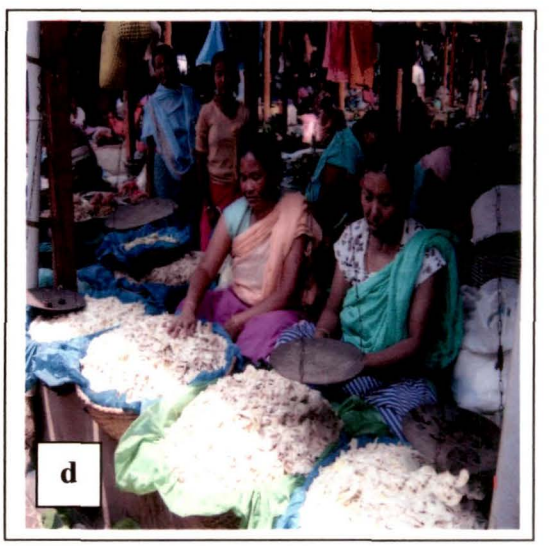


Photo 12. (a) Soibum; **(b)** Soidon; **(c)** Soijim; **(d)** marketing of soibum and soidon at Imphal market

Microbial population

Twelve types of fermented vegetable and bamboo shoot products were collected from different places of Sikkim, Manipur and Arunachal Pradesh in North East India (Fig 12). A total of 225 samples of goyang (20), gundruk (25), inziangsang (11), khalpi (25), sinki (21), ekung (16), eup (13), hirring (15), mesu (46), soibum (12), soidon (11) and soijim (10) were analysed for microbial counts (Table 2). In all fermented vegetable and bamboo shoot products, the population of LAB as well as aerobic mesophilic counts was in the range of 10^5 to 10^8 cfu/g. Count of LAB in goyang and soidon was the lowest 10^6 cfu/g. In some samples of sinki the LAB numbers exceeded 10^8 cfu/g. Yeasts were detected in goyang, sinki and khalpi in numbers ranging between 10^4 and 10^6 cfu/g. Yeast population was at the range of 10^3-10^5 cfu/g in ekung, hirring and mesu. Filamentous moulds were not detected in any of the samples analysed.

The pH of the samples ranged from 3.8 to 6.5, with soijim exhibiting the lowest pH (mean value of 3.8), while goyang has the highest pH value of 6.5 (Table 2).

Fig 12. Collection sites of fermented vegetable and bamboo shoots from North East India

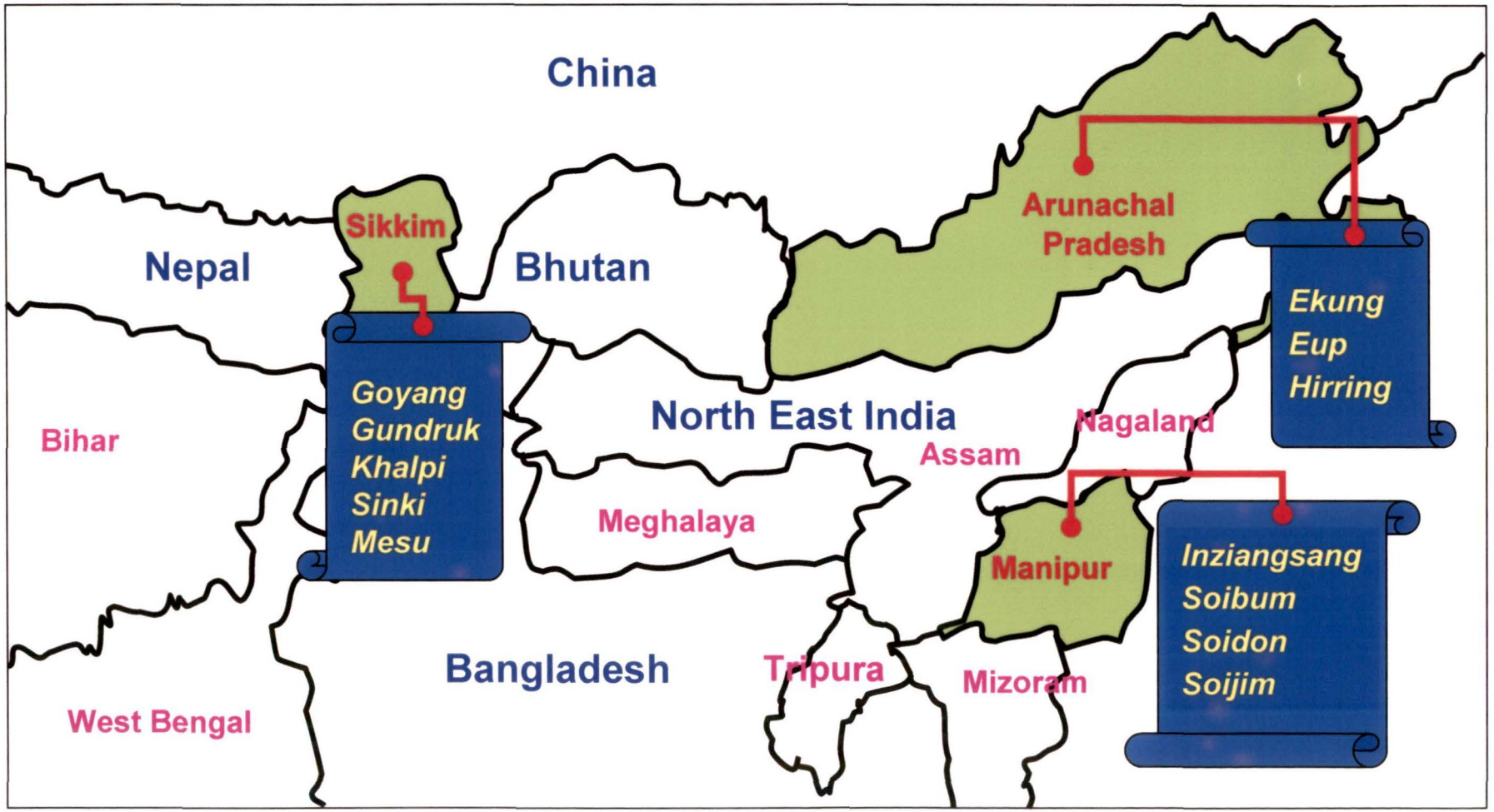


Table 2. Microbiological populations of fermented vegetables products collected from different places of North East India

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)	6.9 ± 0.1	5.8 ± 0.1	8.0 ± 0.1
Gundruk (pH 5.0 ± 0.1)	East Sikkim	Khamdong (n = 5)	7.4 ± 0.2	<DL	7.4 ± 0.1
	East Sikkim	Lingmoo (n = 5)	7.2 ± 0.2	<DL	7.3 ± 0.2
	East Sikkim	Samdong (n = 5)	7.1 ± 0.1	<DL	7.2 ± 0.1
	South Sikkim	Sumbuk (n = 5)	7.3 ± 0.2	<DL	7.4 ± 0.1
	South Sikkim	Lungchok (n = 5)	7.3 ± 0.1	<DL	7.4 ± 0.1
Inziangsang (pH 4.8 ± 0.2)	Manipur	Bishnupur (n = 6)	7.1 ± 0.1	<DL	7.1 ± 0.2
	Manipur	Imphal (n = 5)	7.4 ± 0.2	<DL	7.4 ± 0.2
Khalpi (pH 3.9 ± 0.2)	West Sikkim	Bermiok (n = 5)	6.0 ± 0.7	5.5 ± 0.5	7.0 ± 0.3
	West Sikkim	Geyzing (n = 5)	7.6 ± 0.2	5.0 ± 0.2	7.8 ± 0.1
	East Sikkim	Bhusuk (n = 5)	7.8 ± 0.1	5.0 ± 0.4	7.9 ± 0.2
	East Sikkim	Khamdong (n = 5)	7.8 ± 0.1	4.8 ± 0.2	7.8 ± 0.2
	South Sikkim	Sumbuk (n = 5)	7.8 ± 0.2	4.9 ± 0.3	7.9 ± 0.2

Continued (Table 2)

Product	Region	Place of collection	Log cfu/g sample		
			LAB	Yeast	AMC
Sinki (pH 4.1 ± 0.1)	East Sikkim	Luing (n = 6)	8.0 ± 0.3	4.7 ± 0.2	8.4 ± 0.3
	East Sikkim	Khamdong (n = 6)	7.3 ± 0.4	5.0 ± 0.2	7.9 ± 0.4
	West Sikkim	Bermiok (n = 3)	7.0 ± 0.2	4.7 ± 0.1	7.2 ± 0.2
	West Sikkim	Geyzing (n = 3)	6.9 ± 0.4	<DL	7.6 ± 0.5
	South Sikkim	Sumbuk (n = 3)	6.9 ± 0.1	4.7 ± 0.1	7.1 ± 0.1
Ekung (pH 3.9 ± 0.4)	Papum Pare (AP)	Itanagar (n = 5)	7.8 ± 0.1	<DL	7.9 ± 0.1
	Papum Pare (AP)	Naharlogun (n = 6)	7.8 ± 0.1	2.6 ± 0.2	8.1 ± 0.2
	Lower Subansiri (AP)	Ziro (n = 5)	7.9 ± 0.2	2.4 ± 0.1	8.0 ± 0.1
Eup (pH 4.1 ± 0.2)	Papum Pare (AP)	Itanagar (n = 6)	7.8 ± 0.1	<DL	7.9 ± 0.1
	Papum Pare (AP)	Naharlogun (n = 3)	7.8 ± 0.1	<DL	7.9 ± 0.1
	Lower Subansiri (AP)	Ziro (n = 4)	7.7 ± 0.1	<DL	7.8 ± 0.1
Hirring (pH 4.0 ± 0.2)	Papum Pare (AP)	Itanagar (n = 4)	7.9 ± 0.1	2.4 ± 0.1	8.0 ± 0.1
	Papum Pare (AP)	Naharlogun (n = 5)	7.9 ± 0.1	<DL	8.0 ± 0.1
	Lower Subansiri (AP)	Ziro (n = 6)	7.9 ± 0.1	<DL	7.9 ± 0.1

Continued (Table 2)

Product	Region	Source	Log cfu/g sample		
			LAB	Yeast	AMC
Mesu (pH 3.9 ± 0.4)	East Sikkim	Navey (n = 5)	7.1 ± 0.3	<DL	7.2 ± 0.3
	East Sikkim	Naitam (n = 4)	8.0 ± 0.4	5.5 ± 0.2	8.2 ± 0.5
	South Sikkim	Biring (n = 6)	7.4 ± 0.2	5.6 ± 0.2	7.6 ± 0.2
	East Sikkim	Lingtam (n = 7)	7.6 ± 0.5	4.6 ± 0.4	7.7 ± 0.4
	East Sikkim	Ragdong (n = 6)	7.1 ± 0.1	4.8 ± 0.2	7.4 ± 0.2
	East Sikkim	Tintek (n = 8)	7.1 ± 0.1	3.8 ± 0.2	7.4 ± 0.2
	East Sikkim	Ranka (n = 5)	7.2 ± 0.3	3.7 ± 0.2	7.4 ± 0.3
	West Sikkim	Bermiok (n = 5)	7.3 ± 0.2	3.3 ± 0.3	7.5 ± 0.1
Soibum (pH 3.9 ± 0.1)	Manipur	Bishnupur (n = 6)	5.8 ± 0.3	<DL	5.8 ± 0.3
	Manipur	Imphal (n = 6)	7.1 ± 0.1	<DL	7.1 ± 0.1
Soidon (pH 4.0 ± 0.1)	Manipur	Bishnupur (n = 7)	6.0 ± 0.2	<DL	6.1 ± 0.2
	Manipur	Imphal (n = 4)	5.8 ± 0.1	<DL	5.8 ± 0.1
Soijim (pH 3.8 ± 0.2)	Manipur	Bishnupur (n = 5)	7.1 ± 0.2	<DL	7.2 ± 0.2
	Manipur	Imphal (n = 5)	7.2 ± 0.4	<DL	7.3 ± 0.4

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; AP, Arunachal Pradesh; DL, Less than detection limit (10 cfu/g).

Grouping of representative strains

A total of 547 bacterial isolates were isolated from goyang (37 isolates), gundruk (95), inziangsang (19), khalpi (112), sinki (56), ekung (43), eup (40), hirring (45), mesu (51), soibum (15), soidon (20) and soijim (14), collected from different places of Sikkim, Manipur and Arunachal Pradesh. All isolates were purified in MRS broth, and their cell morphology and preliminary taxonomical tests were performed. All 547 bacterial isolates were considered lactic acid bacteria (LAB) 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 3 and 4). The 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. A total of 251 representative strains of LAB were grouped for detailed identification. Representative strains were assigned the strain code number indicating the sample names and source such as Go (goyang), G (gundruk), I (inziangsang), K (khalpi), S (sinki), Ek (ekung), Ep (eup), Hr (hirrng), Me (mesu), Sb (soibum), Sd (soidon) and Sj (soijim).

Table 3. Grouping of representative strains of the LAB isolated from fermented vegetables

Product[a]	Cell shape	Gas from glucose	Arginine hydrolysis	Grouped Strains	Representative strains	
					Total No	Strain code
Goyang (37)	Rod	–	–	19	12	Go6, Go12, Go14, Go16, Go22, Go23, Go25, Go30, Go31, Go37, Go39, Go40
	Rod	+	+	3	3	Go11, Go24, Go41
	Coccoid rod	–	–	3	2	Go7, Go8
	Coccoid rod	–	+	1	1	Go5
	Coccus	–	+	7	5	Go9, Go20, Go34, Go43, Go45
	Coccus/ tetrad	–	+	4	3	Go1, Go17, Go29
Gundruk (95)	Rod	–	+	7	4	GK:C1, GLn:R1, GSd:R5, GLc:B1
	Rod	–	–	15	6	GLn:C2, GSd:R1, GS:R1, GLn:R5, GLn:R6, GSd:R4
	Coccus/ tetrad	–	+	39	9	GLn:R2, GK:C3, GLn:C5, GS:C1, GLc:C1, GK:B3, GK:B6, GS:B1, GS:B2
	Coccus/ tetrad	–	–	34	8	GLn:R3, GLn:C1, GLn:C3, GSd:C1, GSd:C2, GS:B7, GLc:C4, GK:C2

Continued (Table 3)

Product[a]	Cell shape	Gas from glucose	Arginine hydrolysis	Grouped Strains	Representative strains	
					Total No.	Strain Code
Inziang-sang (19)	Rod	+	+	4	2	IB1, IB9
	Rod	–	–	10	5	IB2, IB3, IB11, IB12, IB13
	Coccus/ tetrad	–	+	5	2	IB5, IB14
Khalpi (112)	Rod	+	+	31	11	KG:B2, KG:B8, KB:B5, KK:B2, KBe:B2, KG:R1, KG:R2, KS:R2, KK:R1, KS:R1, KS:B1
	Rod	–	+	5	2	KG:B7, KB:B9
	Rod	–	–	71	9	KG:B1, KG:B3, KG:B9, KB:B4, KS:B2, KK:B3, KK:B4, KK:B5, KS:B9
	Coccus/ ovoid	+	–	5	3	KB:C1, KS:C1, KS:C2
Sinki (56)	Rod	+	+	5	4	SL:B2, SBe:B5, SS:B2, SL:B8
	Rod	+	–	30	13	SL:B1, SL:B6, SL:B7, SK:B1, SK:B2, SK:B3, SBe:B2, SBe:B6, SBe:B7, SS:B1, SS:B5, SS:B6, SS:B7
	Rod	–	–	13	6	SBe:B1, SG:B1, SG:B4, SG:B5, SG:B7, SS:B4
	Coccus/ ovoid	+	–	8	4	SL:B3, SL:B4, SL:B5, SK:C1

[a]Total number of isolates in each product are given in parenthesis.

All strains of LAB were Gram-positive, catalase-negative, non-motile and non-sporing.

Table 4. Grouping of representative strains of the LAB isolated from fermented bamboo shoots

Product[a]	Cell shape	Gas from glucose	Arginine hydrolysis	Grouped Strains	Representative Strains	
					Total no.	Strain code
Ekung (43)	Rod	–	–	36	22	Ek1, Ek4, Ek5, Ek6, Ek7, Ek10, Ek11, Ek12, Ek15, Ek18, Ek19, Ek20, Ek21, Ek23, Ek24, Ek25, Ek29, Ek34, Ek35 Ek38, Ek39, Ek40
	Rod	+	+	3	1	Ek8
	Coccus/ tetrad	–	+	4	2	Ek28, Ek22
Eup (40)	Rod	–	–	35	22	Ep81, Ep82, Ep84, Ep86, Ep87, Ep89, Ep90, Ep91, Ep94, Ep95, Ep96, Ep97, Ep98, Ep99, Ep1, Ep102, Ep103, Ep106, Ep110, Ep111, Ep112, Ep115,
	Rod	+	+	5	3	Ep92, Ep93, Ep85
Hirring (45)	Rod	–	–	41	24	Hr41, Hr42, Hr45, Hr47, Hr48, Hr49, Hr50, Hr55, Hr57, Hr58, Hr59, Hr60, Hr64, Hr65, Hr66, Hr67, Hr68, Hr70, Hr71, Hr74, Hr75, Hr76, Hr77, Hr80
	Coccoid rod	–	+	4	2	Hr56, Hr62

Continued. (Table 4)

Product[a]	Cell shape	Gas from glucose	Arginine hydrolysis	Grouped Strains	Representative Strains	
					Total no.	Strain code
Mesu (51)	Rod	–	–	21	16	MeL2, MeR1, MeB3, MeL3, MeBe3, MeNt1, MeB1, MeB2, MeB5, MeN4, MeN6, MeR2, MeT1, MeT2, MeT3, MeK3
	Rod	+	–	5	3	MeB4, MeR6, MeN5
	Rod	+	+	10	7	MeN3, MeTR, MeN7, MeN2, MeNt2, MeNt3, MeR7
	Coccoid rod	+	–	6	4	MeN8, MeN9, MeK1, MeK2
	Coccus/ tetrad	–	+	9	5	MeB:C3, MeB:C4, MeT5, MeT6, MeT7
Soibum (15)	Rod	–	–	4	2	SbB2, SbB3
	Coccoid rod	+	–	5	3	SbB1, SbC1, SbC2
	Rod	+	–	3	2	SbB5, SbB6
	Coccus	–	–	3	1	SbB4
Soidon (20)	Rod	+	–	7	4	SdR3, SdR4, SdR5, SdB4
	Coccoid rod	+	–	13	6	SdC1, SdR2, SdB1, SdB2, SdB3, SdB5
Soijim (14)	Rod	+	–	4	2	SjR7, SjB1
	Coccoid rod	+	–	10	6	SjC1, SjR3, SjB2, SjB4, SjB5, SjB8

[a]Total number of isolates in each product are given in parenthesis.

All strains of LAB were Gram-positive, catalase-negative, non-motile and non-sporing.

Characteristics and identity of LAB

All 251 representative strains were phenotypically characterised including determination of the sugar fermentation pattern by API system, lactic acid configuration, determination of *meso*-diaminopimelic acid (DAP), and also Biolog system for few selected strains. Of the 37 strains of LAB isolated from goyang, 3 were heterofermentative rods, 19 were homofermentative rods, 7 were homofermentative cocci, 4 coccoid rod and 4 tetrad-forming cocci (Table 3). Out of 95 strains of LAB isolated from gundruk, 22 strains were homofermentative rods, and 73 were tetrad-forming cocci. Of the 19 LAB strains from inziangsang, 4 were heterofermentative rods, 10 were homofermentative rods and 5 were tetrad-forming cocci. Of the 112 strains of LAB isolated from khalpi, 31 strains were heterofermentative rods, 76 were homofermentative rods and 5 were gas-producing ovoid cocci. Out of 56 strains of LAB, isolated from sinki, comprised 35 heterofermentative rods, 13 homofermentative rods and 8 gas-producing ovoid cocci or coccoid cells (Table 3). All cocci forming tetrads were presumptively grouped as pediococci. Further differentiation of all tetrad forming representative strains; 3 strains (goyang), 17 strains (gundruk) and 2 strains (inziangsang) 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 5) and all 17 strains from gundruk (Table 6) were identified

as *Pediococcus pentosaceus* (*Plate a*). Tetrad strains IB5 and IB14 (inziangsang) were the only strains growing at 50° C and were thus classified as *Pediococcus acidilactici* (Table 7). The sugar fermentation profiles using API system also confirmed the identity of 20 strains (goyang and gundruk) as *Pediococcus pentosaceus*, and 2 strains (inziangsang) as *Pediococcus acidilactici.*

All 44 representative homofermentative rods strains isolated from goyang, gundruk, inziangsang, khalpi and sinki shared the ability to grow at 15° C, contained DAP in the cell wall and produced DL lactate from glucose (Table 5-9). They were able to ferment the majority of the 16 carbohydrates shown in Table 5-9 and were presumptively identified as *Lactobacillus plantarum* (*Plate b*), except SS:B4 (sinki) which was identified as *Lactobacillus casei* (Table 9). The heterofermetative rods (3 strains from goyang, 2 strains from inziangsang, 11 strains from khalpi and 17 strains from sinki) produced DL lactate from glucose and were able to grow at 15° C, but not at 45° C (Table 5-9). The sugar fermentation profiles of these heterofermentative lactobacilli was similar to a number of closely related species of the *Lactobacillus (Lb.) brevis* "group" (*Lb. brevis, Lb. buchneri, Lb. hilgardii, Lb. collinoides* and *Lb. diolivorans*), and did not allow further differentiation on the basis of phenotypic characters.

The 3 strains (KB:C1, KS:C1, KS:C2) from khalpi and 4 strains (SL:B3, SL:B4, SL:B5, SK:C1) from sinki showing ovoid cell morphology, producing gas from glucose and unable to hydrolyse arginine, were

presumptively classified as belonging to the genus *Leuconostoc* (Table 3). Production of dextran from sucrose and D(-)-lactate from glucose by the 7 representative strains and also based on sugar fermentation profiles confirmed the identity of *Leuconostoc* strains as *Leuconostoc fallax* (Table 8-9).

The 3 coccoid rod strains (Go5, Go7, Go8) isolated from goyang (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; while 5 cocci strains (Go9, Go20, Go34, Go43, Go45) from goyang growing at 6.5% NaCl, at 45° C and on the basis of sugar fermentation profiles (Table 5) were identified as *Enterococcus faecium* (*Plate c*).

The representative strains of LAB isolated from bamboo shoots (ekung, eup, hirring, mesu, soibum, soidon and soijim) were phenotypically characterized based on cell morphology, gas production from glucose, arginine hydrolysis, lactate configuration, growth at different temperatures, pH, sugar fermentation patterns and DAP (Table 10-14). Phenotypic characterisation including sugar fermentation pattern, arginine hydrolysis and growth behaviour at 50°C justified the identification of 5 tetrad strains (MeB:C3, MeB:C4, MeT5, MeT6, MeT7) from mesu were identified as *Pediococcus pentosaceus* (Table 13). The two tetrad-forming cocci (Ek22, Ek28) from ekung grew well at 15° C, 6.5 % NaCl and 18 % NaCl, but did not grow at 45° C. Extreme salt tolerance (growth at 18 %

NaCl) differentiated Ek22 and Ek28 from other tetrad-forming cocci, and were identified as *Tetragenococcus halophilus* (Table 10) (*Plate d*), based on the taxonomical keys of Simpson and Taguchi (1995).

A total of 22 representative strains including 1 strain (Ek8) from ekung, 3 strains (Ep85, Ep92, Ep93) from eup, 10 strains (MeN3, MeTR, MeN7, MeN2, MeNt2, MeNt3, MeR7, MeB4, MeR6, MeN5) from mesu, 2 strains (SbB5, SbB6) from soibum, 4 strains (SdR3, SdR4, SdR5, SdB4) from soidon and 2 strains (SjR7, SjB1) from soijim were CO_2 forming DL lactate producers and therefore classified as heterofermentative lactobacilli. Out of 22 heterofermentative lactobacilli, 19 strains except strains from eup were identified as *Lactobacillus brevis* (Table 10-14). While 3 strains Ep85, Ep92, Ep93 from eup unlike *Lactobacillus brevis* did not grow in 6.5 % NaCl, at 15° C but grew at 45° C. Using API system they were identified as *Lactobacillus fermentum* (Table 11).

Of the 86 representative homofermentative rods isolated from fermented bamboo shoots (ekung, eup, hirring, mesu and soibum) 83 strains were arginine negative and shared ability to grow in pH 3.9, 6.5 % NaCl, at 10° C and 15° C but not at 45° C, produced both isomers of lactate from glucose and contained DAP in their cell walls. Sugar fermentation profile confirmed their identity as *Lactobacillus plantarum* (Table 10-14). Among the remaining 3 homofermentative rods, 2 strains (Ek34, Ek35) from ekung and 1 strain (MeK3) from mesu which had similar growth behaviour at different temperatures, pH and NaCl with that

of *Lb. plantarum* but differed due to lack of DAP in their cell wall, produced L(+)- isomer of lactate from glucose and few sugar fermentation profiles (Table 10-13). Thus, strains Ek34 and Ek35 (ekung) were identified as *Lactobacillus casei* (Table 10). Strain MeK3 (mesu) produced both the isomers of lactate from glucose but lacked DAP. Based on sugar fermentation profiles using API system, strain MeK3 (mesu) was identified as *Lactobacillus curvatus* (Table 13).

Two homofermentative coccoid rod strains (Hr56, Hr62) from hirring were arginine positive, grew well in pH 9.6 but not in pH 3.9, growth in 6.5 % NaCl and at 45° C. They were identified following the API system as *Lactococcus lactis* (Table 12) (*Plate e*).

A total of 19 coccoid strains isolated from mesu, soibum, soidon and soijim produced D(-) lactate from glucose, were arginine-negative and showed the typical leuconostoc-like ovoid cells. Four of them (SbC1, SdC1, SjC1, SjB2) isolated from soidon, soibum and soijim fermented a small range of carbohydrates. However, they fermented sucrose, trehalose and mannose but not raffinose. Sugar fermentation profiles using API confirmed their identity as *Leuconostoc fallax* (Table 14) (*Plate f*). The application of the Biolog system based on the utilisation patterns of 95 single carbon sources confirmed this result. The Biolog system also allowed identifying the gas-producing ovoid strain SbC2 (soibum) as *Leuconostoc mesenteroides* subsp. *mesenteroides*. While remaining 4 coccoid strains (MeN8, MeN9, MeK1, MeK2) isolated from mesu were

unable to ferment lactose. Sugar fermentation profile using API system identified these strains as *Leuconostoc citreum* (Table 13).

Another 11 heterofermentative strains, 1 strain (SbB1) from soibum, 6 strains (SdR2, SdB1, SdB2, SdB3, SdB4, SdB5) from soidon and 4 strains (SjR3, SjB4, SjB5, SjB8) from soijim produced only D (-)-lactate from glucose and were unable to hydrolyse arginine as it is known for *Leuconostoc* and some *Weissella* species. They produced acid from lactate and formed dextran from sucrose as well. Sugar fermentation profiles of API system, however, identified them as *Leuconostoc lactis* (Table 14). Coccus strain SbB4 from soibum produced only L lactate from glucose, arginine negative, grew at 15° C, in 6.5 % NaCl was assigned to the genus *Enterococcus*. Using sugar fermentation profile strain SbB4 was identified as *Enterococcus durans* (Table 14).

In the next step, genomic DNA was prepared from the selected strains, and fingerprinting analysis by the RAPD PCR was conducted to reveal similarities among the strains of each group and the possible assignment of the strains to a certain species by including the respective reference strains in the assay. The RAPD profiles generated with the M13 primer produced the dendrogram shown in Fig 13. The 7 strains of pediococci from gundruk were grouped into one cluster at a similarity level above 75 % confirming them as *P. pentosaceus*, whereas the *Pediococcus* IB5 (inziangsang) showed a different profiles, confirmed it as *P. acidilactici.*

Based on the rep-PCR using the $(GTG)_5$ primer, all DAP-positive homofermentative strains (gundruk, khalpi, inziangsang, mesu, soibum) were confirmed as *Lactobacillus plantarum* (Fig 14-16). DAP negative homofermentative strains MeK3 (mesu) was genotypically identified as *Lb. curvatus* (Fig 16). The 12 strains of heterofermentative rods (from khalpi and sinki), phenotypically identified as the *Lb. brevis* "group" on the basis of sugar fermentation, were genotypically confimed the identity of *Lactobacillus brevis* using the results of RAPD-PCR (Fig 17).

Based on the detailed characterisations and identification profiles of dominant LAB isolated from traditional fermented vegetables and bamboo shoots of North East India, species of LAB were summarised as follows: *Lb. plantarum, Lb. brevis*, *Lc. lactis, E. faecium, P. pentosaceus* (goyang); *Lb. plantarum, P. pentosaceus* (gundruk); *Lb. plantarum, Lb. brevis, P. acidilactici* (inziangsang); *Lb. plantarum, Lb. brevis*, *Leuc. fallax* (khalpi); *Lb. plantarum, Lb. brevis, Lb. casei*, *Leuc. fallax* (sinki); *Lb. plantarum, Lb. brevis, Lb. casei*, *Tetragenococcus halophilus* (ekung); *Lb. plantarum, Lb. fermentum* (eup); *Lb. plantarum*, *Lc. lactis* (hirring); *Lb. plantarum, Lb. brevis, Lb. curvatus, Leuc. citreum*, *P. pentosaceus* (mesu); *Lb. plantarum, Lb. brevis, Leuc. fallax, Leuc. lactis, Leuc. mesenteroides, E. durans* (soibum); *Lb. brevis, Leuc. fallax, Leuc. lactis* (soidon); *Lb. brevis, Leuc. fallax, Leuc. lactis* (soijim).

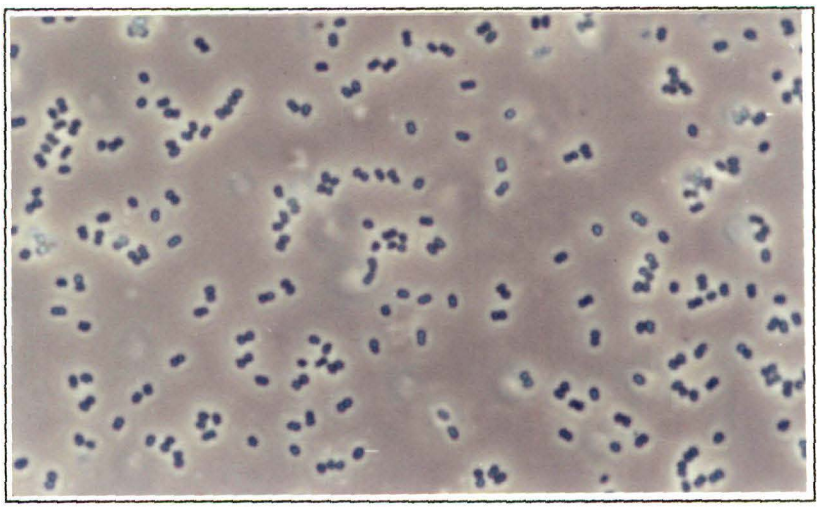


Plate (a). *Pediococcus pentosaceus* Go17 (MRS broth, 3 d, 30° C), isolated from goyang, showing cocci in a phase contrast micrograph (× 825).

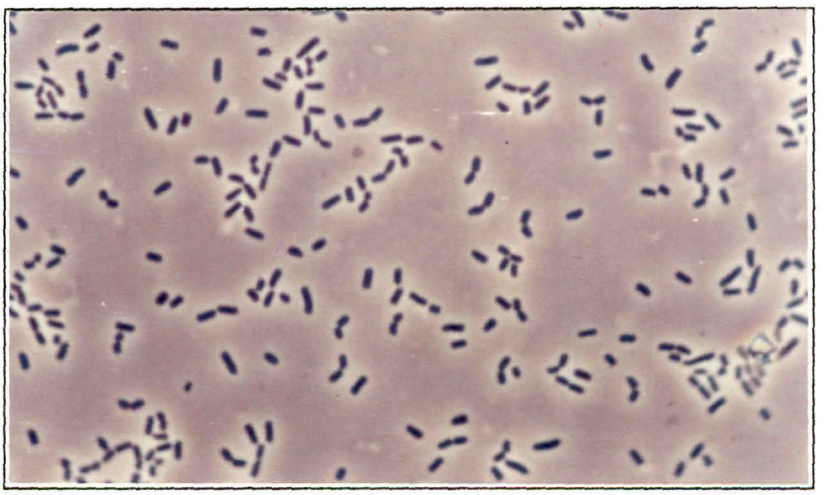


Plate (b). *Lactobacillus plantarum* GS:R1 (MRS broth, 3 d, 30° C), isolated from gundruk, showing non-sporing rods in a phase contrast micrograph (× 825).

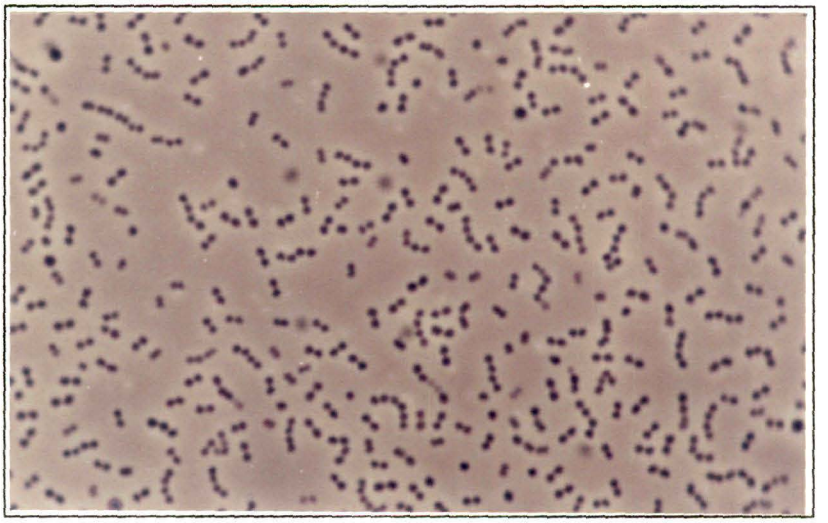


Plate (c). *Enterococcus faecium* Go9 (MRS broth, 3d, 30° C), isolated from goyang, cocci in a phase contrast micrograph (× 825).

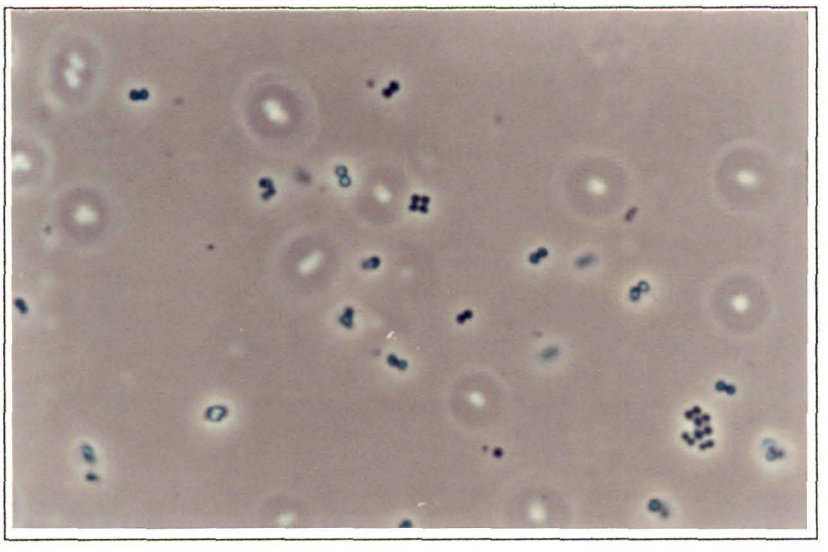


Plate (d). *Tetragenococcus halophilus* Ek28 (MRS broth, 3 d, 30° C), isolated from ekung, showing cocci in a phase contrast micrograph (× 825).

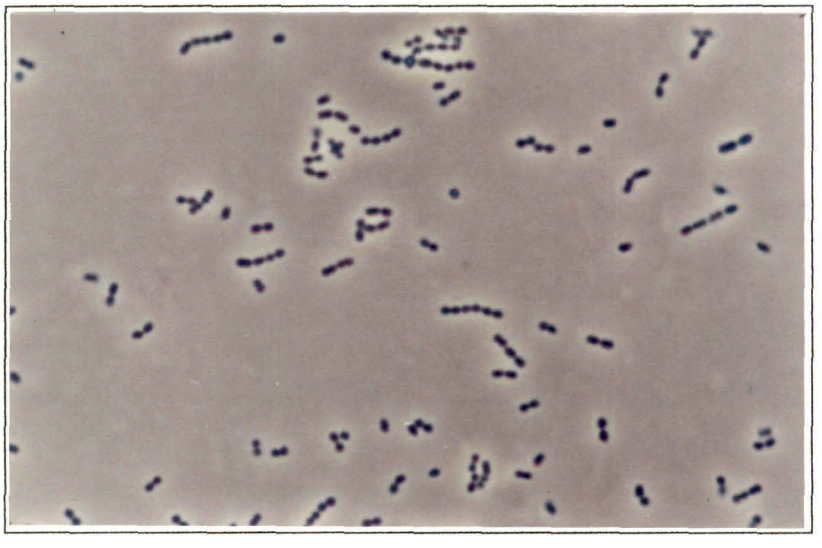


Plate (e). *Lactococcus lactis* Hr56 (MRS broth, 3 d, 30° C), isolated from hirring, showing coccoid cells in a phase contrast micrograph (× 825).

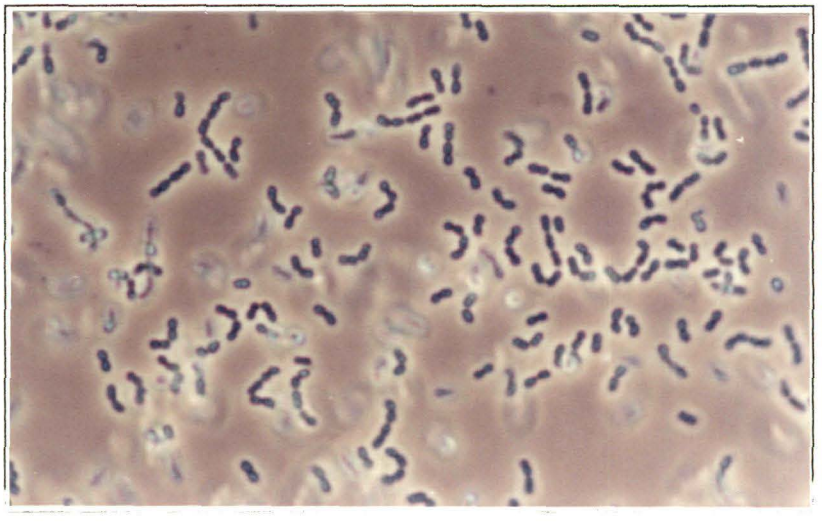


Plate (f). *Leuconostoc fallax* SjC1 (MRS broth, 3 d, 30° C), isolated from soijim, showing coccoid rod in phase contrast micrograph (× 825).

Table 5. Phenotypic characteristics of the LAB from goyang of Sikkim

Strain Code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in									DAP	Lactate isomer	Sugars Fermented																	Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%				Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
Go1	Coccus/tetrad	d = 0.7 (0.6 - 0.8)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
Go5	Coccoid rod	d = 0.9 (0.6 - 1.2)	+	–	+	+	–	–	+	–	–	–	–	L	+	–	+	+	+	–	+	+	–	+	–	+	+	+	–	–	–	*Lactococcus lactis*
Go6	Rod	l = 1.7 (1.5 - 1.9) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	–	+	*Lactobacillus plantarum*
Go7	Coccoid rod	l = 1.2 (1.1 - 1.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	–	+	–	–	–	–	L	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactococcus lactis*
Go8	Coccoid rod	d = 0.8 (0.6 - 1.2)	–	–	+	+	–	–	+	–	–	–	–	L	+	+	+	+	–	–	–	+	–	+	+	+	+	+	–	–	–	*Lactococcus lactis*
Go9	Coccus	l = 1.3 (1.2- 1.6) b = 0.9 (0.8 - 1.0)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	*Enterococcus faecium*
Go11	Rod	l = 2.1 (1.4 - 3.2) b =0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	–	–	–	–	DL	–	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
Go12	Rod	l = 1.6 (1.4 - 1.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	+	–	+	–	+	+	–	–	–	*Lactobacillus plantarum*
Go14	Rod	l = 1.6 (1.5 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	–	+	–	–	–	*Lactobacillus plantarum*
Go16	Rod	l = 1.5 (1.4 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Lactobcillus plantarum*
Go17	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	–	–	–	–	–	*Pediococcus pentosaceus*
Go20	Coccus	l = 1.2 (1.1 - 1.3) b = 0.6 (0.5 - 0.7)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	*Enterococcus faecium*
Go22	Rod	l = 1.5 (1.4 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*

d, diameter; l, length; b, breadth

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 5)

Strain Code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																	Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
Go23	Rod	l = 1.4 (1.3 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	–	–	*Lactobacillus plantarum*
Go24	Rod	l = 1.4 (1.3 - 1.6) b = 0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	–	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
Go25	Rod	l = 1.6 (1.4 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go29	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
Go30	Rod	l = 1.6 (1.4 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go31	Rod	l = 1.5 (1.4 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go34	Coccus	l = 1.2 (1.0 - 1.3) b = 0.6 (0.5 - 0.8)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	–	*Enterococcus faecium*
Go37	Rod	l = 1.5 (1.4 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go39	Rod	l = 1.5 (1.4 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go40	Rod	l = 1.1 (1.0 - 1.2) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go41	Rod	l = 1.4 (1.2 - 1.6) b = 0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	+	–	–	–	DL	–	+	+	+	+	+	–	+	+	–	–	+	+	+	+	+	+	–	*Lactobacillus brevis*
Go43	Coccus	l = 1.2 (1.1 - 1.3) b= 0.7 (0.6 - 0.8)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	–	+	–	–	+	+	+	+	+	+	+	–	*Enterococcus faecium*
Go45	Coccus	l = 1.1 (1.0 - 1.2) b = 0.5 (0.4 - 0.6)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–	–	*Enterococcus faecium*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.
DAP, *meso*-diaminopimelic acid

Table 6. Phenotypic characteristics of the LAB isolated from gundruk of Sikkim

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
GK:C1	Rod	l = 2.0 (1.6 - 3.0) b = 0.7 (0.5 - 0.9)	+	–	+	+	+	+	–	+	+	–	+	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	+	–	–	–	–	*Lactobacillus plantarum*
GK:C2	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	–	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GK:B3	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GK:B6	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GK:C3	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLn:R1	Rod	l = 2.1 (1.6 - 3.0) b = 0.8 (0.6 - 0.9)	+	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GLn:R5	Rod	l = 3.1 (2.3 - 4.2) b = 0.6 (0.5- 0.8)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GLn:R6	Rod	l = 3.3 (2.8 - 4.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
GLn:C1	Coccus/tetrad	d = 0.8 (0.6 - 0.9)	–	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLn:C2	Rod	l = 3.2 (2.8 - 3.5) b = 0.6 (0.5 - 0.7)	–	–	+	+	+	+	–	+	+	–	+	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Lactobacillus plantarum*
GLn:C3	Coccus/tetrad	d =0.8 (0.6 - 0.9)	–	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLn:C5	Coccus/tetrad	d =0.8 (0.6 - 0.9)	+	–	+	+	–	+	–	+	+	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLn:R2	Coccus/tetrad	d = 1.0 (0.8 - 1.1)	+	–	+	+	–	+	–	+	–	–	–	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	–	+	*Pediococcus pentosaceus*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 6)

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
GLn:R3	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	–	–	+	+	–	+	–	+	–	–	–	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	–	+	*Pediococcus pentosaceus*
GSd:R1	Rod	l = 2.0 (1.6 - 3.0) b = 0.7 (0.5 - 0.9)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GSd:R4	Rod	l = 2.0 (1.6 - 3.0) b = 0.7 (0.5 - 0.9)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GSd:R5	Rod	l = 2.0 (1.6 - 3.0) b = 0.6 (0.5 - 0.7)	+	–	+	+	–	+	–	+	–	–	+	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GSd:C1	Coccus/tetrad	d = 0.9 (0.6 - 1.1)	–	–	–	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GSd:C2	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	–	–	+	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GS:R1	Rod	l = 1.9 (1.6 - 2.1) b = 0.9 (0.8 - 0.9)	–	–	+	+	–	+	–	+	+	–	+	DL	–	–	–	+	+	+	–	+	–	+	+	+	+	–	+	–	+	*Lactobacillus plantarum*
GS:B1	Coccus/tetrad	d = 1.0 (0.9 - 1.1)	+	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GS:B2	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	+	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GS:B7	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	–	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GS:C1	Coccus/tetrad	d = 0.9 (0.7 - 1.1)	+	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLc:B1	Rod	l = 2.0 (1.6 - 3.0) b = 0.6 (0.5 - 0.7)	+	–	+	+	–	+	–	+	–	–	+	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GLc:C1	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLc:C4	Coccus/tetrad	d = 1.0 (0.9 - 1.1)	–	–	+	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*

d, diameter; l, length; b, breadth

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 7. Phenotypic characteristics of the LAB isolated from Inziangsang of Manipur

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in									DAP	Lactate isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%				Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
IB1	Rod	l = 2.6 (1.9 - 3.2) b = 0.7 (0.6 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	L(D)	+	+	+	+	–	–	–	–	V	–	–	–	–	+	+	–	–		*Lactobacillus brevis*
IB2	Rod	l = 1.9 (1.6 - 2.4) b = 0.7 (0.6 - 0.8)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus plantarum*
IB3	Rod	l = 1.9 (1.6 - 2.4) b = 0.8 (0.7 - 0.9)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus plantarum*
IB5	Coccus/tetrad	d = 0.8 (0.7 - 0.9)	+	–	–	+	+	+	+	+	–	–	–	DL	+	+	+	+	–	+	–	+	–	+	+	+	–	–	–	–	–		*Pediococcus acidilactici*
IB9	Rod	l = 1.9 (1.6 - 2.4) b = 0.8 (0.7 - 0.9)	+	+	–	+	–	+	–	+	–	–	–	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus brevis*
IB11	Rod	l = 3.9 (3.2 - 4.4) b = 0.7 (0.5 - 0.9)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+		*Lactobacillus plantarum*
IB12	Rod	l = 2.9 (1.6 - 3.4) b = 0.7 (0.5 - 0.9)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+		*Lactobacillus plantarum*
IB13	Rod	l = 3.4 (2.6 - 4.0) b = 0.6 (0.5 - 0.8)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus plantarum*
IB14	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	–	+	+	+	+	+	–	–	–	DL	+	+	+	+	–	+	–	+	–	+	+	+	–	–	–	–	–		*Pediococcus acidilactici*

l, length; b, breadth; d, diameter.

All strains were Gram-positive and catalase-negative, non-motile and non-sp

DAP, *meso*-diaminopimelic acid

Table 8. Phenotypic characteristics of the LAB isolated from khalpi of Sikkim

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																	Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
KG:B1	Rod	l = 2.7 (1.4 - 3.5) b = 0.4 (0.3 - 0.5)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	–	+	+	+	+	+	+	*Lactobacillus plantarum*
KG:B2	Rod	l = 2.3 (1.8 - 3.2) b = 0.4 (0.3 - 0.5)	+	+	–	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KG:B3	Rod	l = 1.6 (1.1 - 1.9) b = 0.5 (0.3 - 0.6)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
KG:B7	Rod	l = 1.6 (1.2 - 1.9) b = 0.8 (0.6 - 0.9)	+	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KG:B8	Rod	l = 1.6 (1.3 - 1.9) b = 0.6 (0.5 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KG:B9	Rod	l = 1.7 (1.3 - 1.8) b = 0.7 (0.6 - 0.8)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KG:R1	Rod	l = 3.2 (2.2 - 4.7) b = 0.6 (0.5 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KG:R2	Rod	l = 3.6 (2.3 - 4.8) b = 0.6 (0.5 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KB:B4	Rod	l = 1.7 (1.3 - 1.8) b = 0.5 (0.3 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KB:B5	Rod	l = 1.5 (1.4 - 1.6) b = 0.6 (0.4 - 0.7)	+	+	–	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KB:C1	Coccus/Ovoid	d = 1.0 (0.9 - 1.2)	–	+	–	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–	*Leuconostoc fallax*
KB:B9	Rod	l = 2.5 (2.0 - 3.1) b = 0.5 (0.4 - 0.6)	+	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
KK:B2	Rod	l = 3.2 (2.4 - 4.2) b = 0.4 (0.3 - 0.5)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 8)

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
KK:B3	Rod	l = 2.6 (2.0 - 3.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KK:B4	Rod	l = 2.8 (2.0 - 3.4) b = 0.6 (0.4 - 0.7)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KK:B5	Rod	l = 2.3 (1.9 - 3.3) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KK:R1	Rod	l = 2.6 (2.0 - 3.8) b = 0.4 (0.3 - 0.5)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
KS:R1	Rod	l = 3.6 (2.2 - 4.2) b = 0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KS:R2	Rod	l = 3.6 (2.8 - 3.9) b = 0.5 (0.5 - 0.7)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KS:B1	Rod	l = 3.0 (2.8 - 3.6) b = 0.6 (0.5 - 0.7)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
KS:B2	Rod	l = 1.5 (1.3 - 1.8) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	–	–	–	+	DL	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KS:B9	Rod	l = 1.5 (1.3 - 1.8) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	–	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KS:C1	Coccus/Ovoid	l = 1.5 (1.3 - 1.8) b = 0.4 (0.3 - 0.5)	–	+	–	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–	*Leuconostoc fallax*
KS:C2	Coccus/Ovoid	l = 1.5 (1.3 - 1.8) b = 0.4 (0.3 - 0.5)	–	+	–	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–	*Leuconostoc fallax*
KBe:B2	Rod	l = 2.5 (2.1 - 3.2) b = 0.4 (0.3 - 0.5)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	–	+	–	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 9. Phenotypic characteristics of the LAB isolated from sinki of Sikkim

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
SL:B1	Rod	l = 1.3 (1.2 - 1.6) b = 0.9 (0.8 - 1.2)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SL:B2	Rod	l = 1.7 (1.6 - 2.0) b = 0.6 (0.5 - 0.7)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SL:B3	Coccus/ovoid	d = 1.0 (0.7 - 1.2)	–	+	+	+	–	–	–	–	–	–	–	D	–	+	–	–	+	+	–	+	–	–	–	–	–	–	+	–	–		*Leuconostoc fallax*
SL:B4	Coccus/ovoid	d = 1.2 (1.0 - 1.3)	–	+	+	+	–	–	–	–	–	–	–	D	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–		*Leuconostoc fallax*
SL:B5	Coccus/ovoid	d = 0.9 (0.8 - 1.2)	–	+	+	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–		*Leuconostoc fallax*
SL:B6	Rod	l = 1.6 (1.4 - 1.7) b = 0.7 (0.6 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SL:B7	Rod	l = 1.7 (1.6 - 2.0) b = 0.8 (0.6 - 0.9)	–	+	+	+	–	+	–	–	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SL:B8	Rod	l = 2.6 (2.0 - 3.0) b = 0.5 (0.4 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SK:B1	Rod	l = 1.6 (1.2 - 1.8) b = 0.7 (0.6 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SK:B2	Rod	l = 1.3 (1.1 - 1.5) b = 0.6 (0.5 - 0.8)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SK:B3	Rod	l = 1.6 (1.3 - 1.7) b = 0.5 (0.4 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SK:C1	Coccus/ovoid	d = 0.9 (0.8 - 1.2)	–	+	+	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–		*Leuconostoc fallax*
SBe:B1	Rod	l = 1.0 (1.2 - 1.4) b = 0.5 (0.4 - 0.9)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus plantarum*
SBe:B2	Rod	l = 2.0 (1.8 - 2.4) b = 0.5 (0.4 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	+	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 9)

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
SBe:B5	Rod	l = 2.0 (1.8 - 2.4) b = 0.5 (0.4 - 0.9)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
SBe:B6	Rod	l = 2.0 (1.8 - 2.4) b = 0.5 (0.4 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
SBe:B7	Rod	l = 2.2 (1.9 - 2.5) b = 0.5 (0.4 - 0.6)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B1	Rod	l = 2.6 (2.2 - 3.2) b = 0.5 (0.4 - 0.7)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B2	Rod	l = 2.4 (1.8 - 2.8) b = 0.5 (0.4 - 0.9)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B4	Rod	l = 1.4 (1.2 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	–	L	–	–	–	–	+	+	–	+	–	+	+	+	+	–	–	–	+	*Lactobacillus casei*
SS:B5	Rod	l = 2.6 (2.2 - 3.0) b = 0.5 (0.4 - 0.6)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B6	Rod	l = 2.9 (2.6 - 3.8) b = 0.5 (0.4 - 0.6)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B7	Rod	l = 3.3 (2.8 - 3.7) b = 0.6 (0.4 - 0.7)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
SG:B1	Rod	l = 1.3 (1.2 - 1.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
SG:B4	Rod	l = 1.4 (1.3 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
SG:B5	Rod	l = 1.4 (1.3 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
SG:B7	Rod	l = 1.5 (1.3 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 5. Phenotypic characteristics of the LAB from goyang of Sikkim

Strain Code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in									DAP	Lactate isomer	Sugars Fermented																	Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%				Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
Go1	Coccus/tetrad	d = 0.7 (0.6 - 0.8)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
Go5	Coccoid rod	d = 0.9 (0.6 - 1.2)	+	–	+	+	–	–	+	–	–	–	–	L	+	–	+	+	+	–	+	+	–	+	–	+	+	+	–	–	–	*Lactococcus lactis*
Go6	Rod	l = 1.7 (1.5 - 1.9) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	–	+	*Lactobacillus plantarum*
Go7	Coccoid rod	l = 1.2 (1.1 - 1.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	–	+	–	–	–	–	L	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactococcus lactis*
Go8	Coccoid rod	d = 0.8 (0.6 - 1.2)	–	–	+	+	–	–	+	–	–	–	–	L	+	+	+	+	–	–	–	+	–	+	+	+	+	+	–	–	–	*Lactococcus lactis*
Go9	Coccus	l = 1.3 (1.2- 1.6) b = 0.9 (0.8 - 1.0)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	*Enterococcus faecium*
Go11	Rod	l = 2.1 (1.4 - 3.2) b =0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	–	–	–	–	DL	–	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
Go12	Rod	l = 1.6 (1.4 - 1.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	+	–	+	–	+	+	–	–	–	*Lactobacillus plantarum*
Go14	Rod	l = 1.6 (1.5 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	–	+	–	–	–	*Lactobacillus plantarum*
Go16	Rod	l = 1.5 (1.4 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Lactobcillus plantarum*
Go17	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	–	–	–	–	–	*Pediococcus pentosaceus*
Go20	Coccus	l = 1.2 (1.1 - 1.3) b = 0.6 (0.5 - 0.7)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	*Enterococcus faecium*
Go22	Rod	l = 1.5 (1.4 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*

d, diameter; l, length; b, breadth

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 5)

Strain Code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																	Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
Go23	Rod	l = 1.4 (1.3 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	–	–	*Lactobacillus plantarum*
Go24	Rod	l = 1.4 (1.3 - 1.6) b = 0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	–	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
Go25	Rod	l = 1.6 (1.4 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go29	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
Go30	Rod	l = 1.6 (1.4 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go31	Rod	l = 1.5 (1.4 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go34	Coccus	l = 1.2 (1.0 - 1.3) b = 0.6 (0.5 - 0.8)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	–	*Enterococcus faecium*
Go37	Rod	l = 1.5 (1.4 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go39	Rod	l = 1.5 (1.4 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go40	Rod	l = 1.1 (1.0 - 1.2) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go41	Rod	l = 1.4 (1.2 - 1.6) b = 0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	+	–	–	–	DL	–	+	+	+	+	+	–	+	+	–	–	+	+	+	+	+	+	–	*Lactobacillus brevis*
Go43	Coccus	l = 1.2 (1.1 - 1.3) b= 0.7 (0.6 - 0.8)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	–	+	–	–	+	+	+	+	+	+	+	–	*Enterococcus faecium*
Go45	Coccus	l = 1.1 (1.0 - 1.2) b = 0.5 (0.4 - 0.6)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–	–	*Enterococcus faecium*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.
DAP, *meso*-diaminopimelic acid

Table 6. Phenotypic characteristics of the LAB isolated from gundruk of Sikkim

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
GK:C1	Rod	l = 2.0 (1.6 - 3.0) b = 0.7 (0.5 - 0.9)	+	–	+	+	+	+	–	+	+	–	+	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	+	–	–	–	–	*Lactobacillus plantarum*
GK:C2	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	–	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GK:B3	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GK:B6	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GK:C3	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLn:R1	Rod	l = 2.1 (1.6 - 3.0) b = 0.8 (0.6 - 0.9)	+	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GLn:R5	Rod	l = 3.1 (2.3 - 4.2) b = 0.6 (0.5- 0.8)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GLn:R6	Rod	l = 3.3 (2.8 - 4.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
GLn:C1	Coccus/tetrad	d = 0.8 (0.6 - 0.9)	–	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLn:C2	Rod	l = 3.2 (2.8 - 3.5) b = 0.6 (0.5 - 0.7)	–	–	+	+	+	+	–	+	+	–	+	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Lactobacillus plantarum*
GLn:C3	Coccus/tetrad	d =0.8 (0.6 - 0.9)	–	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLn:C5	Coccus/tetrad	d =0.8 (0.6 - 0.9)	+	–	+	+	–	+	–	+	+	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLn:R2	Coccus/tetrad	d = 1.0 (0.8 - 1.1)	+	–	+	+	–	+	–	+	–	–	–	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	–	+	*Pediococcus pentosaceus*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 6)

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
GLn:R3	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	–	–	+	+	–	+	–	+	–	–	–	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	–	+	*Pediococcus pentosaceus*
GSd:R1	Rod	l = 2.0 (1.6 - 3.0) b = 0.7 (0.5 - 0.9)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GSd:R4	Rod	l = 2.0 (1.6 - 3.0) b = 0.7 (0.5 - 0.9)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GSd:R5	Rod	l = 2.0 (1.6 - 3.0) b = 0.6 (0.5 - 0.7)	+	–	+	+	–	+	–	+	–	–	+	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GSd:C1	Coccus/tetrad	d = 0.9 (0.6 - 1.1)	–	–	–	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GSd:C2	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	–	–	+	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GS:R1	Rod	l = 1.9 (1.6 - 2.1) b = 0.9 (0.8 - 0.9)	–	–	+	+	–	+	–	+	+	–	+	DL	–	–	–	+	+	+	–	+	–	+	+	+	+	–	+	–	+	*Lactobacillus plantarum*
GS:B1	Coccus/tetrad	d = 1.0 (0.9 - 1.1)	+	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GS:B2	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	+	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GS:B7	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	–	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GS:C1	Coccus/tetrad	d = 0.9 (0.7 - 1.1)	+	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLc:B1	Rod	l = 2.0 (1.6 - 3.0) b = 0.6 (0.5 - 0.7)	+	–	+	+	–	+	–	+	–	–	+	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GLc:C1	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLc:C4	Coccus/tetrad	d = 1.0 (0.9 - 1.1)	–	–	+	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*

d, diameter; l, length; b, breadth

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 7. Phenotypic characteristics of the LAB isolated from Inziangsang of Manipur

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in									DAP	Lactate isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%				Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
IB1	Rod	l = 2.6 (1.9 - 3.2) b = 0.7 (0.6 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	L(D)	+	+	+	+	–	–	–	–	V	–	–	–	–	+	+	–	–		*Lactobacillus brevis*
IB2	Rod	l = 1.9 (1.6 - 2.4) b = 0.7 (0.6 - 0.8)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus plantarum*
IB3	Rod	l = 1.9 (1.6 - 2.4) b = 0.8 (0.7 - 0.9)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus plantarum*
IB5	Coccus/tetrad	d = 0.8 (0.7 - 0.9)	+	–	–	+	+	+	+	+	–	–	–	DL	+	+	+	+	–	+	–	+	–	+	+	+	–	–	–	–	–		*Pediococcus acidilactici*
IB9	Rod	l = 1.9 (1.6 - 2.4) b = 0.8 (0.7 - 0.9)	+	+	–	+	–	+	–	+	–	–	–	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus brevis*
IB11	Rod	l = 3.9 (3.2 - 4.4) b = 0.7 (0.5 - 0.9)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+		*Lactobacillus plantarum*
IB12	Rod	l = 2.9 (1.6 - 3.4) b = 0.7 (0.5 - 0.9)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+		*Lactobacillus plantarum*
IB13	Rod	l = 3.4 (2.6 - 4.0) b = 0.6 (0.5 - 0.8)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus plantarum*
IB14	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	–	+	+	+	+	+	–	–	–	DL	+	+	+	+	–	+	–	+	–	+	+	+	–	–	–	–	–		*Pediococcus acidilactici*

l, length; b, breadth; d, diameter.

All strains were Gram-positive and catalase-negative, non-motile and non-sp

DAP, *meso*-diaminopimelic acid

Table 8. Phenotypic characteristics of the LAB isolated from khalpi of Sikkim

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																	Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
KG:B1	Rod	l = 2.7 (1.4 - 3.5) b = 0.4 (0.3 - 0.5)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	–	+	+	+	+	+	+	*Lactobacillus plantarum*
KG:B2	Rod	l = 2.3 (1.8 - 3.2) b = 0.4 (0.3 - 0.5)	+	+	–	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KG:B3	Rod	l = 1.6 (1.1 - 1.9) b = 0.5 (0.3 - 0.6)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
KG:B7	Rod	l = 1.6 (1.2 - 1.9) b = 0.8 (0.6 - 0.9)	+	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KG:B8	Rod	l = 1.6 (1.3 - 1.9) b = 0.6 (0.5 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KG:B9	Rod	l = 1.7 (1.3 - 1.8) b = 0.7 (0.6 - 0.8)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KG:R1	Rod	l = 3.2 (2.2 - 4.7) b = 0.6 (0.5 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KG:R2	Rod	l = 3.6 (2.3 - 4.8) b = 0.6 (0.5 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KB:B4	Rod	l = 1.7 (1.3 - 1.8) b = 0.5 (0.3 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KB:B5	Rod	l = 1.5 (1.4 - 1.6) b = 0.6 (0.4 - 0.7)	+	+	–	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KB:C1	Coccus/Ovoid	d = 1.0 (0.9 - 1.2)	–	+	–	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–	*Leuconostoc fallax*
KB:B9	Rod	l = 2.5 (2.0 - 3.1) b = 0.5 (0.4 - 0.6)	+	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
KK:B2	Rod	l = 3.2 (2.4 - 4.2) b = 0.4 (0.3 - 0.5)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 8)

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
KK:B3	Rod	l = 2.6 (2.0 - 3.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KK:B4	Rod	l = 2.8 (2.0 - 3.4) b = 0.6 (0.4 - 0.7)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KK:B5	Rod	l = 2.3 (1.9 - 3.3) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KK:R1	Rod	l = 2.6 (2.0 - 3.8) b = 0.4 (0.3 - 0.5)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
KS:R1	Rod	l = 3.6 (2.2 - 4.2) b = 0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KS:R2	Rod	l = 3.6 (2.8 - 3.9) b = 0.5 (0.5 - 0.7)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KS:B1	Rod	l = 3.0 (2.8 - 3.6) b = 0.6 (0.5 - 0.7)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
KS:B2	Rod	l = 1.5 (1.3 - 1.8) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	–	–	–	+	DL	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KS:B9	Rod	l = 1.5 (1.3 - 1.8) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	–	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KS:C1	Coccus/Ovoid	l = 1.5 (1.3 - 1.8) b = 0.4 (0.3 - 0.5)	–	+	–	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–	*Leuconostoc fallax*
KS:C2	Coccus/Ovoid	l = 1.5 (1.3 - 1.8) b = 0.4 (0.3 - 0.5)	–	+	–	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–	*Leuconostoc fallax*
KBe:B2	Rod	l = 2.5 (2.1 - 3.2) b = 0.4 (0.3 - 0.5)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	–	+	–	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 9. Phenotypic characteristics of the LAB isolated from sinki of Sikkim

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
SL:B1	Rod	l = 1.3 (1.2 - 1.6) b = 0.9 (0.8 - 1.2)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SL:B2	Rod	l = 1.7 (1.6 - 2.0) b = 0.6 (0.5 - 0.7)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SL:B3	Coccus/ovoid	d = 1.0 (0.7 - 1.2)	–	+	+	+	–	–	–	–	–	–	–	D	–	+	–	–	+	+	–	+	–	–	–	–	–	–	+	–	–		*Leuconostoc fallax*
SL:B4	Coccus/ovoid	d = 1.2 (1.0 - 1.3)	–	+	+	+	–	–	–	–	–	–	–	D	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–		*Leuconostoc fallax*
SL:B5	Coccus/ovoid	d = 0.9 (0.8 - 1.2)	–	+	+	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–		*Leuconostoc fallax*
SL:B6	Rod	l = 1.6 (1.4 - 1.7) b = 0.7 (0.6 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SL:B7	Rod	l = 1.7 (1.6 - 2.0) b = 0.8 (0.6 - 0.9)	–	+	+	+	–	+	–	–	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SL:B8	Rod	l = 2.6 (2.0 - 3.0) b = 0.5 (0.4 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SK:B1	Rod	l = 1.6 (1.2 - 1.8) b = 0.7 (0.6 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SK:B2	Rod	l = 1.3 (1.1 - 1.5) b = 0.6 (0.5 - 0.8)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SK:B3	Rod	l = 1.6 (1.3 - 1.7) b = 0.5 (0.4 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SK:C1	Coccus/ovoid	d = 0.9 (0.8 - 1.2)	–	+	+	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–		*Leuconostoc fallax*
SBe:B1	Rod	l = 1.0 (1.2 - 1.4) b = 0.5 (0.4 - 0.9)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus plantarum*
SBe:B2	Rod	l = 2.0 (1.8 - 2.4) b = 0.5 (0.4 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	+	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 9)

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
SBe:B5	Rod	l = 2.0 (1.8 - 2.4) b = 0.5 (0.4 - 0.9)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
SBe:B6	Rod	l = 2.0 (1.8 - 2.4) b = 0.5 (0.4 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
SBe:B7	Rod	l = 2.2 (1.9 - 2.5) b = 0.5 (0.4 - 0.6)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B1	Rod	l = 2.6 (2.2 - 3.2) b = 0.5 (0.4 - 0.7)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B2	Rod	l = 2.4 (1.8 - 2.8) b = 0.5 (0.4 - 0.9)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B4	Rod	l = 1.4 (1.2 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	–	L	–	–	–	–	+	+	–	+	–	+	+	+	+	–	–	–	+	*Lactobacillus casei*
SS:B5	Rod	l = 2.6 (2.2 - 3.0) b = 0.5 (0.4 - 0.6)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B6	Rod	l = 2.9 (2.6 - 3.8) b = 0.5 (0.4 - 0.6)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B7	Rod	l = 3.3 (2.8 - 3.7) b = 0.6 (0.4 - 0.7)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
SG:B1	Rod	l = 1.3 (1.2 - 1.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
SG:B4	Rod	l = 1.4 (1.3 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
SG:B5	Rod	l = 1.4 (1.3 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
SG:B7	Rod	l = 1.5 (1.3 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 10. Phenotypic characteristics of the LAB isolated from ekung of Arunachal Pradesh

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
Ek1	Rod	l = 1.4 (1.2 - 1.6) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek4	Rod	l = 1.3 (1.2 - 1.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek5	Rod	l = 1.4 (1.3 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek6	Rod	l = 1.5 (1.2 - 1.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	LD	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Ek7	Rod	l = 1.4 (1.3 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek8	Rod	l = 1.3 (1.2 - 1.4) b = 0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	+	–	–	–	DL	+	–	–	+	+	+	–	+	–	–	+	+	+	–	–	–	–	*Lactobacillus brevis*
Ek10	Rod	l = 1.3 (1.1 - 1.5) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek11	Rod	l = 1.3 (1.2 - 1.4) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek12	Rod	l = 1.4 (1.3 - 1.5) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek15	Rod	l = 1.2 (1.0 - 1.4) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Ek18	Rod	l = 1.4 (1.3 - 1.5) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek19	Rod	l = 1.2 (1.0 - 1.3) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 10. Phenotypic characteristics of the LAB isolated from ekung of Arunachal Pradesh

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
Ek1	Rod	l = 1.4 (1.2 - 1.6) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek4	Rod	l = 1.3 (1.2 - 1.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek5	Rod	l = 1.4 (1.3 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek6	Rod	l = 1.5 (1.2 - 1.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	LD	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Ek7	Rod	l = 1.4 (1.3 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek8	Rod	l = 1.3 (1.2 - 1.4) b = 0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	+	–	–	–	DL	+	–	–	+	+	+	–	+	–	–	+	+	+	–	–	–	–	*Lactobacillus brevis*
Ek10	Rod	l = 1.3 (1.1 - 1.5) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek11	Rod	l = 1.3 (1.2 - 1.4) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek12	Rod	l = 1.4 (1.3 - 1.5) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek15	Rod	l = 1.2 (1.0 - 1.4) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Ek18	Rod	l = 1.4 (1.3 - 1.5) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek19	Rod	l = 1.2 (1.0 - 1.3) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 5. Phenotypic characteristics of the LAB from goyang of Sikkim

Strain Code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in									DAP	Lactate isomer	Sugars Fermented																	Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%				Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
Go1	Coccus/tetrad	d = 0.7 (0.6 - 0.8)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
Go5	Coccoid rod	d = 0.9 (0.6 - 1.2)	+	–	+	+	–	–	+	–	–	–	–	L	+	–	+	+	+	–	+	+	–	+	–	+	+	+	–	–	–	*Lactococcus lactis*
Go6	Rod	l = 1.7 (1.5 - 1.9) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	+	+	+	+	+	+	+	+	–	–	+	*Lactobacillus plantarum*
Go7	Coccoid rod	l = 1.2 (1.1 - 1.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	–	+	–	–	–	–	L	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactococcus lactis*
Go8	Coccoid rod	d = 0.8 (0.6 - 1.2)	–	–	+	+	–	–	+	–	–	–	–	L	+	+	+	+	–	–	–	+	–	+	+	+	+	+	–	–	–	*Lactococcus lactis*
Go9	Coccus	l = 1.3 (1.2- 1.6) b = 0.9 (0.8 - 1.0)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	*Enterococcus faecium*
Go11	Rod	l = 2.1 (1.4 - 3.2) b =0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	–	–	–	–	DL	–	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
Go12	Rod	l = 1.6 (1.4 - 1.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	+	–	+	–	+	+	–	–	–	*Lactobacillus plantarum*
Go14	Rod	l = 1.6 (1.5 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	–	+	–	–	–	*Lactobacillus plantarum*
Go16	Rod	l = 1.5 (1.4 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Lactobcillus plantarum*
Go17	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	–	–	–	–	–	*Pediococcus pentosaceus*
Go20	Coccus	l = 1.2 (1.1 - 1.3) b = 0.6 (0.5 - 0.7)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	–	*Enterococcus faecium*
Go22	Rod	l = 1.5 (1.4 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*

d, diameter; l, length; b, breadth

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 5)

Strain Code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																	Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
Go23	Rod	l = 1.4 (1.3 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	–	–	*Lactobacillus plantarum*
Go24	Rod	l = 1.4 (1.3 - 1.6) b = 0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	–	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
Go25	Rod	l = 1.6 (1.4 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go29	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
Go30	Rod	l = 1.6 (1.4 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go31	Rod	l = 1.5 (1.4 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go34	Coccus	l = 1.2 (1.0 - 1.3) b = 0.6 (0.5 - 0.8)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	–	*Enterococcus faecium*
Go37	Rod	l = 1.5 (1.4 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go39	Rod	l = 1.5 (1.4 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go40	Rod	l = 1.1 (1.0 - 1.2) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Go41	Rod	l = 1.4 (1.2 - 1.6) b = 0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	+	–	–	–	DL	–	+	+	+	+	+	–	+	+	–	–	+	+	+	+	+	+	–	*Lactobacillus brevis*
Go43	Coccus	l = 1.2 (1.1 - 1.3) b= 0.7 (0.6 - 0.8)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	–	+	–	–	+	+	+	+	+	+	+	–	*Enterococcus faecium*
Go45	Coccus	l = 1.1 (1.0 - 1.2) b = 0.5 (0.4 - 0.6)	+	–	+	+	+	–	+	+	–	–	–	L	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–	–	*Enterococcus faecium*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.
DAP, *meso*-diaminopimelic acid

Table 6. Phenotypic characteristics of the LAB isolated from gundruk of Sikkim

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
GK:C1	Rod	l = 2.0 (1.6 - 3.0) b = 0.7 (0.5 - 0.9)	+	–	+	+	+	+	–	+	+	–	+	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	+	–	–	–	–	*Lactobacillus plantarum*
GK:C2	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	–	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GK:B3	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GK:B6	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GK:C3	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLn:R1	Rod	l = 2.1 (1.6 - 3.0) b = 0.8 (0.6 - 0.9)	+	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GLn:R5	Rod	l = 3.1 (2.3 - 4.2) b = 0.6 (0.5- 0.8)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GLn:R6	Rod	l = 3.3 (2.8 - 4.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
GLn:C1	Coccus/tetrad	d = 0.8 (0.6 - 0.9)	–	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLn:C2	Rod	l = 3.2 (2.8 - 3.5) b = 0.6 (0.5 - 0.7)	–	–	+	+	+	+	–	+	+	–	+	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Lactobacillus plantarum*
GLn:C3	Coccus/tetrad	d =0.8 (0.6 - 0.9)	–	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLn:C5	Coccus/tetrad	d =0.8 (0.6 - 0.9)	+	–	+	+	–	+	–	+	+	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLn:R2	Coccus/tetrad	d = 1.0 (0.8 - 1.1)	+	–	+	+	–	+	–	+	–	–	–	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	–	+	*Pediococcus pentosaceus*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 6)

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
GLn:R3	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	–	–	+	+	–	+	–	+	–	–	–	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	–	+	*Pediococcus pentosaceus*
GSd:R1	Rod	l = 2.0 (1.6 - 3.0) b = 0.7 (0.5 - 0.9)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GSd:R4	Rod	l = 2.0 (1.6 - 3.0) b = 0.7 (0.5 - 0.9)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GSd:R5	Rod	l = 2.0 (1.6 - 3.0) b = 0.6 (0.5 - 0.7)	+	–	+	+	–	+	–	+	–	–	+	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GSd:C1	Coccus/tetrad	d = 0.9 (0.6 - 1.1)	–	–	–	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GSd:C2	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	–	–	+	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GS:R1	Rod	l = 1.9 (1.6 - 2.1) b = 0.9 (0.8 - 0.9)	–	–	+	+	–	+	–	+	+	–	+	DL	–	–	–	+	+	+	–	+	–	+	+	+	+	–	+	–	+	*Lactobacillus plantarum*
GS:B1	Coccus/tetrad	d = 1.0 (0.9 - 1.1)	+	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GS:B2	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	+	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GS:B7	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	–	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GS:C1	Coccus/tetrad	d = 0.9 (0.7 - 1.1)	+	–	+	+	+	+	–	+	+	–	–	DL	+	+	–	+	–	+	–	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLc:B1	Rod	l = 2.0 (1.6 - 3.0) b = 0.6 (0.5 - 0.7)	+	–	+	+	–	+	–	+	–	–	+	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
GLc:C1	Coccus/tetrad	d = 0.9 (0.8 - 1.1)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
GLc:C4	Coccus/tetrad	d = 1.0 (0.9 - 1.1)	–	–	+	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	–	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*

d, diameter; l, length; b, breadth

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 7. Phenotypic characteristics of the LAB isolated from Inziangsang of Manipur

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in									DAP	Lactate isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%				Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
IB1	Rod	l = 2.6 (1.9 - 3.2) b = 0.7 (0.6 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	L(D)	+	+	+	+	–	–	–	–	V	–	–	–	–	+	+	–	–		*Lactobacillus brevis*
IB2	Rod	l = 1.9 (1.6 - 2.4) b = 0.7 (0.6 - 0.8)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus plantarum*
IB3	Rod	l = 1.9 (1.6 - 2.4) b = 0.8 (0.7 - 0.9)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus plantarum*
IB5	Coccus/tetrad	d = 0.8 (0.7 - 0.9)	+	–	–	+	+	+	+	+	–	–	–	DL	+	+	+	+	–	+	–	+	–	+	+	+	–	–	–	–	–		*Pediococcus acidilactici*
IB9	Rod	l = 1.9 (1.6 - 2.4) b = 0.8 (0.7 - 0.9)	+	+	–	+	–	+	–	+	–	–	–	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus brevis*
IB11	Rod	l = 3.9 (3.2 - 4.4) b = 0.7 (0.5 - 0.9)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+		*Lactobacillus plantarum*
IB12	Rod	l = 2.9 (1.6 - 3.4) b = 0.7 (0.5 - 0.9)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+		*Lactobacillus plantarum*
IB13	Rod	l = 3.4 (2.6 - 4.0) b = 0.6 (0.5 - 0.8)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus plantarum*
IB14	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	–	+	+	+	+	+	–	–	–	DL	+	+	+	+	–	+	–	+	–	+	+	+	–	–	–	–	–		*Pediococcus acidilactici*

l, length; b, breadth; d, diameter.

All strains were Gram-positive and catalase-negative, non-motile and non-sp

DAP, *meso*-diaminopimelic acid

Table 8. Phenotypic characteristics of the LAB isolated from khalpi of Sikkim

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																	Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
KG:B1	Rod	l = 2.7 (1.4 - 3.5) b = 0.4 (0.3 - 0.5)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	–	+	+	+	+	+	+	*Lactobacillus plantarum*
KG:B2	Rod	l = 2.3 (1.8 - 3.2) b = 0.4 (0.3 - 0.5)	+	+	–	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KG:B3	Rod	l = 1.6 (1.1 - 1.9) b = 0.5 (0.3 - 0.6)	–	–	–	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
KG:B7	Rod	l = 1.6 (1.2 - 1.9) b = 0.8 (0.6 - 0.9)	+	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KG:B8	Rod	l = 1.6 (1.3 - 1.9) b = 0.6 (0.5 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KG:B9	Rod	l = 1.7 (1.3 - 1.8) b = 0.7 (0.6 - 0.8)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KG:R1	Rod	l = 3.2 (2.2 - 4.7) b = 0.6 (0.5 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KG:R2	Rod	l = 3.6 (2.3 - 4.8) b = 0.6 (0.5 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KB:B4	Rod	l = 1.7 (1.3 - 1.8) b = 0.5 (0.3 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KB:B5	Rod	l = 1.5 (1.4 - 1.6) b = 0.6 (0.4 - 0.7)	+	+	–	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
KB:C1	Coccus/Ovoid	d = 1.0 (0.9 - 1.2)	–	+	–	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–	*Leuconostoc fallax*
KB:B9	Rod	l = 2.5 (2.0 - 3.1) b = 0.5 (0.4 - 0.6)	+	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
KK:B2	Rod	l = 3.2 (2.4 - 4.2) b = 0.4 (0.3 - 0.5)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 8)

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
KK:B3	Rod	l = 2.6 (2.0 - 3.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KK:B4	Rod	l = 2.8 (2.0 - 3.4) b = 0.6 (0.4 - 0.7)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KK:B5	Rod	l = 2.3 (1.9 - 3.3) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KK:R1	Rod	l = 2.6 (2.0 - 3.8) b = 0.4 (0.3 - 0.5)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	+	–	–	+	–	+	–	–		*Lactobacillus brevis*
KS:R1	Rod	l = 3.6 (2.2 - 4.2) b = 0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
KS:R2	Rod	l = 3.6 (2.8 - 3.9) b = 0.5 (0.5 - 0.7)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
KS:B1	Rod	l = 3.0 (2.8 - 3.6) b = 0.6 (0.5 - 0.7)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	+	–	–	+	–	+	–	–		*Lactobacillus brevis*
KS:B2	Rod	l = 1.5 (1.3 - 1.8) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	–	–	–	+	DL	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KS:B9	Rod	l = 1.5 (1.3 - 1.8) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	–	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
KS:C1	Coccus/Ovoid	l = 1.5 (1.3 - 1.8) b = 0.4 (0.3 - 0.5)	–	+	–	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–		*Leuconostoc fallax*
KS:C2	Coccus/Ovoid	l = 1.5 (1.3 - 1.8) b = 0.4 (0.3 - 0.5)	–	+	–	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–		*Leuconostoc fallax*
KBe:B2	Rod	l = 2.5 (2.1 - 3.2) b = 0.4 (0.3 - 0.5)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	–	+	–	–	–	–	–	+	–	–	+	–	+	–	–		*Lactobacillus brevis*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 9. Phenotypic characteristics of the LAB isolated from sinki of Sikkim

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
SL:B1	Rod	l = 1.3 (1.2 - 1.6) b = 0.9 (0.8 - 1.2)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SL:B2	Rod	l = 1.7 (1.6 - 2.0) b = 0.6 (0.5 - 0.7)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SL:B3	Coccus/ovoid	d = 1.0 (0.7 - 1.2)	–	+	+	+	–	–	–	–	–	–	–	D	–	+	–	–	+	+	–	+	–	–	–	–	–	–	+	–	–		*Leuconostoc fallax*
SL:B4	Coccus/ovoid	d = 1.2 (1.0 - 1.3)	–	+	+	+	–	–	–	–	–	–	–	D	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–		*Leuconostoc fallax*
SL:B5	Coccus/ovoid	d = 0.9 (0.8 - 1.2)	–	+	+	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–		*Leuconostoc fallax*
SL:B6	Rod	l = 1.6 (1.4 - 1.7) b = 0.7 (0.6 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SL:B7	Rod	l = 1.7 (1.6 - 2.0) b = 0.8 (0.6 - 0.9)	–	+	+	+	–	+	–	–	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SL:B8	Rod	l = 2.6 (2.0 - 3.0) b = 0.5 (0.4 - 0.8)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SK:B1	Rod	l = 1.6 (1.2 - 1.8) b = 0.7 (0.6 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SK:B2	Rod	l = 1.3 (1.1 - 1.5) b = 0.6 (0.5 - 0.8)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SK:B3	Rod	l = 1.6 (1.3 - 1.7) b = 0.5 (0.4 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
SK:C1	Coccus/ovoid	d = 0.9 (0.8 - 1.2)	–	+	+	+	–	–	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	+	–	–	–	–		*Leuconostoc fallax*
SBe:B1	Rod	l = 1.0 (1.2 - 1.4) b = 0.5 (0.4 - 0.9)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+		*Lactobacillus plantarum*
SBe:B2	Rod	l = 2.0 (1.8 - 2.4) b = 0.5 (0.4 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	+	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 9)

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
SBe:B5	Rod	l = 2.0 (1.8 - 2.4) b = 0.5 (0.4 - 0.9)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
SBe:B6	Rod	l = 2.0 (1.8 - 2.4) b = 0.5 (0.4 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
SBe:B7	Rod	l = 2.2 (1.9 - 2.5) b = 0.5 (0.4 - 0.6)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B1	Rod	l = 2.6 (2.2 - 3.2) b = 0.5 (0.4 - 0.7)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B2	Rod	l = 2.4 (1.8 - 2.8) b = 0.5 (0.4 - 0.9)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B4	Rod	l = 1.4 (1.2 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	–	L	–	–	–	–	+	+	–	+	–	+	+	+	+	–	–	–	+	*Lactobacillus casei*
SS:B5	Rod	l = 2.6 (2.2 - 3.0) b = 0.5 (0.4 - 0.6)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B6	Rod	l = 2.9 (2.6 - 3.8) b = 0.5 (0.4 - 0.6)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
SS:B7	Rod	l = 3.3 (2.8 - 3.7) b = 0.6 (0.4 - 0.7)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	–	–	–	–	+	–	–	+	–	+	–	–	*Lactobacillus brevis*
SG:B1	Rod	l = 1.3 (1.2 - 1.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
SG:B4	Rod	l = 1.4 (1.3 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
SG:B5	Rod	l = 1.4 (1.3 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
SG:B7	Rod	l = 1.5 (1.3 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 10. Phenotypic characteristics of the LAB isolated from ekung of Arunachal Pradesh

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
Ek1	Rod	l = 1.4 (1.2 - 1.6) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek4	Rod	l = 1.3 (1.2 - 1.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek5	Rod	l = 1.4 (1.3 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek6	Rod	l = 1.5 (1.2 - 1.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	LD	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Ek7	Rod	l = 1.4 (1.3 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek8	Rod	l = 1.3 (1.2 - 1.4) b = 0.5 (0.4 - 0.6)	+	+	+	+	–	+	–	+	–	–	–	DL	+	–	–	+	+	+	–	+	–	–	+	+	+	–	–	–	–	*Lactobacillus brevis*
Ek10	Rod	l = 1.3 (1.1 - 1.5) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek11	Rod	l = 1.3 (1.2 - 1.4) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek12	Rod	l = 1.4 (1.3 - 1.5) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek15	Rod	l = 1.2 (1.0 - 1.4) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Ek18	Rod	l = 1.4 (1.3 - 1.5) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek19	Rod	l = 1.2 (1.0 - 1.3) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 10)

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
Ek20	Rod	l = 1.2 (1.1 - 1.3) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Ek21	Rod	l = 1.3 (1.2 - 1.4) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Ek22	Coccus/tetrad	d = 1.0 (0.9 - 1.2)	+	–	+	+	–	+	–	+	+	+	–	L	–	–	–	+	+	–	–	+	–	–	–	–	+	+	–	–	+	*Tetragenococcus halophilus*
Ek23	Rod	l = 3.7 (2.8 - 4.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	+	+	–	+	–	+	–	+	+	+	+	+	–	–	–	*Lactobacillus plantarum*
Ek24	Rod	l = 1.5 (1.3 - 1.7) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	–	*Lactobacillus plantarum*
Ek25	Rod	l = 1.2 (1.1 - 1.3) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek28	Coccus/tetrad	d = 1.0 (0.9 - 1.2)	+	–	+	+	–	+	–	+	+	+	–	L	–	–	–	+	+	–	–	+	–	–	–	–	+	+	–	–	+	*Tetragenococcus halophilus*
Ek29	Rod	l = 1.4 (1.2 - 1.6) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek34	Rod	l = 1.4 (1.2 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	–	L	–	–	–	+	+	–	–	+	–	+	–	+	+	+	–	–	+	*Lactobacillus casei*
Ek35	Rod	l = 1.4 (1.2 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	–	L	–	–	–	+	–	–	–	+	–	–	–	–	–	+	–	–	–	*Lactobacillus casei*
Ek38	Rod	l = 1.2 (1.0 - 1.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek39	Rod	l = 1.2 (1.0 - 1.4) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	–	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ek40	Rod	l = 1.5 (1.2 - 1.7) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 11. Phenotypic characteristics of the LAB from eup collected from Arunachal Pradesh

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																	Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
Ep81	Rod	l = 1.7 (1.6 - 1.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep82	Rod	l = 1.6 (1.4 - 1.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep84	Rod	l = 1.4 (1.3 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep85	Rod	l = 2.0 (1.6 - 2.4) b = 0.5 (0.4 - 0.6)	+	+	–	–	+	+	–	–	–	–	–	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	*Lactobacillus fermentum*
Ep86	Rod	l = 1.8 (1.7 - 2.0) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	–	*Lactobacillus plantarum*
Ep87	Rod	l = 1.7 (1.5 - 1.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep89	Rod	l = 1.8 (1.7 - 2.0) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep90	Rod	l = 1.9 (1.7 - 2.2) b = 0.6 (0.5 - 0.7)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	*Lactobacillus plantarum*
Ep91	Rod	l = 1.2 (1.0 - 1.3) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep92	Rod	l = 2.0 (1.6 - 2.4) b = 0.5 (0.4 - 0.6)	+	+	–	–	+	+	–	–	–	–	–	DL	–	+	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–	*Lactobacillus fermentum*
Ep93	Rod	l = 2.2 (1.7 - 2.8) b = 0.6 (0.5 - 0.7)	+	+	–	–	+	+	–	–	–	–	–	DL	–	+	+	+	+	+	–	+	–	–	–	–	+	+	+	+	–	*Lactobacillus fermentum*
Ep94	Rod	l = 2.0 (1.6 - 2.3) b = 0.6 (0.5 - 0.7)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep95	Rod	l = 1.5 (1.4 - 1.6) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	–	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 11)

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in									DAP	Lactate isomer	Sugars Fermented																	Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%				Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
Ep96	Rod	l = 1.8 (1.6 - 1.9) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep97	Rod	l = 1.5 (1.4 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep98	Rod	l = 1.3 (1.2 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep99	Rod	l = 1.5 (1.2 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep101	Rod	l = 1.7 (1.4 - 1.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep102	Rod	l = 1.5 (1.4 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep103	Rod	l = 1.6 (1.5 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep106	Rod	l = 1.7 (1.4 - 1.9) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep110	Rod	l = 1.4 (1.3 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep111	Rod	l = 1.5 (1.2 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep112	Rod	l = 1.7 (1.5 - 1.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Ep115	Rod	l = 1.6 (1.4 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 12. Phenotypic characteristics of the LAB isolated from hirring of Arunachal Pradesh

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
Hr41	Rod	l = 1.8 (1.6 - 2.1) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Hr42	Rod	l = 1.7 (1.6 - 1.9) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Hr45	Rod	l = 1.2 (1.0 - 1.4) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Hr47	Rod	l = 1.4 (1.2 - 1.6) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Hr48	Rod	l = 1.2 (1.0 - 1.4) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Hr49	Rod	l = 1.2 (1.0 - 1.6) b = 0.5 (0.3 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	–	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Hr50	Rod	l = 1.6 (1.4 - 1.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	–	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Hr55	Rod	l = 1.3 (1.1 - 1.5) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	–	*Lactobacillus plantarum*
Hr56	Coccoid rod	l = 1.0 (0.9 - 1.1) b = 0.7 (0.6 - 0.8)	+	–	+	+	–	–	+	–	–	–	–	L	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactococcus lactis*
Hr57	Rod	l = 1.3 (1.1 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Hr58	Rod	l = 1.3 (1.1 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Hr59	Rod	l = 1.4 (1.2 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Hr60	Rod	l = 1.5 (1.2 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Continued (Table 12)

Strain code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
Hr62	Coccoid rod	l = 1.0 (0.9 - 1.2) b = 0.7 (0.6 - 0.8)	+	–	+	+	–	–	+	–	–	–	–	L	+	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactococcus lactis*
Hr64	Rod	l = 1.4 (1.2 - 1.6) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Hr65	Rod	l = 1.5 (1.2 - 1.7) b = 0.5 (0.4 - 0.7)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Hr66	Rod	l = 1.4 (1.1 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Hr67	Rod	l = 1.5 (1.2 - 1.6) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	–	+	–	+	*Lactobacillus plantarum*
Hr68	Rod	l = 1.3 (1.1 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	–	+	+	+	+	*Lactobacillus plantarum*
Hr70	Rod	l = 1.3 (1.2 - 1.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	–	+	+	+	+	*Lactobacillus plantarum*
Hr71	Rod	l = 1.6 (1.4 - 1.8) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
Hr74	Rod	l = 1.3 (1.1 - 1.5) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Hr75	Rod	l = 1.4 (1.2 - 1.7) b = 0.4 (0.3- 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	–	*Lactobacillus plantarum*
Hr76	Rod	l = 1.5 (1.3 - 1.7) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	–	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus planterum*
Hr77	Rod	l = 2.2 (1.8 - 2.9) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
Hr80	Rod	l = 1.7 (1.5 - 1.9) b = 0.6 (0.4 - 0.7)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus planterum*

l, length; b, breadth; d, diameter

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

DAP, *meso*-diaminopimelic acid

Table 13. Phenotypic properties of the LAB isolated from mesu of Sikkim

Strain Code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in									DAP	Lactate isomer	Sugars Fermented																	Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%				Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose	
MeL2	Rod	l = 1.9 (0.8 - 3.0) b = 0.9 (0.6 - 1.3)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
MeB4	Rod	l = 1.6 (0.8 - 2.4) b = 0.4 (0.3 - 0.5)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–	*Lactobacillus brevis*
MeR1	Rod	l = 1.3 (1.2 - 1.6) b = 0.5 (0.4 - 0.8)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
MeB3	Rod	l = 0.8 (0.6 - 0.8) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus plantarum*
MeR6	Rod	l = 0.7 (0.5 - 0.8) b = 0.3 (0.2 - 0.6)	–	+	+	+	–	+	–	–	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
MeN3	Rod	l = 0.9 (0.8 - 1.3) b = 0.5 (0.3 - 0.6)	+	+	+	+	–	+	–	–	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–	*Lactobacillus brevis*
MeTR	Rod	l = 0.8 (0.5 - 1.0) b = 0.4 (0.3 - 0.5)	+	+	+	+	–	+	–	–	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
MeN7	Rod	l = 1.1 (0.8 - 1.4) b = 0.5 (0.3 - 0.6)	+	+	+	+	–	+	–	–	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–	*Lactobacillus brevis*
MeN5	Rod	l = 1.2 (1.0 - 1.6) b = 0.6 (0.5 - 0.6)	–	+	+	+	–	+	–	–	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
MeL3	Rod	l = 0.9 (0.6 - 1.5) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
MeBe3	Rod	l = 1.2 (0.8 - 1.4) b = 0.5 (0.4 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	–	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
MeN2	Rod	l = 2.4 (1.6 - 3.2) b = 0.7 (0.5 - 0.9)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
MeNt1	Rod	l = 1.6 (0.8 - 2.4) b = 0.4 (0.3 - 0.5)	–	–	+	–	–	+	–	+	–	–	+	DL	–	–	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
MeB1	Rod	l = 2.1 (1.6 - 2.5) b = 0.5 (0.3 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	–	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
MeB2	Rod	l = 2.1 (1.5 - 2.2) b = 0.5 (0.3 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	–	–	+	*Lactobacillus plantarum*
MeNt2	Rod	l = 0.9 (0.8 - 1.2) b = 0.3 (0.2 - 0.5)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus brevis*
MeB5	Rod	l = 0.8 (0.5 - 0.8) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*

l, length; b, breadth; d, diameter

DAP, *meso*-diaminopimelic acid

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

Continued (Table 13)

Strain Code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate isomer	Sugars Fermented																		Identity
					10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
MeNt3	Rod	l = 0.8 (0.6 - 1.0) b = 0.4 (0.3 - 0.5)	+	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	+	*Lactobacillus brevis*
MeN4	Rod	l = 1.5 (1.0 - 1.6) b = 0.4 (0.3 - 0.5)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	+	+	+	+	+	+	–	–	+	*Lactobacillus plantarum*
MeN6	Rod	l = 1.6 (1.3 - 1.9) b = 0.6 (0.3 - 0.9)	–	–	+	+	–	+	–	+	–	–	+	DL	–	–	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
MeR2	Rod	l = 1.3 (1.0 - 1.6) b = 0.5 (0.3 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
MeT1	Rod	l = 1.1 (0.8 - 1.5) b = 0.5 (0.3 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
MeT2	Rod	l = 1.2 (0.8 - 1.4) b = 0.5 (0.5 - 0.6)	–	–	+	+	–	+	–	+	–	–	+	DL	–	–	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
MeT3	Rod	l = 0.9 (0.6 - 1.3) b = 0.4 (0.3 - 0.4)	–	–	+	+	–	+	–	+	–	–	+	DL	–	–	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
MeN8	Rod	l = 2.6 (1.9 - 3.5) b = 0..5 (0.3 - 0.6)	–	+	+	+	–	+	–	+	–	–	–	D	+	–	–	–	+	+	–	+	–	+	+	+	+	–	–	–	–	*Leuconostoc citreum*
MeN9	Rod	l = 3.6 (2.7 - 4.2) b = 0.6 (0.4 - 0.7)	–	+	+	+	–	+	–	+	–	–	–	D	+	–	–	–	+	+	–	+	–	–	+	+	+	–	–	–	+	*Leuconostoc citreum*
MeK1	Rod	l = 2.9 (2.3 - 3.5) b = 0.4 (0.3 - 0.5)	–	+	+	+	–	+	–	+	–	–	–	D	+	–	–	+	+	+	–	+	–	–	–	+	+	–	–	–	–	*Leuconostoc citreum*
MeK2	Rod	l = 3.2 (2.9 - 3.8) b = 0.6 (0.5 - 0.8)	–	+	+	+	–	+	–	+	–	–	–	D	+	–	+	+	+	+	–	+	–	+	+	+	+	–	–	–	–	*Leuconostoc citreum*
MeR7	Rod	l = 2.8 (2.4 - 3.5) b = 0.4 (0.3 - 0.5)	+	+	+	+	–	+	–	+	–	–	–	DL	–	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
MeK3	Rod	l = 3.9 (0.8 - 1.0) b = 0.5 (0.3 - 0.6)	–	–	+	+	–	+	–	+	–	–	–	DL	–	+	–	+	+	+	–	+	–	–	+	–	+	–	–	–	–	*Lactobacillus curvatus*
MeB:C3	Coccus/tetrad	d = 1.0 (0.8 - 1.3)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	–	–	–	+	–	–	–	–	*Pediococcus pentosaceus*
MeB:C4	Coccus/tetrad	d = 1.0 (0.9 - 1.6)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	+	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
MeT5	Coccus/tetrad	d = 1.0 (0.8 - 1.2)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
MeT6	Coccus/tetrad	d = 0.9 (0.8 - 1.0)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*
MeT7	Coccus/tetrad	d = 0.9 (0.8 - 1.2)	+	–	+	+	+	+	–	+	–	–	–	DL	+	+	–	+	–	+	+	+	–	+	+	+	+	–	–	–	–	*Pediococcus pentosaceus*

l, length; b, breadth; d, diameter

DAP, *meso*-diaminopimelic acid

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

Table 14. Phenotypic characterstics of LAB Isolated from fermented bamboo shoots of Manipur

Product	Isolate code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in								DAP	Lactate Isomer	Sugars Fermented																	Identification
						10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%			Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Celloblose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
Soibum	SbB1	Coccoid rod	l = 2.1 (2.0 - 2.4) b = 0.8 (0.6 - 1.4)	–	+	+	+	–	+	–	+	–	–	–	D	–	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	–	*Leuconostoc lactis*
	SbC1	Coccoid rod	d = 0.9 (0.8 - 1.0)	–	+	+	+	–	+	–	–	–	–	–	D	–	–	+	–	+	+	–	+	–	+	+	–	–	–	+	–	–	*Leuconostoc fallax*
	SbC2	Coccoid rod	d = 1.0 (0.8 - 1.3)	–	+	+	+	–	+	–	+	–	–	–	D	+	–	–	+	+	+	–	+	–	–	+	+	–	+	+	–	–	*Leuconostoc mesenteroides*
	SbB2	Rod	l = 1.0 (0.9 - 1.1) b = 0.8 (0.7 - 0.9)	–	–	+	+	–	+	–	+	–	–	+	DL	–	+	–	+	+	+	–	+	–	–	+	+	+	–	–	–	–	*Lactobacillus plantarum*
	SbB3	Rod	l = 1.5 (1.2 - 1.6) b = 0.8 (0.7 - 0.9)	–	–	+	+	–	+	–	+	–	–	+	D(L)	–	+	–	+	+	+	–	+	+	–	+	+	+	–	–	–	–	*Lactobacillus plantarum*
	SbB4	Coccus	l = 1.6 (1.5 - 1.9) b = 0.9 (0.7 - 1.1)	–	–	+	+	–	+	–	+	–	–	–	L	–	+	–	+	–	+	–	+	–	+	+	+	+	+	+	–	–	*Enterococcus durans*
	SbB5	Rod	l = 2.1 (2.0 - 2.4) b = 0.8 (0.7 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
	SbB6	Rod	l = 2.1 (2.0 - 2.4) b = 0.8 (0.7 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Lactobacillus brevis*
Soldon	SdC1	Coccoid rod	d = 0.9 (0.7 - 1.4)	–	+	+	+	–	+	–	–	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	–	–	–	–	–	*Leuconostoc fallax*
	SdR2	Coccoid rod	l = 1.4 (1.2 - 1.6) b = 0.5 (0.4 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	D	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–	*Leuconostoc lactis*
	SdR3	Rod	l = 1.4 (1.2 - 1.6) b = 0.5 (0.4 - 0.8)	–	+	+	+	–	+	–	–	–	–	–	DL	–	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	–	*Lactobacillus brevis*
	SdR4	Rod	l = 1.4 (1.2 - 1.6) b = 0.5 (0.4 - 0.8)	–	+	+	+	–	+	–	–	–	–	–	DL	–	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	–	*Lactobacillus brevis*
	SdR5	Rod	l = 1.4 (1.2 - 1.6) b = 0.5 (0.4 - 0.8)	–	+	+	+	–	+	–	–	–	–	–	DL	–	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	–	*Lactobacillus brevis*

l, length; b, breadth; d, diameter

DAP, *meso*-diaminopimelic acid

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

Continued (Table 14)

Product	Isolate code	Cell Morphology	Cell size (µm)	NH_3 from arginine	CO_2 from glucose	Growth at/in									DAP	Lactate isomer	Sugars Fermented																		Identification
						10° C	15° C	45° C	pH 3.9	pH 9.6	NaCl 6.5%	NaCl 10%	NaCl 18%				Arabinose	Ribose	Xylose	Galactose	Sucrose	Trehalose	Rhamnose	Mannose	Sorbitol	Esculin	Salicin	Cellobiose	Maltose	Lactose	Melibiose	Raffinose	Melezitose		
Soidon	SdB1	Coccoid rod	l = 0.9 (0.8 - 1.2) b = 1.0 (0.8 - 1.4)	–	+	+	+	–	+	–	–	–	–	–	D	–	–	+	+	+	+	–	+	–	–	+	–	+	+	+	+	–		*Leuconostoc lactis*
	SdB2	Coccoid rod	l = 1.7 (1.2 - 1.9) b = 0.8 (0.6 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	D	–	–	+	+	+	+	–	+	–	–	–	–	+	+	+	+	–		*Leuconostoc lactis*
	SdB3	Coccoid rod	l = 2.0 (1.8 - 2.1) b = 0.9 (0.8 - 1.0)	–	+	+	+	–	+	–	+	–	–	–	D	–	–	+	+	+	+	–	+	–	+	+	–	+	+	+	+	–		*Leuconostoc lactis*
	SdB4	Rod	l = 1.7 (1.4 - 2.0) b = 0.7 (0.5 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
	SdB5	Coccoid rod	l = 1.3 (1.0- 1.7) b = 0.9 (0.8 - 1.0)	–	+	+	+	–	+	–	+	–	–	–	D	–	–	+	+	+	+	–	+	–	+	+	–	+	+	+	+	–		*Leuconostoc lactis*
Soijim	SjC1	Coccoid rod	d = 0.9 (0.7 - 1.0)	–	+	+	+	–	+	–	–	–	–	–	D	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–		*Leuconostoc fallax*
	SjR3	Coccoid rod	l = 2.0 (1.8 - 2.3) b = 0.8 (0.7 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	D	–	–	+	+	+	+	–	+	–	–	–	+	+	+	+	+	–		*Leuconostoc lactis*
	SjR7	Rod	l = 1.9 (1.7 - 2.2) b = 0.9 (0.7 - 1.2)	–	+	+	+	–	+	–	–	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
	SjB1	Rod	l = 1.0 (0.8 - 1.1) b = 0.7 (0.6 - 0.8)	–	+	+	+	–	+	–	–	–	–	–	DL	+	+	+	+	–	–	–	–	–	–	–	–	+	–	+	–	–		*Lactobacillus brevis*
	SjB2	Coccoid rod	l = 1.5 (1.2 - 1.8) b = 0.9 (0.8 - 1.1)	–	+	+	+	–	+	–	+	–	–	–	D	–	–	–	–	+	+	–	+	–	–	–	–	–	–	–	–	–		*Leuconostoc fallax*
	SjB4	Coccoid rod	l = 2.3 (2.0 - 2.6) b = 0.8 (0.7 - 0.9)	–	+	+	+	–	+	–	+	–	–	–	D	–	–	+	+	+	+	–	+	–	–	–	–	+	+	+	+	–		*Leuconostoc lactis*
	SjB5	Coccoid rod	l = 1.1 (1.0 - 1.5) b = 0.7 (0.5 - 0.8)	–	+	+	+	–	+	–	–	–	–	–	D	–	–	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–		*Leuconostoc lactis*
	SjB8	Coccoid rod	l = 1.1 (1.0 - 1.5) b = 0.7 (0.6 - 0.9)	–	+	+	+	–	+	–	–	–	–	–	D	–	–	+	+	+	+	–	+	–	+	+	+	+	+	+	+	–		*Leuconostoc lactis*

l, length; b, breadth; d, diameter

DAP, *meso*-diaminopimelic acid

All isolates were Gram-positive, catalase-negative, non-motile and non-sporeformers.

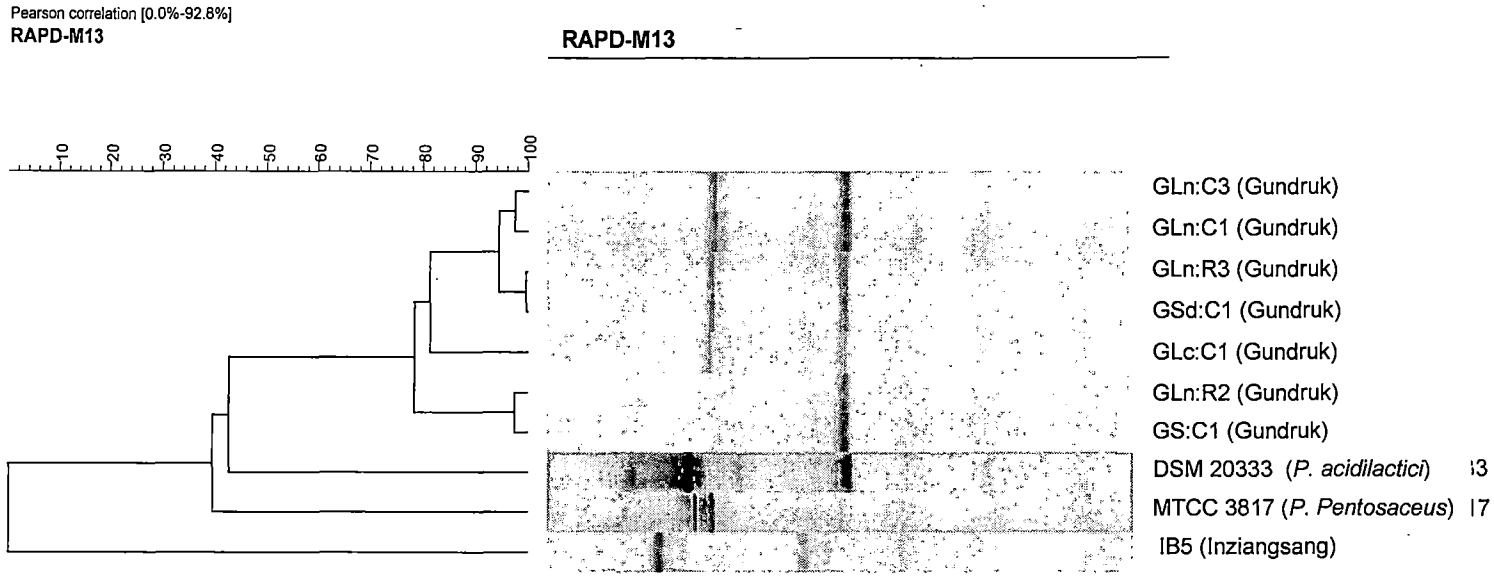


Fig 13. Dendrogram obtained by using RAPD patterns for pediococci generated from M13 primer. Patterns were grouped with the unweighted pair group algorithm with arithmetric averages (UPGMA).

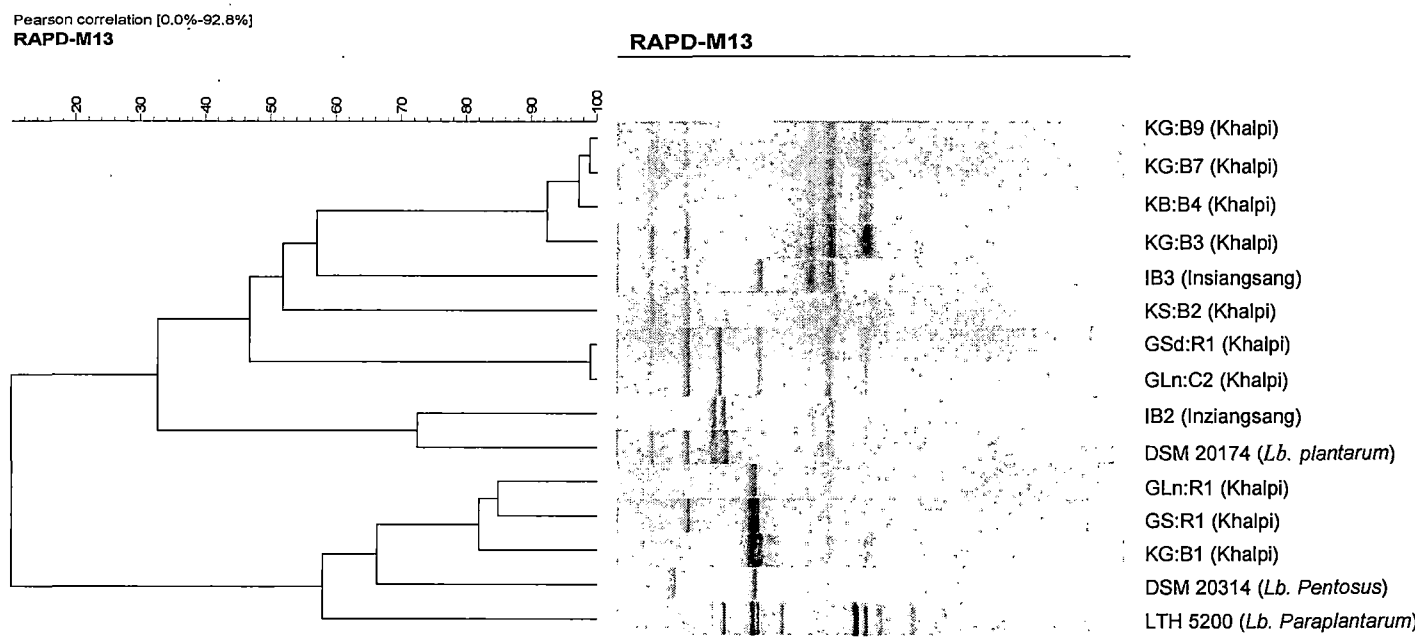


Fig 14. Dendrogram obtained by using RAPD patterns for strains of the *Lactobacillus plantarum* group generated from M13 primer. Patterns were grouped with the unweighted pair group arithmetric average (UPGMA).

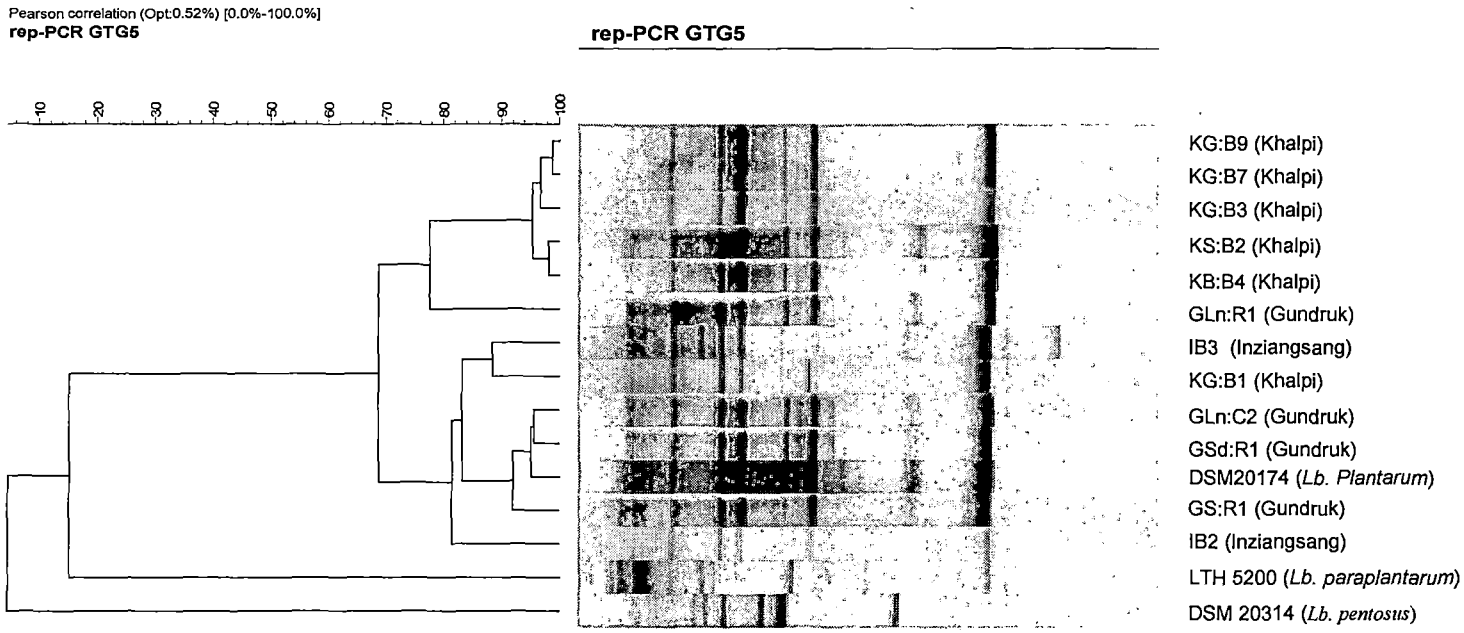


Fig 15. Dendrogram obtained from $(GTG)_5$-PCR fingerprints of strains of the *Lactobacillus plantarum* group. Patterns were grouped with the unweighted pair group algorithm with arithmetric averages (UPGMA).

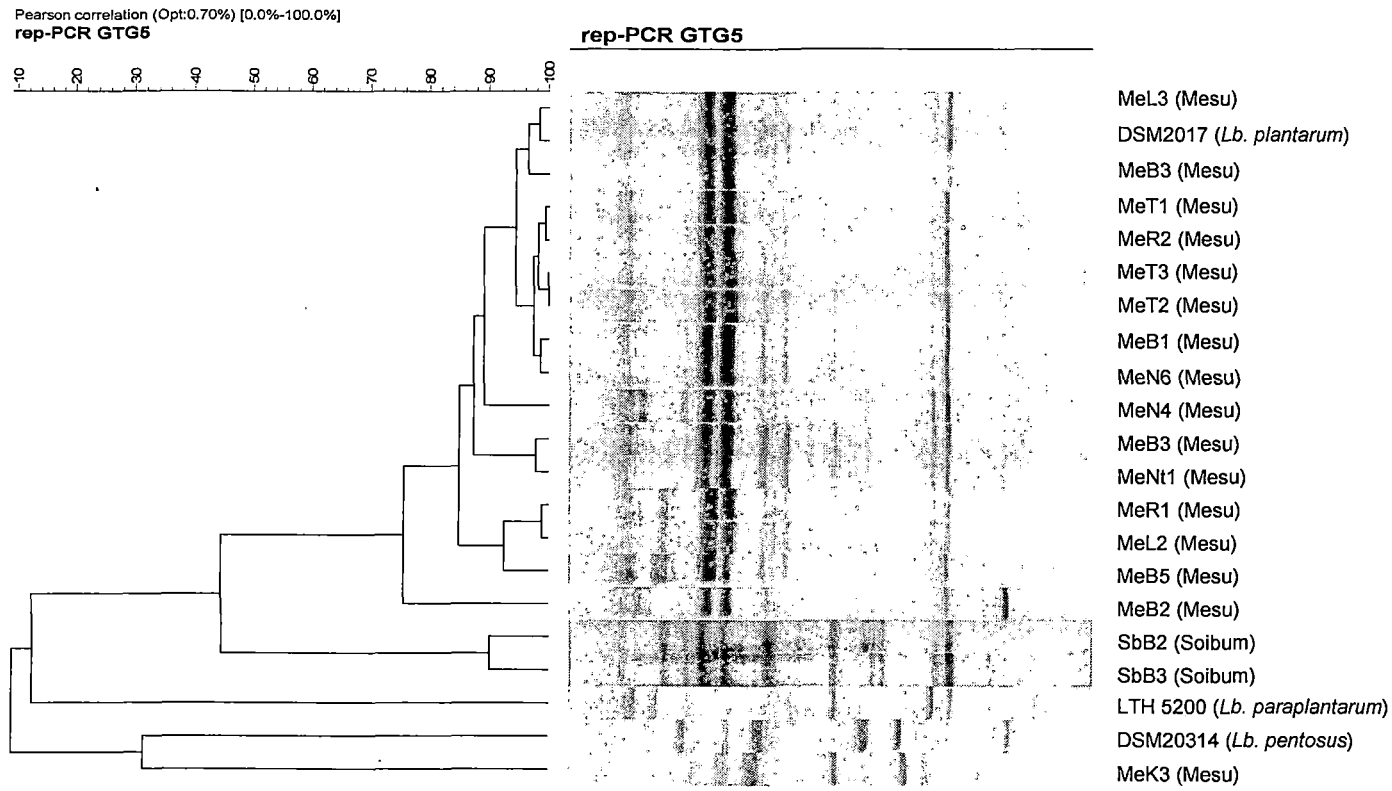


Fig 16. Dendrogram obtained from rep-PCR $(GTG)_5$ fingerprints of strains of DAP-positive homofermentative (isolated from mesu and soibum) *Lactobacillus plantarum* group. Patterns were grouped with the unweighted pair group algorithm with arithmetric averages (UPGMA).

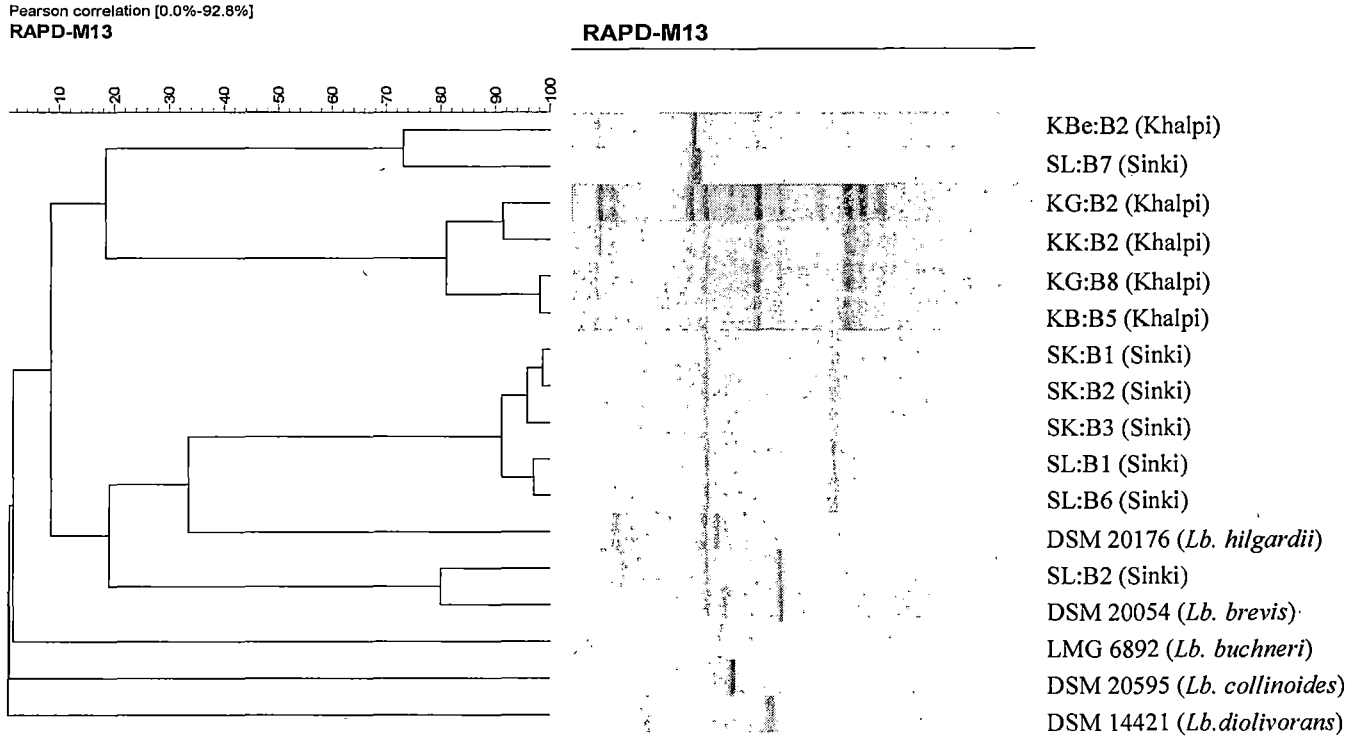


Fig 17. Dendrogram obtained by using RAPD patterns for heterofermentative lactobacilli generated from M13 primer. Patterns were grouped with the unweighted pair group algorithm with arithmetric averages (UPGMA).

Prevalence of LAB

The most dominant LAB in goyang were *Lactobacillus* represented by 59.5 % followed by *Enterococcus* (18.9 %), *Lactococcus* (10.8 %) and *Pediococcus* (10.8 %) (Fig 18). *P. pentosaceus* was the predominant LAB (76.8 %) in gundruk whereas *Lb. plantarum* was represented by 23.2 % (Table 15a). In inziangsang 73.7 % of the LAB were lactobacilli and 26.3 % were pediococci. *Lactobacillus* dominated the microflora in khalpi represented by 95.5 % (out of which, homofermentative rods were 67.9 % and heterofermentative rods 27.7 %), while *Leuconostoc* was represented by just 4.5 %. In sinki, out of 85.7 % lactobacilli, heterofermentatives *Lb. brevis* comprised 62.5 % and homofermentatives *Lb. plantarum* and *Lb. casei* comprised 23.2 %, while 14.3 % were represented by *Leuconostoc* (Fig 18).

About 90.7 % of LAB was represented by lactobacilli in ekung, rest 9.3 % was *Tetragenococcus* (Table 15b). About 87.5 % homofermentative *Lb. plantarum* and 12.5 % heterofermentative *Lb. fermentum* were present in eup (Fig 19). In hirring, the homofermentative *Lactobacillus* dominated the lactic flora (91.1 %), followed by *Lactococcus* (8.9 %). Heterofermentative lactobacilli were not found in hirring. In mesu, lactobacilli were represented by 70.6 % (out of which, homofermentative *Lb. plantarum* and *Lb. curvatus* were 41.2 % and heterofermentative *Lb. brevis* was 29.4 %). Sizeable amount of *Leuconostoc* (11.8 %) and *Pediococcus* (17.6 %) were also present in mesu. In fermented bamboo

shoots of Manipur (soibum, soidon and soijim) *Leuconostoc* was dominant followed by other lactics (Table 5b). *Lactobacillus* was the most dominant lactic flora represented by 65.4 % out of 547 LAB strains isolated from traditional fermented vegetable and bamboo shoots of North East India (Fig 19). Among lactobacilli, homofermentative lactobacilli were represented by 45.4 %, followed by heterofermentative lactobacilli representing 20 %, leuconostoc 16.3%, pediococci 12.7%, lactococci 1.7% and *Tetragenococcus* 0.7 % (Table 15a, b & Fig 20).

Table 15a. Prevalence of functional LAB in fermented vegetable products

Product[a]	Functional LAB	% of Prevalence							
		Lactobcillus							
		Homoferment.	*Heteroferment.*	Total lactobacilli	*Lactococcus*	*Leuconostoc*	*Enterococcus*	*Pediococcus*	*Tetragenococcus*
Goyang (37)	*Lb. plantarum, Lb. brevis, Lc. lactis, E. faecium, P. pentosaceus*	51.4	8.1	59.5	10.8	0	18.9	10.8	0
Gundruk (95)	*Lb. plantarum, P. pentosaceus*	23.2	0	23.2	0	0	0	76.8	0
Inziang-sang (19)	*Lb. plantarum, Lb. brevis, P. acidilactici*	52.6	21.1	73.7	0	0	0	26.3	0
Khalpi (112)	*Lb. plantarum, Lb. brevis, Leuc. fallax*	67.9	27.7	95.5	0	4.5	0	0	0
Sinki (56)	*Lb. plantarum, Lb. brevis, Lb. casei, Leuc. fallax*	23.2	62.5	85.7	0	14.3	0	0	0
Total		43.6	23.9	67.5	2.2	3.8	3.8	22.8	0

[a]Total number of LAB isolates of each products are given in parenthesis.

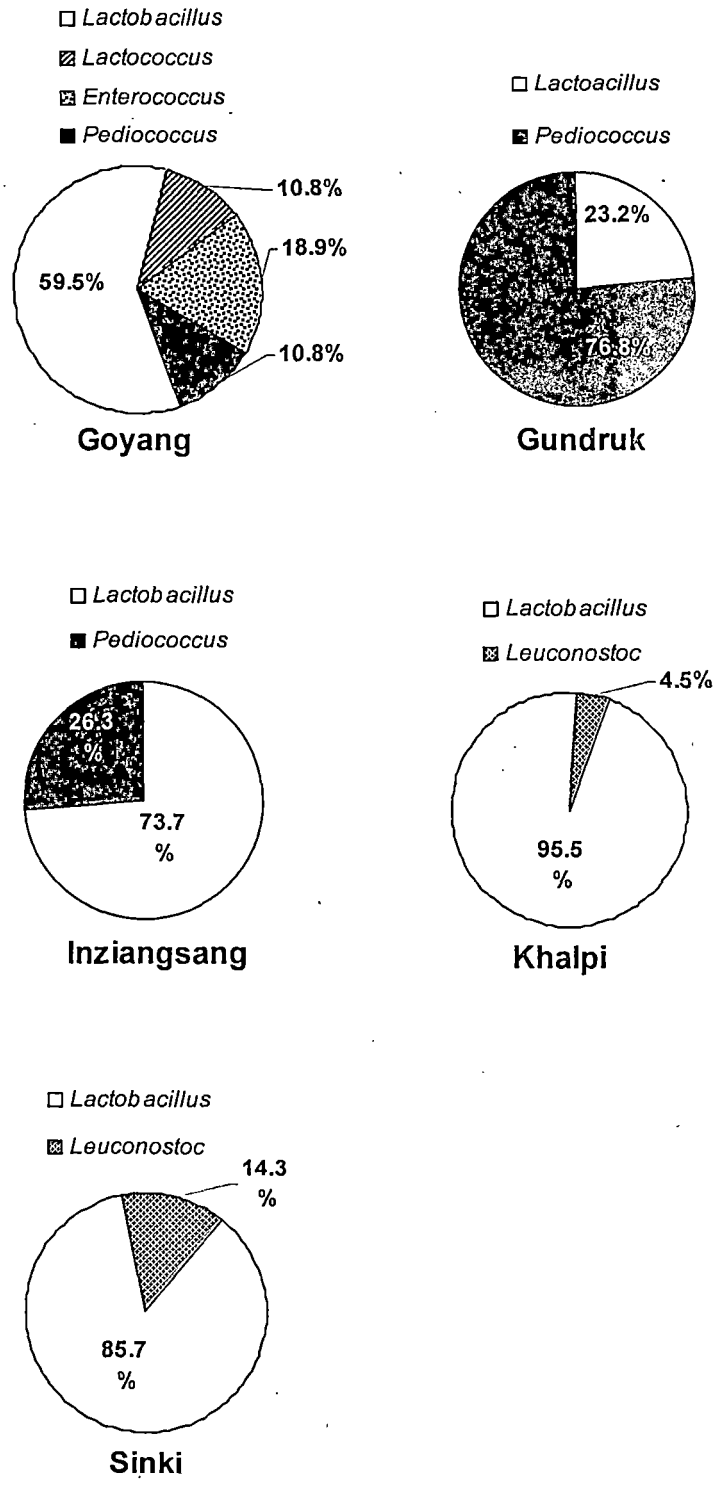


Fig 18. Graphic representation of prevalence of LAB in fermented vegetable products of North East India.

Table 15b. Prevalence of functional LAB in fermented bamboo shoot products

Product[a]	Functional LAB	% of Prevalence							
		Lactobacillus							
		Homofermentative	*Heterofermentative*	Total lactobacilli	*Lactococcus*	*Leuconostoc*	*Enterococcus*	*Pediococcus*	*Tetragenococcus*
Ekung (43)	*Lb. plantarum, Lb. brevis, Lb. casei, T. halophilus*	83.7	7.0	90.7	0	0	0	0	9.3
Eup (40)	*Lb. plantarum, Lb. fermentum*	87.5	12.5	100	0	0	0	0	0
Hirring (45)	*Lb. plantarum, Lc. lactis*	91.1	0	91.1	8.9	0	0	0	0
Mesu (51)	*Lb. plantarum, Lb. brevis, Lb. curvatus, P. pentosaceus, Leuc. citreum*	41.2	29.4	70.6	0	11.8	0	17.6	0
Soibum (15)	*Lb. plantarum, Lb. brevis, Leuc. lactis, Leuc. fallax, Leuc. mesenteroides, E. durans*	26.7	0	26.7	0	53.3	20	0	0
Soidon (20)	*Lb. brevis, Leuc. fallax, Leuc. lactis*	0	35.0	35.0	0	65.0	0	0	0
Soijim (14)	*Lb. brevis, Leuc. fallax, Leuc. lactis*	0	28.0	28.0	0	71.4	0	0	0
Total		47.2	16.0	63.2	1.2	28.8	2.9	2.5	1.3

[a]Total number of LAB isolates of each product is given in parenthesis.

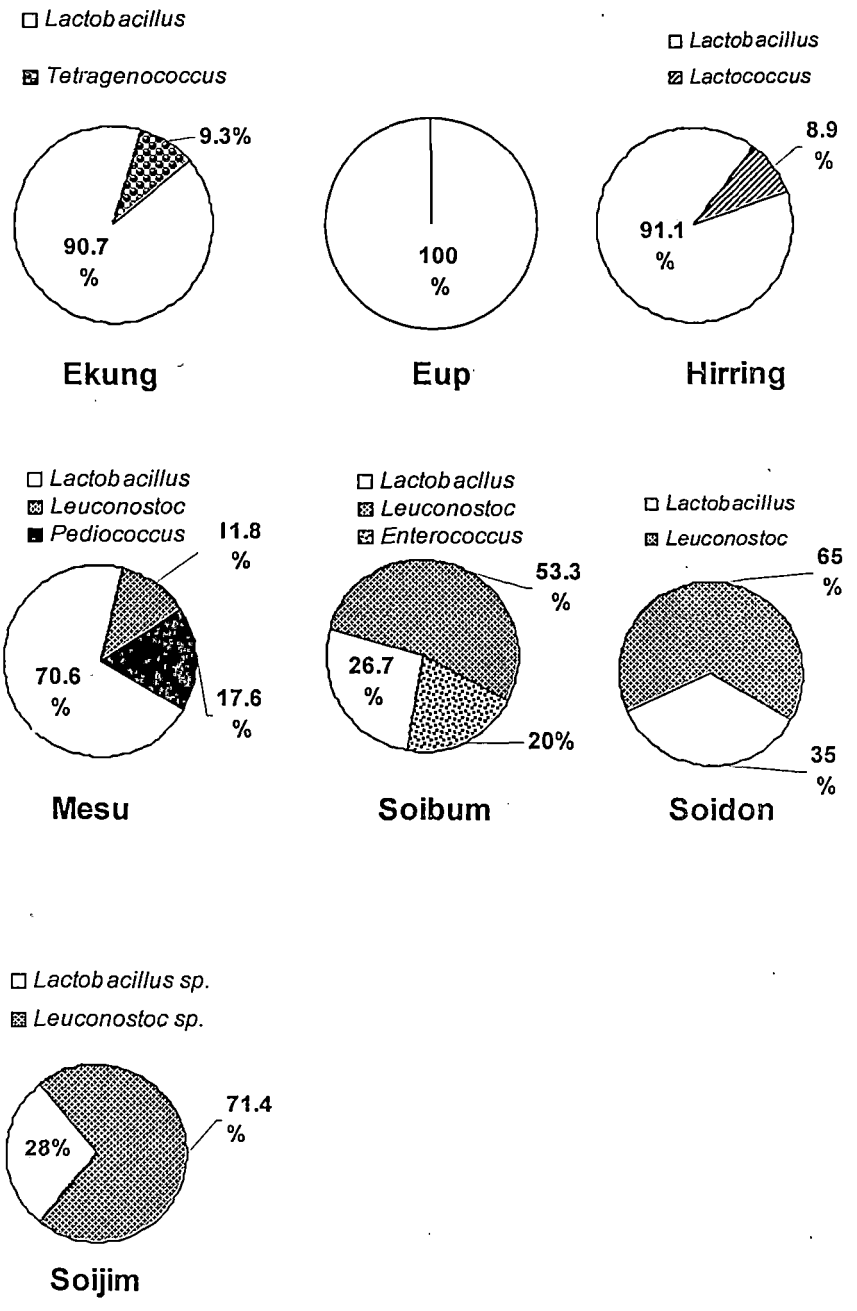


Fig19. Graphic representation of prevalence of LAB in fermented tender bamboo shoot products of North East India.

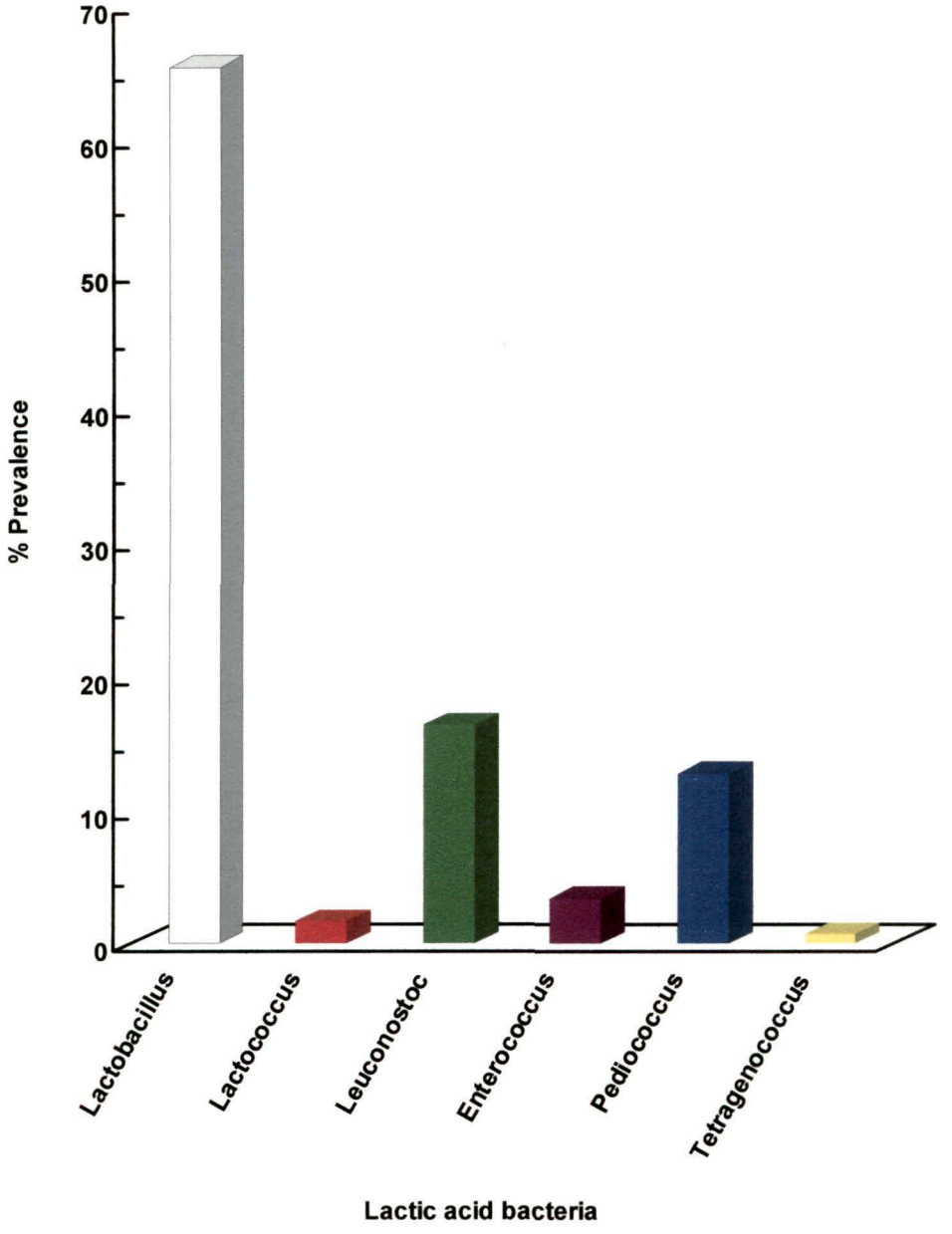


Fig 20. Graphic representation of prevalence of LAB in all 12 samples of fermented vegetable products from North East India

Pathogenic contaminants

Finish products of goyang, gundruk, inziangsang, khalpi, sinki, ekung, eup, hirring, mesu, soibum, soidon and soijim were tested for presence of bacterial contaminants such as *Bacillus cereus, Staphylococcus aureus,* enterobacteriaceae and some pathogenic bacteria such as *Listeria* sp., *Salmonella* sp. and *Shigella* sp. using selective media (Table 16). In sinki, only *Bacillus cereus* and *Staphylococcus aureus* were detected. While *Staphylococcus aureus* and enterobacteriaceae were detected in few samples of goyang and market gundruk (Table 16). In few samples, counts of *Bacillus cereus*, *Staphylococcus aureus* and enterobacteriaceae were less than 10^2 cfu/g, respectively. No bacterial contaminants were detected in any sample of inziangsang, khalpi, ekung, eup, mesu, hirring, soibum, soidon and soijim. Food borne pathogens *Listeria* sp., *Salmonella* sp. and *Shigella* sp. were not detected in any sample of fermented vegetable and bamboo shoot products of North East India.

Table 16. Microbial load of pathogenic bacteria in fermented vegetables and bamboo shoot products collected from different places of North East India

Product	Log cfu/g sample					
	Bacillus cereus	Enterobacteriaceae	*Listeria* sp.	*Salmonella* sp.	*Shigella* sp.	*Staphylococcus aureus*
Goyang (n = 20)	0	2.0 ± 0.6	0	0	0	1.3 ± 0.9
Gundruk (n = 25)	0	2.2 ± 0.1	0	0	0	2.0 ± 0.1
Inziangsang (n = 11)	0	0	0	0	0	0
Khalpi (n = 25)	0	0	0	0	0	0
Sinki (n = 21)	1.2 ± 0.1	0	0	0	0	2.3 ± 0.1
Ekung (n = 16)	0	0	0	0	0	0
Eup (n = 13)	0	0	0	0	0	0
Hirring (n = 15)	0	0	0	0	0	0
Mesu (n = 46)	0	0	0	0	0	0
Soibum (n = 12)	0	0	0	0	0	0
Soidon (n = 11)	0	0	0	0	0	0
Soijim (n = 8)	0	0	0	0	0	0

n, number of samples collected.

Data represents the means (± SD) of number of samples.

Yeasts

Though the dominant microflora in all 12 fermented vegetable and bamboo products was LAB, a sizable number of yeasts were also recovered in some finish products. Yeasts were detected in goyang and in few samples of sinki, khalpi, ekung, hirring and mesu in numbers ranging between 10^4 and 10^6 cfu/g. Yeasts were not detected in gundruk, inziangsang, eup, soibum, soidon and soijim. Of the 43 strains of yeasts, 8 strains were isolated from goyang, 14 from khalpi, 7 from sinki, 3 each from ekung and hirring and 8 strains from mesu. All yeast strains were phenotypically characterized including sugar fermentation and assimilation tests (Table 17). Based on the taxonomic keys of Kurtzman and Fell (1998) and Yarrow (1998), yeasts strains were identified as species of *Candida, Pichia, Saccharomyces* and *Rhodotorula* (Tables 17). Identification to species level was not confirmed.

Table 17. Phenotypic characteristics of yeast from fermented vegetable products

Parameters	Genera			
	Candida	*Pichia*	*Rhodotorula*	*Saccharomyces*
No of strains	14	9	4	16
Origin of strains*	Go (8); K (6)	K (4); S (3); Me (2)	S (2); Me (2)	K (4); S (2); Me (4); Ek (3); Hr(3)
Cell morphology	Oval to cylindrical	Oval to cylindrical	Oval	Oval to cylindrical
Cell size (µm)	l = 11.0 (5.4 – 21.2)	l = 8.4 (4.5 – 12.3)	l = 4.2 (2.4 – 6.2)	l = 10.2 (6.4 – 15.6)
	b = 4.0 (3.1 – 6.8)	b = 4.5 (3.0 – 5.6)	b = 3.8 (3.0 – 5.8)	b = 6.0 (3.2 – 8.3)
Mycelium	Pseudo	Pseudo	Pseudo	Pseudo
Ascus and ascospores	Globose	Spherical	Globose	Globose
Nitrate reduction	–	2/7	2/2	–
Growth at 37^0 C	–	2/7	–	4/12
Sugars fermented:				
Glucose	10/4	2/7	–	+
Galactose	9/5	1/8	–	+
Lactose	–	–	–	+
Maltose	–	–	–	+
Raffinose	–	–	–	+
Sucrose	7/7	–	–	–
Starch	–	–	–	–
Trehalose	–	–	–	–
Sugars assimilated:				
Arabinose	6/8	+	+	14/2
Cellobiose	–	5/4	2/2	–
Galactose	+	8/1	+	+
Glycerol	+	+	+	10/6
Inositol	–	2/7	1/3	–
Lactose	–	5/4	3/1	–
Maltose	1/13	5/4	3/1	10/6
Melibiose	8/6	6/3	1/3	10/6
Mannitol	+	6/3	3/1	9/7
Rhamnose	2/12	–	–	4/12
Raffinose	9/5	7/2	3/1	9/7
Sucrose	+	+	+	–
Starch	1/13	5/4	3/1	4/12
Trehalose	2/12	5/4	2/2	5/11
xylose	+	7/2	3/1	–

*Origin of strains: Go, goyang; K, khalpi; S, sinki, Me, mesu. Number of strains isolated from the respective product are given in parenthesis;

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

l, length; b, breadth.

Technological properties of LAB strains

Acidification

Effect of acidification and coagulation by the LAB strains isolated from fermented vegetables and bamboo shoots were tested (Table 18 and 19). *Lb. plantarum* Ek6 (ekung) showed the lowest acidification value of pH 3.9 among all the tested strains of LAB, followed by *Lb. plantarum* Go25 (goyang), *Lb. plantarum* strains GLn:R5, GLn:R6 (gundruk); *Lb. plantarum* strains KG:B1, KK:B3, KS:B9, KBe:B2 (khalpi), *Lb. plantarum* SG:B1 (sinki), *Lb. plantarum* Ek7 (ekung), *Lb. plantarum* Ep103 (Eup) dropping the pH to 4.0.

About 76 % of LAB strains caused coagulation of milk at 30° C with a significant drop in pH, as shown in Table 18 and 19. About 91 % *Lb. plantarum*, 58 % of *Lb. brevis,* 38 % of *Leuconostoc* sp. and 80 % of *Lactococcus lactis* and all strains of *Enterococcus faecium* coagulated skim milk. The coagulation time ranged from 19 to 44 hour at 30° C. The fastest coagulation time of 19-24 hour was observed in several strains of LAB such as *Lc. lactis* Go8 (goyang), *Lb. plantarum* strains GLn:R1, GS:R1 (gundruk), *Lb. plantarum* strains IB2, IB3 (inziangsang), *Lactobacillus plantarum* KG:B1 (khalpi), *Lb. brevis* KG:B2 (khalpi), *Lb. plantarum* strains Ek4, Ek6 (ekung), *Lb. plantarum* Ep81 (eup), *Lb. plantarum* strains Hr41, Hr42 (hirring), *Lc. lactis* Hr56 (hirring), *Lb. plantarum* strains MeL2, MeR1, MeB3 and MeL3 (mesu) and *Leuc. lactis* SbB1 (soibum), *P. pentosaceus* GLn:R2 and GLn:R3 (gundruk).

Table 18. Effect of LAB strains from fermented vegetables on acidification and coagulation

Product	Strains	30° C	
		Acidification (pH)	Coagulation
	Skim milk powder (Control)	6.8	–
Goyang	*P. pentosaceus* Go1	5.1	+
	Lc. lactis Go5	4.6	+
	Lb. plantarum Go6	4.5	+
	Lc. lactis Go7	5.4	+
	Lc. lactis Go8	5.6	–
	E. faecium Go9	4.9	+
	Lb. brevis Go11	6.0	+
	Lb. plantarum Go12	5.0	+
	Lb. plantarum Go14	5.0	+
	Lb. plantarum Go16	4.5	+
	P. pentosaceus Go17	4.9	+
	E. faecium Go20	4.9	+
	Lb. plantarum Go22	4.8	+
	Lb. plantarum Go23	4.1	+
	Lb. brevis Go24	4.2	+
	Lb. plantarum Go25	4.0	+
	P. pentosaceus Go29	4.7	+
	Lb. plantarum Go30	4.2	+
	Lb. plantarum Go31	4.2	+
	E. faecium Go34	4.8	+
	Lb. plantarum Go37	4.6	+
	Lb. plantarum Go39	4.3	+
	Lb. plantarum Go40	4.3	+
	Lb. brevis Go41	5.0	+
	E. faecium Go43	4.8	+
	E. faecium Go45	4.9	+

Continued (Table 18)

Product	Strains	30° C	
		Acidification (pH)	Coagulation
	Lb. plantarum GK:C1	5.7	–
	P. pentosaceus GK:C2	5.4	+
	P. pentosaceus GK:B3	5.5	–
	P. pentosaceus GK:B6	5.7	–
	P. pentosaceus GK:C3	5.0	+
	Lb. plantarum GLn:R1	4.3	+
	Lb. plantarum GLn:R5	4.0	+
	Lb. plantarum GLn:R6	4.0	+
	P. pentosaceus GLn:C1	5.8	–
	Lb. plantarum GLn:C2	5.8	–
	P. pentosaceus GLn:C3	5.7	–
	P. pentosaceus GLn:C5	5.6	+
	P. pentosaceus GLn:R2	4.1	+
Gundruk	*P. pentosaceus* GLn:R3	4.7	+
	Lb. plantarum GSd:R1	6.1	–
	Lb. plantarum GSd:R4	4.6	+
	Lb. plantarum GSd:R5	4.4	+
	P. pentosaceus GSd:C1	5.6	–
	P. pentosaceus GSd:C2	5.6	+
	Lb. plantarum GS:R1	4.4	+
	P. pentosaceus GS:B1	4.9	+
	P. pentosaceus GS:B2	4.9	+
	P. pentosaceus GS:B7	4.9	+
	P. pentosaceus GS:C1	5.6	–
	Lb. plantarum GLc:B1	4.9	+
	P. pentosaceus GLc:C1	5.5	–
	P. pentosaceus GLc:C4	5.0	+

Continued (Table 18)

Product	Strains	30° C	
		Acidification (pH)	Coagulation
	Lb. brevis IB1	5.6	–
	Lb. plantarum IB2	4.4	+
	Lb. plantarum IB3	4.8	+
	P. acidilactici IB5	6.0	–
Inziangsang	*Lb. brevis* IB9	4.5	+
	Lb. plantarum IB11	4.2	+
	Lb. plantarum IB12	4.7	+
	Lb. plantarum IB13	4.6	+
	P. acidilactici IB14	5.7	+
	Lb. plantarum KG:B1	4.0	+
	Lb. brevis KG:B2	4.7	+
	Lb. plantarum KG:B3	6.1	–
	Lb. plantarum KG:B7	4.2	+
	Lb. brevis KG:B8	4.2	+
	Lb. plantarum KG:B9	6.1	–
	Lb. brevis KG:R1	5.3	+
	Lb. brevis KG:R2	5.0	+
	Lb. plantarum KB:B4	4.2	+
	Lb. brevis KB:B5	4.0	+
Khalpi	*Leuc. fallax* KB:C1	4.7	+
	Lb. plantarum KB:B9′	4.8	+
	Lb. brevis KK:B2	6.1	–
	Lb. plantarum KK:B3	4.0	+
	Lb. plantarum KK:B4	4.9	+
	Lb. plantarum KK:B5	4.6	+
	Lb. brevis KK:R1	4.9	+
	Lb. brevis KS:R1	5.3	+
	Lb. brevis KS:R2	5.7	+
	Lb. brevis KS:B1	4.9	–
	Lb. plantarum KS:B2	6.2	+
	Lb. plantarum KS:B9	4.0	+

Continued (Table 18)

Product	Strains	30° C	
		Acidification (pH)	Coagulation
Khalpi	*Leuc. fallax* KS:C1	5.6	+
	Leuc. fallax KS:C2	5.6	–
	Lb. brevis KBe:B2	4.0	+
Sinki	*Lb. brevis* SL:B1	5.9	–
	Lb. brevis SL:B2	4.4	+
	Leuc. fallax SL:B3	6.0	–
	Leuc. fallax SL:B4	6.0	–
	Leuc. fallax SL:B5	6.0	–
	Lb. brevis SL:B6	5.9	–
	Lb. brevis SL:B7	5.8	–
	Lb. brevis SL:B8	6.0	–
	Lb. brevis SK:B1	6.0	–
	Lb. brevis SK:B2	5.9	–
	Lb. brevis SK:B3	5.8	–
	Leuc. fallax SK:C1	5.5	–
	Lb. plantarum SBe:B1	5.1	+
	Lb. brevis SBe:B2	5.5	–
	Lb. brevis SBe:B5	4.9	+
	Lb. brevis SBe:B6	5.0	+
	Lb. brevis SBe:B7	5.2	+
	Lb. brevis SS:B1	4.7	–
	Lb. brevis SS:B2	4.8	+
	Lb. casei SS:B4	5.2	+
	Lb. brevis SS:B5	5.6	+
	Lb. brevis SS:B6	5.6	–
	Lb. brevis SS:B7	5.5	+
	Lb. plantarum SG:B1	4.0	+
	Lb. plantarum SG:B4	4.2	+
	Lb. plantarum SG:B5	4.4	+
	Lb. plantarum SG:B7	4.2	+

Data represents the mean value of 3 sets of experiment.
Coagulation occurred between 19-44 h.
+, indicates coagulation; -, indicates absence of coagulation.

Table 19. Effect of LAB strains isolated from fermented bamboo shoot products on acidification and coagulation

Product	Strains	30°C	
		Acidification (pH)	Coagulation
Ekung	*Lb. plantarum* Ek1	4.2	+
	Lb. plantarum Ek4	4.2	+
	Lb. plantarum Ek5	4.4	+
	Lb. plantarum Ek6	3.9	+
	Lb. plantarum Ek7	4.0	+
	Lb. brevis Ek8	4.2	+
	Lb. plantarum Ek10	5.5	+
	Lb. plantarum Ek11	4.8	+
	Lb. plantarum Ek12	4.4	+
	Lb. plantarum Ek15	4.5	+
	Lb. plantarum Ek18	4.2	+
	Lb. plantarum Ek19	4.4	+
	Lb. plantarum Ek20	4.3	+
	Lb. plantarum Ek21	4.2	+
	T. halophilus Ek 22	4.4	+
	Lb. plantarum Ek23	5.9	+
	Lb. plantarum Ek24	4.5	+
	Lb. plantarum Ek25	4.4	+
	T. halophilus Ek28	4.7	+
	Lb. plantarum Ek29	4.3	+
	Lb. casei Ek34	5.9	+
	Lb. casei Ek35	5.7	+
	Lb. plantarum Ek38	5.1	–
	Lb. plantarum Ek39	4.6	+
	Lb. plantarum Ek40	4.6	+
Eup	*Lb. plantarum* Ep81	4.1	+
	Lb. plantarum Ep82	4.2	+
	Lb. plantarum Ep84	5.6	–

Continued (Table 19)

Product	Strains	30° C	
		Acidification (pH)	Coagulation
Eup	*Lb. fermentum* Ep85	4.6	+
	Lb. plantarum Ep86	4.6	+
	Lb. plantarum Ep87	4.1	+
	Lb. plantarum Ep89	4.1	+
	Lb. plantarum Ep90	4.1	+
	Lb. plantarum Ep91	5.8	–
	Lb. fermentum Ep92	4.7	+
	Lb. fermentum Ep93	5.7	+
	Lb. plantarum Ep94	5.8	–
	Lb. plantarum Ep95	4.6	+
	Lb. plantarum Ep96	4.4	+
	Lb. plantarum Ep97	4.4	+
	Lb. plantarum Ep98	4.1	+
	Lb. plantarum Ep99	4.3	+
	Lb. plantarum Ep101	4.2	+
	Lb. plantarum Ep102	4.2	+
	Lb. plantarum Ep103	4.0	+
	Lb. plantarum Ep106	4.4	+
	Lb. plantarum Ep110	4.6	+
	Lb. plantarum Ep111	4.3	+
	Lb. plantarum Ep112	4.3	+
	Lb. plantarum Ep115	4.3	+
Hirring	*Lb. plantarum* Hr41	4.2	+
	Lb. plantarum Hr42	4.4	+
	Lb. plantarum Hr45	4.2	+
	Lb. plantarum Hr47	4.3	+
	Lb. plantarum Hr48	4.2	+
	Lb. plantarum Hr49	4.6	+
	Lb. plantarum Hr50	4.6	+
	Lb. plantarum Hr55	4.4	+

Continued (Table 19)

Product	Strains	30° C	
		Acidification (pH)	Coagulation
	Lc. lactis Hr56	5.1	+
	Lb. plantarum Hr57	5.0	+
	Lb. plantarum Hr58	4.8	+
	Lb. plantarum Hr59	4.1	+
	Lb. plantarum Hr60	4.5	+
	Lc. lactis Hr62	5.6	+
	Lb. plantarum Hr64	4.6	+
	Lb. plantarum Hr65	4.2	+
	Lb. plantarum Hr66	4.4	+
	Lb. plantarum Hr67	4.4	+
	Lb. plantarum Hr68	4.3	+
	Lb. plantarum Hr70	4.6	+
	Lb. plantarum Hr71	4.5	+
	Lb. plantarum Hr74	4.6	+
	Lb. plantarum Hr75	4.7	+
	Lb. plantarum Hr76	4.3	+
	Lb. plantarum Hr77	4.8	–
	Lb. plantarum Hr80	4.6	+
Mesu	*Lb. plantarum* MeL2	4.2	+
	Lb. brevis MeB4	6.0	–
	Lb. plantarum MeR1	4.3	+
	Lb. plantarum MeB3	4.7	+
	Lb. brevis MeR6	6.1	–
	Lb. brevis MeN3	6.1	–
	Lb. brevis MeTR	6.1	–
	Lb. brevis MeN7	6.1	–
	Lb. brevis MeN5	5.8	–
	Lb. plantarum MeL3	4.8	+

Continued (Table 19)

Product	Strains	30° C	
		Acidification (pH)	Coagulation
Mesu	*Lb. plantarum* MeBe3	4.4	+
	Lb. brevis MeN2	4.9	+
	Lb. plantarum MeNt1	4.4	+
	Lb. plantarum MeB1	4.4	+
	Lb. plantarum MeB2	4.5	+
	Lb. brevis MeNt2	4.8	+
	Lb. plantarum MeB5	4.4	+
	Lb. brevis MeNt3	5.5	–
	Lb. plantarum MeN4	5.6	+
	Lb. plantarum MeN6	4.5	+
	Lb. plantarum MeR2	4.3	–
	Lb. plantarum MeT1	4.7	+
	Lb. plantarum MeT2	5.0	+
	Lb. plantarum MeT3	5.1	+
	Leuc. citreum MeN8	6.1	+
	Leuc. citreum MeN9	6.1	–
	Leuc. citreum MeK1	6.0	+
	Leuc. citreum MeK2	6.0	–
	Lb. brevis MeR7	5.6	+
	Lb. curvatus MeK3	5.5	+
	P. pentosaceus MeB:C3	6.0	–
	P. pentosaceus MeB:C4	5.9	–
	P. pentosaceus MeT5	5.5	+
	P. pentosaceus MeT6	5.0	+
	P. pentosaceus MeT7	5.1	+
Soibum	*Leuc. lactis* SbB1	5.2	+
	Leuc. fallax SbC1	6.1	–
	Leuc. mesenteroides SbC2	5.5	–
	Lb. plantarum SbB2	4.7	+
	Lb. plantarum SbB3	4.4	+

Continued (Table 19)

Product	Strains	30° C	
		Acidification (pH)	Coagulation
Soibum	*E. durans* SbB4	5.6	+
	Lb. brevis SbB5	**5.5**	+
	Lb. brevis SbB6	5.6	+
Soidon	*Leuc. fallax* SdC1	6.2	–
	Leuc. lactis SdR2	**6.1**	–
	Lb. brevis SdR3	**5.6**	–
	Lb. brevis SdR4	5.3	+
	Lb. brevis SdR5	**5.4**	+
	Leuc. lactis SdB1	5.4	+
	Leuc. lactis SdB2	5.6	+
	Leuc. lactis SdB3	5.7	–
	Lb. brevis SdB4	5.6	+
	Leuc. lactis SdB5	5.0	+
Soijim	*Leuc. fallax* SjC1	6.1	–
	Leuc. lactis SjR3	**5.4**	–
	Lb. brevis SjR7	**6.1**	–
	Lb. brevis SjB1	**5.1**	+
	Leuc. fallax SjB2	5.7	–
	Leuc. lactis SjB4	5.7	–
	Leuc. lactis SjB5	4.9	+
	Leuc. lactis SjB8	5.2	+

Data represents the mean value of 3 sets of experiment.

Coagulation occurred between 19-44 hour.

+, indicates coagulation; -, indicates absence of coagulation.

Antimicrobial activities

Tables 20 and 21 showed the antagonistic activities of LAB strains, isolated from fermented vegetable and bamboo shoot products against different indicator strains used such as *Listeria innocua* DSM 20649, *Listeria 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, *Enterobacter agglomerans* BFE 154 and *Pseudomonas aeruginosa* BFE 162. Most of the LAB strains showed the clear inhibition zones in agar-spot plates against the used indicator strains, showing antagonisms. However, only 71 strains of LAB out of total 251 strains of LAB tested for antagonism, showed the clear inhibition zones measurements by scale of more than 4 mm in agar-spot plates.

The LAB strains showing the inhibition zones of >4 mm in agar-spot plates were *Lb. plantarum* Go12, Go14, Go22, Go40 (goyang), *Lb. plantarum* Gk:C1, GLn:R1, GLn:C2, GSd:R1, GS:R1, GLc:B1 (gundruk), *Lb. plantarum* IB2, IB3 (inziangsang), *Lb. plantarum* KG:B1, KG:B3, KG:B7, KG:B9, KB:B4, KS:B2 (khalpi). *Lb. plantarum* Ek1, Ek4, Ek5, Ek7, Ek12, Ek15, Ek20, Ek21, Ek24, Ek25 (ekung), *Lb. plantarum* Ep84, Ep86, Ep101, Ep106 (eup), *Lb. plantarum* Hr42, Hr47 (hirring), *Lb. plantarum* MeL2, MeR1, MeB3, MeL3 (mesu), *P. pentosaceus* Go29 (goyang), *P. pentosaceus* GK:C2, GLn:C1, GLn:C3, GLn:R2, GSd:C1, GLn:R3,

GS:C1, GLc:C1 (gundruk), *P. pentosaceus* MeB:C3, MeB:C4 (mesu), *P. acidilactici* IB5 (inziangsang), *Lb. brevis* IB1 (inziangsang), *Lb. brevis* KG:B8, KBe:B2 (khalpi), *Lb. brevis* SL:B1, SL:B2, SL:B6 (sinki), *Lb. brevis* MeB4, MeN7, MeN5 (mesu), *Lb. brevis* SdR3 (soidon), *Lb. brevis* SjR7 (soijim), *E. faecium* Go34 (goyang), *Leuc. fallax* SL:B3, SL:B4 (sinki), *Leuc. fallax* SbC1 (soibum), *Leuc. fallax* SdC1 (soidon), *Leuc. fallax* SjC1 (soijim), *Leuc. lactis* SbB1 (soibum), *Leuc. lactis* SdR2 (soidon), *Leuc. lactis* SjR3 (soijim) and *Leuc. mesenteroides* SbC2 (soibum) (Table 20 and 21). None of LAB species from sinki showed >4 mm clear zones of inhibition in agar-spot plates, though few strains (Table 20) did show inhibition zones against indicator strains.

Table 20. Antimicrobial activity of the LAB strains from fermented vegetable products

Strains	Indicator strains									
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*E. faecium* DSM 20477	*Strep. mutans* DSM 6178	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. cloacae* BFE 282	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Goyang										
Go1	–	–	–	–	–	–	–	–	–	–
Go5	–	–	–	–	–	–	–	–	–	–
Go6	–	–	–	–	–	–	–	–	–	–
Go7	–	–	–	–	–	–	–	–	–	–
Go8	–	–	–	–	–	–	–	–	–	–
Go9	–	–	–	–	–	–	–	–	–	–
Go11	–	–	–	–	–	–	–	–	–	–
Go12	++	–	–	–	–	–	–	–	–	–
Go14	+++	–	–	–	–	–	–	–	–	–
Go16	–	–	–	–	–	–	–	–	–	–
Go17	–	–	–	–	–	–	–	–	–	–
Go20	–	–	–	–	–	–	–	–	–	–
Go22	++	–	–	–	–	–	–	–	–	–
Go23	–	–	–	–	–	–	–	–	–	–
Go24	–	–	–	–	–	–	–	–	–	–
Go25	–	–	–	–	–	–	–	–	–	–
Go29	++	–	–	–	–	–	–	–	–	–
Go30	–	–	–	–	–	–	–	–	–	–
Go31	–	–	–	–	–	–	–	–	–	–

Continued (Table 20)

Strains	Indicator strains									
	***L. innocua* DSM 20649**	***L. monocytogenes* DSM 20600**	***B. cereus* CCM 2010**	***S. aureus* S1**	***E. faecium* DSM 20477**	***Strep. mutans* DSM 6178**	***K. pneumoniae* subsp. *pneumoniae* BFE 147**	***Ent. cloacae* BFE 282**	***Ent. agglomerans* BFE 154**	***Pseud. aeruginosa* BFE 162**
Goyang										
Go34	++	–	–	–	–	–	–	–	–	–
Go37	–	–	–	–	–	–	–	–	–	–
Go39	–	–	–	–	–	–	–	–	–	–
Go40	++	–	–	–	–	–	–	–	–	–
Go41	–	–	–	–	–	–	–	–	–	–
Go43	–	–	–	–	–	–	–	–	–	–
Go45	–	–	–	–	–	–	–	–	–	–
Gundruk										
GK:C1	+	–	+	++	–	–	+	–	–	–
GK:C2	+	–	+	++	–	–	+	–	–	–
GK:B3	+	–	+	–	–	–	+	–	–	–
GK:B6	+	–	+	+	–	–	+	–	–	–
GK:C3	+	–	+	+	–	+	+	–	–	–
GLn:R1	+	–	+++	++	–	+	+	–	+++	–
GLn:R5	–	–	–	–	–	–	–	–	–	–
GLn:R6	–	–	–	–	–	–	–	–	–	–

Continued (Table 20)

Strains	Indicator strains									
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*E. faecium* DSM 20477	*Strep. mutans* DSM 6178	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. cloacae* BFE 282	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Gundruk										
GLn:C1	+	+	+	++	–	++	+	–	+++	–
GLn:C2	+	+	–	++	–	+	+	++	–	–
GLn:C3	+	–	+	++	+	+	+	++	–	–
GLn:C5	+	–	+	–	+	+	+	–	–	–
GLn:R2	+	–	+	+	+	+++	+	+	++	–
GLn:R3	+	+	+	++	+	+++	+	+	++	+
GSd:R1	–	+	+	++	+	–	–	+	+	+
GSd:R4	–	+	+	–	–	–	–	–	–	–
GSd:R5	–	+	+	–	–	–	–	–	–	–
GSd:C1	++	+	+	++	+	–	++	+	+	+
GSd:C2	–	+	+	–	–	–	–	–	–	–
GS:R1	+	–	+	+	+	++	+	–	++	+
GS:B1	+	–	+	+	+	+	+	–	+	+
GS:B2	+	–	+	+	+	+	+	–	+	+
GS:B7	+	–	+	+	+	+	+	–	+	+
GS:C1	++	–	+	++	–	+	++	–	+	++
GLc:B1	++	++	+	+	–	+	+	–	+	+
GLc:C1	+	+	+	++	–	++	++	–	++	++
GLc:C4	+	–	–	+	–	+	+	–	+	+

Continued (Table 20)

Strains	Indicator strains									
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*E. faecium* DSM 20477	*Strep. mutans* DSM 6178	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. cloacae* BFE 282	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Inziangsang										
IB1	–	–	–	+	–	–	+	–	++	–
IB2	+	+	+	++	+	–	+	–	+	++
IB3	+	+	+	+	+	+++	+	+	–	+
IB5	+	–	–	–	+	++	+	–	–	+
IB9	+	–	–	–	+	+	+	–	–	+
IB11	+	–	–	–	+	+	+	–	–	+
IB12	+	–	–	–	+	+	+	–	–	+
IB13	+	–	–	–	+	+	+	–	–	+
IB14	+	–	–	–	+	+	+	–	–	+
Khalpi										
KG:B1	+	+	+	+	–	+++	+	–	–	–
KG:B2	–	–	–	+	–	–	–	–	+	+
KG:B3	++	+	+	++	+	–	+	+	–	+
KG:B7	++	+	++	+	+	+++	+	–	–	+
KG:B8	–	–	–	+	–	–	–	–	+	++
KG:B9	+	–	+	++	+	+	+	–	++	++
KG:R1	+	–	+	+	+	+	+	–	+	+
KG:R2	+	–	+	+	+	+	+	–	+	+
KB:B4	+	+	+	++	+	++	+	–	+	+
KB:B5	+	–	–	–	–	–	+	–	–	+

Continued (Table 20)

Strains	Indicator strains									
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*E. faecium* DSM 20477	*Strep. mutans* DSM 6178	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. cloacae* BFE 282	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Khalpi										
KB:C1	+	–	+	+	–	–	–	–	–	+
KB:B9	+	+	+	–	–	–	–	–	–	–
KK:B2	–	+	–	–	–	+	–	+	–	–
KK:B3	–	+	–	+	–	–	+	–	–	–
KK:B4	–	–	–	–	–	–	–	–	–	–
KK:B5	–	–	–	–	–	–	–	–	–	–
KK:R1	–	–	–	–	–	–	–	–	–	–
KS:R1	–	–	–	–	–	–	–	–	–	–
KS:R2	–	–	+	++	–	–	–	–	–	+
KS:B1	–	–	–	–	–	–	–	–	–	–
KS:B2	–	–	+	–	–	–	–	–	–	+
KS:B9	–	+	+	–	–	–	–	–	–	+
KS:C1	+	+	–	+	+	+	+	+	–	–
KS:C2	–	+	+	–	+	–	+	–	–	–
KBe:B2	–	–	++	–	–	–	–	++	–	–
Sinki										
SL:B1	–	–	–	+	–	–	–	–	–	++
SL:B2	–	–	–	+	–	–	–	–	–	++
SL:B3	–	–	–	–	–	++	+	–	–	–
SL:B4	+	–	–	+	–	++	+	–	–	+
SL:B5	+	–	–	–	–	–	–	–	–	–
SL:B6	++	–	–	–	–	–	–	–	–	+
SL:B7	–	–	–	–	–	–	–	–	–	+

Continued (Table 20)

Strains	Indicator strains									
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*E. faecium* DSM 20477	*Strep. mutans* DSM 6178	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. cloacae* BFE 282	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Sinki										
SL:B8	–	–	–	+	–	–	–	–	–	–
SK:B1	–	–	–	–	–	–	–	–	–	+
SK:B2	–	–	–	–	–	–	–	–	–	+
SK:B3	–	–	–	+	–	–	–	–	–	+
SK:C1	–	–	+	+	–	–	+	–	–	–
SBe:B1	–	–	+	+	–	–	+	–	–	–
SBe:B2	–	–	+	+	–	–	+	–	–	–
SBe:B5	–	–	–	–	–	–	+	–	–	–
SBe:B6	–	+	–	+	–	–	–	–	–	–
SBe:B7	+	–	+	+	–	–	–	–	–	–
SS:B1	+	–	+	–	–	–	–	–	–	–
SS:B2	+	–	+	–	–	–	–	+	–	–
SS:B4	–	–	–	+	–	–	–	–	–	–
SS:B5	–	–	–	+	–	–	–	–	–	–
SS:B6	–	–	+	+	–	–	–	–	–	–
SS:B7	+	–	–	–	–	–	+	–	–	+
SG:B1	–	–	+	+	–	–	+	–	–	–
SG:B4	–	+	+	+	–	–	–	–	+	–
SG:B5	–	–	+	+	–	–	+	–	–	–
SG:B7	–	–	+	+	–	–	+	–	–	–

Data shows 3 sets of experiments.

–, no zone of inhibition; +, 2mm <zone <4mm; ++, 4mm <zone <6mm; +++, >6mm

Table 21. Antimicrobial activity of LAB strains from fermented bamboo shoot products

Strains	Indicator strains									
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*E. faecium* DSM 20477	*Strep. mutans* DSM 6178	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. cloacae* BFE 282	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Ekung										
EK1	++	++	–	+	+	+	–	+	+	–
EK4	++	++	–	++	+	+	–	–	–	++
EK5	++	++	–	–	–	–	–	–	–	–
EK6	–	+	+	+	+	+	+	+	+	+
EK7	++	++	–	–	–	++	–	–	–	–
EK10	–	–	–	–	–	+	–	–	–	–
EK11	–	–	–	–	–	+	–	–	–	–
EK12	++	–	–	–	–	+	++	–	–	–
EK15	++	–	–	–	–	+	–	–	++	–
EK18	–	–	–	–	–	+	–	–	–	–
EK19	–	–	–	–	–	–	–	–	–	–
EK20	++	–	–	–	–	–	–	–	–	–
EK21	++	–	++	–	–	+	–	–	–	–
EK22	+	–	–	–	–	+	–	–	–	–
EK23	–	–	–	–	–	–	–	–	–	–
EK24	++	–	–	–	–	+	–	+	–	–

Continued (Table 21)

Strains	Indicator strains									
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*E. faecium* DSM 20477	*Strep. mutans* DSM 6178	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. cloacae* BFE 282	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Ekung										
EK25	++	–	–	–	–	+	–	–	–	–
EK28	–	–	+	–	+	+	–	–	–	–
EK29	–	–	–	–	–	+	–	–	–	–
EK34	–	–	–	–	–	–	–	–	–	–
EK35	–	–	–	–	–	–	–	–	–	–
EK38	–	–	–	–	–	–	–	–	–	–
EK39	–	–	–	+	–	+	–	–	–	–
EK40	–	–	–	+	–	+	–	–	–	–
Eup										
Ep81	+	+	+	–	–	–	–	+	–	–
Ep82	–	–	–	+	–	–	–	–	–	–
Ep84	–	–	–	++	–	–	–	–	–	–
Ep85	–	–	–	–	–	–	–	–	–	–
Ep86	++	–	–	–	–	++	–	–	–	–
Ep87	–	–	–	+	–	–	–	–	–	–
Ep89	+	–	–	+	+	+	+	–	–	–
Ep90	–	–	–	–	+	–	–	–	–	–

Continued (Table 21)

Strains	Indicator strains									
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*E. faecium* DSM 20477	*Strep. mutans* DSM 6178	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. cloacae* BFE 282	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Eup										
Ep91	–	–	–	–	–	–	–	–	–	–
Ep92	–	–	–	–	–	–	–	–	–	–
Ep93	–	–	–	–	–	–	–	–	–	–
Ep94	–	–	–	–	–	–	–	–	–	–
Ep95	–	–	–	–	–	–	–	–	–	–
Ep96	–	–	–	–	–	–	–	–	–	–
Ep97	–	–	–	–	–	–	–	–	–	–
Ep98	–	–	–	–	–	–	–	–	–	–
Ep99	–	–	–	–	–	–	–	–	–	–
Ep101	++	–	–	–	–	–	–	–	–	–
Ep102	–	–	–	–	–	–	–	–	–	–
Ep103	–	–	–	–	–	–	–	–	–	–
Ep106	–	–	++	–	–	–	–	–	–	–
Ep110	–	–	–	+	–	–	+	–	+	–
Ep111	–	–	–	–	–	–	–	–	–	–
Ep112	–	–	–	–	–	–	–	–	–	–
Ep115	+	–	–	–	–	–	–	–	–	–

Continued (Table 21)

Strains	Indicator strains									
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*E. faecium* DSM 20477	*Strep. mutans* DSM 6178	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. cloacae* BFE 282	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Hirring										
Hr41	+	+	+	+	+	+	–	–	+	+
Hr42	++	+	+	+	+	+	–	–	+	+
Hr47	++	–	–	++	+	+	–	–	–	–
Hr48	–	–	–	–	+	+	–	–	–	–
Hr49	+	+	–	+	–	–	+	+	–	–
Hr50	–	–	–	–	–	–	–	–	–	–
Hr55	–	–	–	–	–	–	–	–	–	–
Hr56	–	–	–	–	–	–	–	–	–	–
Hr57	–	–	–	–	–	–	–	–	–	–
Hr58	–	–	–	–	–	–	–	–	–	–
Hr59	–	–	–	–	–	–	–	–	–	–
Hr60	–	–	–	–	–	–	–	–	–	–
Hr62	–	+	+	+	+	+	–	–	–	–
Hr64	–	–	–	–	–	–	–	–	–	–
Hr65	–	–	–	–	–	–	–	–	–	–
Hr66	–	–	–	–	–	–	–	–	–	–
Hr67	+	–	–	–	–	–	–	–	–	+

Continued (Table 21)

Strains	Indicator strains									
	***L. innocua* DSM 20649**	***L. monocytogenes* DSM 20600**	***B. cereus* CCM 2010**	***S. aureus* S1**	***E. faecium* DSM 20477**	***Strep. mutans* DSM 6178**	***K. pneumoniae* subsp. *pneumoniae* BFE 147**	***Ent. cloacae* BFE 282**	***Ent. agglomerans* BFE 154**	***Pseud. aeruginosa* BFE 162**
Hirring										
Hr68	–	–	–	–	–	–	–	–	–	–
Hr70	–	+	–	–	–	–	–	–	–	–
Hr71	–	–	–	–	–	–	+	–	–	–
Hr74	–	–	–	–	–	–	–	–	–	–
Hr75	–	–	–	–	–	–	–	–	–	–
Hr76	–	–	–	–	–	–	–	–	–	–
Hr77	–	–	–	–	–	–	–	–	–	–
Hr80	–	–	+	+	–	–	+	–	–	–
Mesu										
MeL2	++	–	–	++	–	–	++	+	+	++
MeB4	–	–	–	+	–	–	+	+	+++	++
MeR1	++	–	–	++	–	–	+	+	+++	++
MeB3	++	–	–	++	–	–	++	+	+++	+
MeR6	–	–	–	–	–	–	–	–	–	+
MeN3	–	–	–	–	–	–	+	+	+	+
MeTR	–	–	–	–	–	–	–	+	–	+
MeN7	–	–	–	–	–	–	+	+	+++	+
MeN5	–	–	–	–	–	–	+	+	+++	+

Continued (Table 21)

Strains	Indicator strains									
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*E. faecium* DSM 20477	*Strep. mutans* DSM 6178	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. cloacae* BFE 282	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Mesu										
MeL3	++	–	–	–	–	–	+	+	+	++
MeBe3	–	–	–	–	–	–	+	–	+	+
MeN2	+	–	–	–	+	–	–	+	+	+
MeNt1	–	–	–	–	–	–	+	+	+	+
MeB1	+	–	–	–	–	–	–	+	+	+
MeB2	–	–	–	–	–	–	+	+	+	+
MeNt2	–	–	–	+	–	–	+	+	+	+
MeB5	+	–	+	–	–	–	+	+	+	+
MeNt3	–	–	–	–	–	–	–	+	–	–
MeN4	–	–	–	–	–	–	–	+	–	–
MeN6	+	–	–	–	–	–	–	+	–	–
MeR2	–	–	–	–	–	–	–	+	–	–
MeT1	–	+	–	–	–	–	–	+	–	–
MeT2	–	–	–	–	–	–	–	+	–	–
MeT3	–	–	–	–	–	–	–	–	–	–
MeN8	+	–	–	–	–	–	–	+	–	–
MeN9	+	+	–	–	–	–	–	+	–	+

Continued (Table 21)

Strains	Indicator strains									
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*E. faecium* DSM 20477	*Strep. mutans* DSM 6178	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. cloacae* BFE 282	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Mesu										
MeK1	+	–	+	–	–	–	–	+	–	–
MeK2	–	–	–	–	–	–	–	+	+	+
MeR7	+	–	–	–	–	–	–	+	–	–
MeK3	–	–	–	–	–	–	–	+	–	–
MeB:C3	+	+	+++	++	–	+++	+	+	+	+
MeB:C4	++	+	++	++	–	+++	+	+	–	++
MeT5	+	–	–	–	–	–	–	+	–	–
MeT6	–	–	+	–	–	+	–	+	–	+
MeT7	+	+	+	–	–	–	–	+	–	–
Soibum										
SbB1	+	–	–	++	–	–	++	+	++	+
SbC1	+	–	–	++	–	–	–	+	+	++
SbC2	+	–	–	+	–	–	+	++	++	++
SbB2	+	–	–	+	–	–	+	+	+	+
SbB3	+	–	–	–	–	–	+	–	–	–
SbB4	–	–	–	+	–	–	+	–	–	–
SbB5	–	–	–	–	–	–	+	–	–	–
SbB6	+	–	–	+	–	–	+	–	–	–

Continued (Table 21)

Strains	Indicator strains									
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*E. faecium* DSM 20477	*Strep. mutans* DSM 6178	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. cloacae* BFE 282	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Soidon										
SdC1	+	–	–	++	–	–	++	++	–	++
SdR2	+	–	–	–	–	–	++	+	–	+
SdR3	+	–	–	++	–	–	++	+	+++	+
SdR4	+	–	–	–	–	–	–	+	–	+
SdR5	+	–	–	–	–	–	–	+	–	+
SdB1	+	–	+	+	+	–	+	–	+	–
SdB2	+	–	+	+	+	–	+	–	+	–
SdB3	+	–	+	+	+	–	+	–	+	–
SdB4	+	–	+	+	+	–	+	–	+	–
SdB5	+	–	+	+	+	–	+	–	+	–

Continued (Table 21)

Strains	Indicator strains									
	L. innocua DSM 20649	*L. monocytogenes* DSM 20600	*B. cereus* CCM 2010	*S. aureus* S1	*E. faecium* DSM 20477	*Strep. mutans* DSM 6178	*K. pneumoniae* subsp. *pneumoniae* BFE 147	*Ent. cloacae* BFE 282	*Ent. agglomerans* BFE 154	*Pseud. aeruginosa* BFE 162
Soijim										
SjC1	+	–	+	++	+	–	+	++	+++	++
SjR3	+	–	–	++	+	–	+	+	–	++
SjR7	+	–	–	–	–	++	–	+++	–	+
SjB1	–	–	–	–	–	–	+	–	–	–
SjB2	+	–	–	–	–	+	–	–	–	–
SjB4	+	–	–	–	–	–	–	–	+	–
SjB5	–	+	–	–	–	–	–	–	–	–
SjB8	–	–	–	–	–	–	–	–	–	+

Data shows 3 sets of experiment.

–, no zone of inhibition; +, 2mm <zone <4mm; ++, 4mm <zone <6mm; +++, >6mm

Bacteriocin Assay

LAB strains showing the inhibition zones of >4 mm against indicator strains in agar-spot plates, were selected for bacteriocin assay against the respective indicator strains. Cell-free supernatant extract of 24 hour old culture of selected LAB strains were spotted onto the plates containing indicator organisms. Among the 71 tested organisms only *Lb. plantarum* IB2 isolated from inziangsang showed a clear zone of >10.5 mm in agar spot against *Staphylococcus aureus* S1 indicating bacteriocin activity. However, this *Lb. plantarum* strain IB2 did not produce bacteriocin against any other indicator strains under the test conditions applied (Table 22 & 23). It could be summarized that *Lb. plantarum* IB2 was the only strain among 251 LAB having bacteriocin activity.

Quantification of bacteriocin

For quantification of bacteriocin from *Lb. plantarum* IB2 two fold dilution of cell-free supernatant extract of the 24 hour old culture was spotted onto agar diffusion assay containing *Staphylococcus aureus* S1 as an indicator strain. The clear halo zone was observed showing inhibition of growth of the indicator organism at dilution 1:32 which was calculated to 32 activity units per ml (32 AU/ml) of bacteriocin (data not shown).

Table 22. Screening of bacteriocin-producing strains of LAB from fermented vegetable products by cell free supernatants

Strains[a]	Indicator strains							
	L. innocua DSM 20649	*S. aureus* S1	*B. cereus* CCM 2010	*Strep. mutans* DSM 6178	*Pseud. aeruginosa* BFE 162	*Ent. agglomerans* BFE 154	*Ent. cloacae* BFE 282	*K. pneumoniae* subsp. *pneumoniae* BFE 147
Goyang								
Go12	–	–	–	–	–	–	–	–
Go14	–	–	–	–	–	–	–	–
Go22	–	–	–	–	–	–	–	–
Go29	–	–	–	–	–	–	–	–
Go34	–	–	–	–	–	–	–	–
Go40	–	–	–	–	–	–	–	–
Gundruk								
GK:C1	–	–	–	–	–	–	–	–
GK:C2	–	–	–	–	–	–	–	–
GLn:R1	–	–	–	–	–	–	–	–
GLn:C1	–	–	–	–	–	–	–	–
GLn:C2	–	–	–	–	–	–	–	–
GLn:C3	–	–	–	–	–	–	–	–
GLn:R2	–	–	–	–	–	–	–	–
GLn:R3	–	–	–	–	–	–	–	–
GSd:R1	–	–	–	–	–	–	–	–
GSd:C1	–	–	–	–	–	–	–	–
GS:R1	–	–	–	–	–	–	–	–
GS:C1	–	–	–	–	–	–	–	–
GLc:B1	–	–	–	–	–	–	–	–
GLc:C1	–	–	–	–	–	–	–	–

Continued (Table 22)

Strains[a]	Indicator strains							
	L. innocua DSM 20649	*S. aureus* S1	*B. cereus* CCM 2010	*Strep. mutans* DSM 6178	*Pseud. aeruginosa* BFE 162	*Ent. agglomerans* BFE 154	*Ent. cloacae* BFE 282	*K. pneumoniae subsp. pneumoniae* BFE 147
Inziangsang								
IB1	–	–	–	–	–	–	–	–
IB2	–	+++ (10.5)	–	–	–	–	–	–
IB3	–	–	–	–	–	–	–	–
IB5	–	–	–	–	–	–	–	–
Khalpi								
KG:B1	–	–	–	–	–	–	–	–
KG:B3	–	–	–	–	–	–	–	–
KG:B7	–	–	–	–	–	–	–	–
KG:B8	–	–	–	–	–	–	–	–
KG:B9	–	–	–	–	–	–	–	–
KB:B4	–	–	–	–	–	–	–	–
KS:B2	–	–	–	–	–	–	–	–
KBe:B2	–	–	–	–	–	–	–	–
Sinki								
SL:B1	–	–	–	–	–	–	–	–
SL:B2	–	–	–	–	–	–	–	–
SL:B3	–	–	–	–	–	–	–	–
SL:B4	–	–	–	–	–	–	–	–
SL:B6	–	–	–	–	–	–	–	–

Data shows 3 sets of experiment.

[a]Strains showing ≥ ++ inhibition zone in the agar spot assay for antagonism were selected for bacteriocin assay.

–, no zone of inhibition; Diameter of clear zone (mm) is given in parenthesis.

Table 23: Screening of bacteriocin-producing strains of LAB from fermented bamboo shoot products by cell-free culture supernatants

Strains[a]	Indicator strains							
	L. innocua DSM 20649	*L. mobocytogenes* DSM 206600	*S. aureus* S1	*B. cereus* CCM 2010	*Strep. mutans* DSM 6178	*Pseud. aeruginosa* BFE 162	*Ent. agglomerans* BFE 154	*K. pneumoniae subsp. pneumoniae* BFE 147
Ekung								
Ek1	–	–	–	–	–	–	–	–
Ek4	–	–	–	–	–	–	–	–
Ek5	–	–	–	–	–	–	–	–
Ek7	–	–	–	–	–	–	–	–
Ek12	–	–	–	–	–	–	–	–
Ek15	–	–	–	–	–	–	–	–
Ek20	–	–	–	–	–	–	–	–
Ek21	–	–	–	–	–	–	–	–
Ek24	–	–	–	–	–	–	–	–
Ek25	–	–	–	–	–	–	–	–
Eup								
Ep84	–	–	–	–	–	–	–	–
Ep86	–	–	–	–	–	–	–	–
Ep101	–	–	–	–	–	–	–	–
Ep106	–	–	–	–	–	–	–	–
Hirring								
Hr42	–	–	–	–	–	–	–	–
Hr47	–	–	–	–	–	–	–	–
Mesu								
MeL2	–	–	–	–	–	–	–	–
MeB4	–	–	–	–	–	–	–	–

Continued (Table 23)

Strains[a]	Indicator strains							
	L. innocua DSM 20649	*L. mobocytogenes* DSM 206600	*S. aureus* S1	*B. cereus* CCM 2010	*Strep. mutans* DSM 6178	*Pseud. aeruginosa* BFE 162	*Ent. agglomerans* BFE 154	*K. pneumoniae* subsp. *pneumoniae* BFE 147
Mesu								
MeR1	–	–	–	–	–	–	–	–
MeB3	–	–	–	–	–	–	–	–
MeN7	–	–	–	–	–	–	–	–
MeN5	–	–	–	–	–	–	–	–
MeL3	–	–	–	–	–	–	–	–
MeB:C3	–	–	–	–	–	–	–	–
MeB:C4	–	–	–	–	–	–	–	–
Soibum								
SbB1	–	–	–	–	–	–	–	–
SbC1	–	–	–	–	–	–	–	–
SbC2	–	–	–	–	–	–	–	–
Soidon								
SdC1	–	–	–	–	–	–	–	–
SdR2	–	–	–	–	–	–	–	–
SdR3	–	–	–	–	–	–	–	–
Soijim								
SjC1	–	–	–	–	–	–	–	–
SjR3	–	–	–	–	–	–	–	–
SjR7	–	–	–	–	–	–	–	–

Data shows 3 sets of experiment.

[a]Strains showing ≥ ++ inhibition zone in the agar spot assay for antagonism were selected for bacteriocin assay.

–, no zone of inhibition

Enzymatic profiles

Enzymatic profiles of LAB strains were assayed using the API-zym (bioMérieux, France) galleries (Tables 24 and 25). Each of the predominant LAB strain produced a wide spectrum of enzymes. These strains showed relatively moderate esterase (C4) and strong arylamidase and phosphatase activities. However, they showed no detectable proteinase activity with the methods applied. Acid phosphatase activity was detected in all the strains tested among which >40 nanomole activities was showed by *Lb. brevis* KG:B2 (khalpi), *Lb. brevis* IB1 (inziangsang), *Lb. brevis* MeB4 (mesu), *Lb. brevis* (SjR7), *Leuc. fallax* SdR2 (soidon), *Leuc. lactis* SbB1 (soibum) and *T. halophilus* Ek28 (ekung). Phosphohydrolase activity was shown by all strains tested except *Leuc. fallax* SjC1 (soijim). The α-glucosidase activity was highest (>40 nanomoles) in *Lb. plantarum* KG:B1 (khalpi), *Lb. plantarum* IB2 (inziangsang) and *Lb. plantarum* MeL2 (mesu), *Lb. brevis* SL:B1 (sinki), *Lb. brevis* KG:B2 (khalpi) and *Lb. brevis* IB1 (inziangsang), *Leuc. fallax* SL:B3 (sinki), *Leuc. fallax* SdC1 (soidon), *Leuc. fallax* SbC1 (soibum), *Leuc. fallax* SjC1 (soijim) and *Leuc. lactis* SdR2 (soidon). Glucosaminidase activity was shown by mostly *Lb. plantarum* strains GK:C1, (gundruk), KG:B1 (khalpi), IB2 (inziangsang), Ek4 (ekung), Hr42 (hirring), MeL2 (mesu), and *P. pentosaceus* strains GLn:C1 (gundruk), MeB:C3 (mesu). None of the strains tested showed mannosidase and fucosidase activities.

Table 24. Enzymatic profiles of LAB strains from fermented vegetable products using API-zym

Enzyme	Strain (Activity in nanomoles)									
	Go7	Go9	GK:C1	GLn:C1	KG:B2	KG:B1	KB:C1	IB1	IB2	IB5
Control (*without enzyme*)	0	0	0	0	0	0	0	0	0	0
Alkaline phosphatase	5	0	0	0	0	5	0	10	5	5
Esterase (C4)	0	0	0	0	5	5	5	5	0	0
Esterase lipase (C8)	5	5	0	0	5	5	0	5	5	0
Lipase (C14)	0	0	5	5	5	5	0	5	5	5
Leucine arylamidase	10	0	>40	>40	>40	>40	5	>40	>40	>40
Valine arylamidase	0	0	>40	>40	>40	>40	0	>40	>40	>40
Cystine arylamidase	0	0	5	5	10	10	0	20	20	30
Trypsin	0	0	0	0	0	0	0	0	0	0
α-chymotrypsin	0	0	0	0	0	0	0	0	0	0
Acid phosphatase	20	5	20	20	>40	10	10	>40	20	20
Napthol-AS-BI-phosphohydrolase	20	10	20	>40	20	10	5	20	20	30
α-galactosidase	0	0	0	0	30	5	0	20	5	0
β-galactosidase	0	0	5	5	30	>40	0	>40	>40	20
β-glucuronidase	0	0	0	0	20	0	0	10	0	0
α-glucosidase	0	0	0	0	>40	>40	>40	>40	>40	0
β-glucosidase	0	20	10	30	30	30	0	>40	30	20
N-acetyl-β-glucosaminidase	0	0	20	>40	0	>40	0	0	30	0
α-mannosidase	0	0	0	0	0	0	0	0	0	0
α-fucosidase	0	0	0	0	0	0	0	0	0	0

Data represents the means of 3 sets of experiment.

Go7 *Lc. lactis* (goyang); Go9 *E. faecium* (goyang); GK:C1 *Lb. plantarum* (gundruk); GLn:C1 *P. pentosaceus* (gundruk); KG:B2 *Lb. brevis* (khalpi); KG:B1 *Lb. plantarum* (khalpi); KB:C1 *Leuc. fallax* (khalpi); IB1 *Lb. brevis* (inziangsang); IB2 *Lb. plantarum* (inziangsang); IB5 *P. acidilactici* (inziangsang).

Table 25. Enzymatic profiles of LAB strains from fermented vegetable and bamboo shoots using API-zym

Enzyme	Strain (Activity in nanomoles)									
	SL:B1	SL:B3	Ek4	Ek23	Ek28	Ek34	Ep92	Hr42	MeB4	MeL2
Control (without enzyme)	0	0	0	0	0	0	0	0	0	0
Alkaline phosphatase	0	0	5	10	2	0	0	5	10	5
Esterase (C4)	10	5	0	0	10	0	0	0	5	0
Esterase lipase (C8)	10	5	5	0	5	0	0	0	5	5
Lipase (C14)	5	0	5	0	0	5	0	0	0	5
Leucine arylamidase	30	5	>40	30	0	30	20	30	>40	>40
Valine arylamidase	>40	0	30	20	0	30	5	30	>40	>40
Cystine arylamidase	>40	0	20	0	0	5	0	5	10	20
Trypsin	0	0	0	0	0	0	0	0	0	0
α-chymotrypsin	0	0	0	0	0	0	0	0	0	0
Acid phosphatase	30	30	20	20	>40	20	5	10	>40	30
Napthol-AS-BI-phosphohydrolase	20	10	20	10	5	20	5	10	>40	20
α-galactosidase	10	0	10	0	0	0	5	5	20	5
β-galactosidase	30	0	>40	0	0	0	10	>40	>40	>40
β-glucuronidase	5	0	0	0	0	0	0	0	0	0
α-glucosidase	>40	>40	20	0	0	0	20	20	30	>40
β-glucosidase	>40	5	30	0	0	30	20	20	20	>40
N-acetyl-β-glucosaminidase	0	0	>40	0	0	30	0	>40	0	>40
α-mannosidase	0	0	0	0	0	0	0	0	0	0
α-fucosidase	0	0	0	0	0	0	0	0	0	0

Data represents the means of 3 sets of experiment.

SL:B1 *Lb. brevis* (sinki); SL:B3 *Leuc. fallax* (sinki);). Ek4 *Lb. plantarum* (ekung); Ek23 *Lb. plantarum* (ekung); Ek28 *T. halophilus* (ekung); Ek34 *Lb. casei* (ekung); Ep92 *Lb. fermentum* (eup); Hr42 *Lb. plantarum* (hirring); MeB4 *Lb. brevis* (mesu); MeL2 *Lb. plantarum* (mesu).

Continued (Table 25)

Enzyme	Strain (Activity in nanomoles)								
	MeB:C3	SbC1	SbB1	SdC1	SdR2	SdR3	SjC1	SjR3	SjR7
Control (without enzyme)	0	0	0	0	0	0	0	0	0
Alkaline phosphatase	0	0	5	0	5	10	0	5	5
Esterase (C4)	0	5	0	5	5	0	5	0	5
Esterase lipase (C8)	5	0	5	0	10	5	0	5	5
Lipase (C14)	5	0	0	0	5	0	0	0	5
Leucine arylamidase	>40	5	5	5	>40	10	5	5	>40
Valine arylamidase	>40	0	0	0	>40	0	0	0	>40
Cystine arylamidase	20	5	0	5	30	0	0	0	10
Trypsin	0	0	0	0	0	0	0	0	0
α-chymotrypsin	0	0	0	0	0	0	0	0	0
Acid phosphatase	20	10	>40	10	>40	20	20	10	>40
Napthol-AS-BI-phosphohydrolase	30	5	5	5	10	5	0	5	5
α-galactosidase	0	0	5	0	20	5	0	5	10
β-galactosidase	5	0	5	0	>40	10	0	5	>40
β-glucuronidase	0	0	0	0	0	0	0	0	0
α-glucosidase	0	>40	20	>40	>40	20	>40	5	30
β-glucosidase	5	0	0	0	>40	0	0	0	>40
N-acetyl-β-glucosaminidase	5	0	0	0	0	0	0	0	0
α-mannosidase	0	0	0	0	0	0	0	0	0
α-fucosidase	0	0	0	0	0	0	0	0	0

Data represents the means of 3 sets of experiment.
MeB:C3 *P. pentosaceus* (mesu); SbC1 *Leuc. fallax* (soibum); SbB1 *Leuc. lactis* (soibum); SdC1 *Leuc. fallax* (soidon); SdR2 *Leuc. lactis* (soidon); SdR3 *Lb. brevis* (soidon); SjC1 *Leuc. fallax* (soijim); SjR3 *Leuc. lactis* (soijim); SjR7 *Lb. brevis* (soijim).

Degradation of antinutritive factors

LAB strains isolated from fermented vegetable products and fermented bamboo shoot products were screened for their ability to degrade antinutritive factors (Tables 26 and 27). Out of 251 LAB strains screened for antinutritive factors 47.4 % of LAB strains degraded phytic acids, 39 % degraded raffinose and 5.2 % degraded stachyose in the applied method. *Lb. plantarum* showed the highest percentage of degradation of anti-nutritive factors; out of which 51.3 % degraded phytic acid, 76.7 % degraded raffinose and 69.2 % degraded stachyose from the positive degradation data (Table 26 and 27).

Among the remaining non-*Lb. plantarum* strains, *Lb. casei*, *Lb. fermentum,* some strains of *Lb. brevis*, *Leuconostoc* spp. and *Enterococcus* spp. degraded phytic acid and oligosaccharides. However, no effect on degradation of antinutritive factors was shown by *Lb. curvatus* (mesu), *T. halophilus* (ekung) and *Leuc. citreum* (mesu).

Table 26. Degradation of phytic acid and flatulence causing oligosaccharides by LAB strains from fermented vegetable products

Product	Strain	Phytic acid[a]	Raffinose[b]	Stachyose[c]
Goyang	*P. pentosaceus* Go1	+	–	–
	Lc. lactis Go5	+	–	–
	Lb. plantarum Go6	–	–	–
	Lc. lactis Go7	–	+	–
	Lc. lactis Go8	+	–	–
	E. faecium Go9	+	+	–
	Lb. brevis Go11	–	–	–
	Lb. plantarum Go12	+	–	–
	Lb. plantarum Go14	–	–	–
	Lb. plantarum Go16	+	+	–
	P. pentosaceus Go17	–	–	–
	E. faecium Go20	–	+	–
	Lb. plantarum Go22	–	–	–
	Lb. plantarum Go23	–	–	–
	Lb. brevis Go24	+	–	–
	Lb. plantarum Go25	+	+	–
	P. pentosaceus Go29	+	–	–
	Lb. plantarum Go30	+	+	–
	Lb. plantarum Go31	+	+	–
	E. faecium Go34	+	–	–
	Lb. plantarum Go37	+	+	–
	Lb. plantarum Go39	–	+	–
	Lb. plantarum Go40	–	+	–
	Lb. brevis Go41	–	+	–
	E. faecium Go43	–	+	–
	E. faecium Go45	–	–	–
Gundruk	*Lb. plantarum* GK:C1	+	+	–
	P. pentosaceus GK:C2	–	–	–
	P. pentosaceus GK:B3	–	–	–
	P. pentosaceus GK:B6	–	–	–

Continued (Table 26)

Product	Strain	Phytic acid[a]	Raffinose[b]	Stachyose[c]
	P. pentosaceus GK:C3	–	–	–
	Lb. plantarum GLn:R1	–	–	–
	Lb. plantarum GLn:R5	–	–	–
	Lb. plantarum GLn:R6	+	+	–
	P. pentosaceus GLn:C1	+	–	–
	Lb. plantarum GLn:C2	+	–	+
	P. pentosaceus GLn:C3	+	–	–
	P. pentosaceus GLn:C5	–	–	–
	P. pentosaceus GLn:R2	+	–	–
	P. pentosaceus GLn:R3	+	–	–
	Lb. plantarum GSd:R1	+	–	–
Gundruk	*Lb. plantarum* GSd:R4	–	–	–
	Lb. plantarum GSd:R5	–	–	–
	P. pentosaceus GSd:C1	+	–	–
	P. pentosaceus GSd:C2	–	–	–
	Lb. plantarum GS:R1	+	–	–
	P. pentosaceus GS:B1	–	–	–
	P. pentosaceus GS:B2	–	–	–
	P. pentosaceus GS:B7	–	–	–
	P. pentosaceus GS:C1	+	–	–
	Lb. plantarum GLc:B1	–	–	–
	P. pentosaceus GLc:C1	–	–	–
	P. pentosaceus GLc:C4	+	–	–
	Lb. brevis IB1	+	–	–
	Lb. plantarum IB2	+	+	–
	Lb. plantarum IB3	+	+	–
Inziang-sang	*P. acidilactici* IB5	+	–	–
	Lb. brevis IB9	–	+	–
	Lb. plantarum IB11	–	–	–
	Lb. plantarum IB12	–	–	–
	Lb. plantarum IB13	–	+	–
	P. acidilactici IB14	–	–	–

Continued (Table 26)

Product	Strain	Phytic acid[a]	Raffinose[b]	Stachyose[c]
	Lb. plantarum KG:B1	+	+	–
	Lb. brevis KG:B2	+	–	–
	Lb. plantarum KG:B3	+	+	–
	Lb. plantarum KG:B7	+	–	–
	Lb. brevis KG:B8	+	–	–
	Lb. plantarum KG:B9	+	–	–
	Lb. brevis KG:R1	–	–	–
	Lb. brevis KG:R2	–	–	–
	Lb. plantarum KB:B4	+	–	–
	Lb. brevis KB:B5	+	–	–
	Leuc. fallax KB:C1	+	–	–
	Lb. plantarum KB:B9	–	+	–
Khalpi	*Lb. brevis* KK:B2	+	–	–
	Lb. plantarum KK:B3	–	–	–
	Lb. plantarum KK:B4	–	–	–
	Lb. plantarum KK:B5	–	–	–
	Lb. brevis KK:R1	+	–	–
	Lb. brevis KS:R1	–	–	–
	Lb. brevis KS:R2	–	–	–
	Lb. brevis KS:B1	–	–	–
	Lb. plantarum KS:B2	+	–	–
	Lb. plantarum KS:B9	–	–	–
	Leuc. fallax KS:C1	–	–	–
	Leuc. fallax KS:C2	–	–	–
	Lb. brevis KBe:B2	+	–	–
	Lb. brevis SL:B1	+	–	–
	Lb. brevis SL:B2	+	–	–
Sinki	*Leuc. fallax* SL:B3	+	–	–
	Leuc. fallax SL:B4	+	–	–
	Leuc. fallax SL:B5	+	–	–

Continued (Table 26)

Product	Strain	Phytic acid[a]	Raffinose[b]	Stachyose[c]
	Lb. brevis SL:B6	+	–	–
	Lb. brevis SL:B7	+	–	–
	Lb. brevis SL:B8	–	–	–
	Lb. brevis SK:B1	+	–	–
	Lb. brevis SK:B2	+	–	–
	Lb. brevis SK:B3	+	–	–
	Leuc. fallax SK:C1	–	–	–
	Lb. plantarum SBe:B1	+	+	–
	Lb. brevis SBe:B2	–	–	–
	Lb. brevis SBe:B5	–	–	–
	Lb. brevis SBe:B6	–	–	–
Sinki	*Lb. brevis* SBe:B7	–	–	–
	Lb. brevis SS:B1	–	–	–
	Lb. brevis SS:B2	–	–	–
	Lb. casei SS:B4	+	–	–
	Lb. brevis SS:B5	–	–	–
	Lb. brevis SS:B6	–	–	–
	Lb. brevis SS:B7	–	–	–
	Lb. plantarum SG:B1	+	+	–
	Lb. plantarum SG:B4	+	+	–
	Lb. plantarum SG:B5	+	+	–
	Lb. plantarum SG:B7	–	–	–

Data represents the means of 3 sets of experiment.

[a]In Ca-phytate agar plates.

[b]Fermentation in MRS broth without beef extract and glucose supplemented with 1% raffinose.

[c]Fermentation in MRS broth without beef extract and glucose supplemented with 1% stachyose.

+, indicates degradation of antinutritive factors; –, indicates non-degradation of antinutritive factors.

Table 27. Degradation of phytic acid and flatulence causing oligosaccharides by LAB strains from fermented bamboo shoot products

Product	Strain	Phytic acid[a]	Raffinose[b]	Stachyose[c]
	Lb. plantarum Ek1	–	+	–
	Lb. plantarum Ek4	+	+	–
	Lb. plantarum Ek5	–	+	–
	Lb. plantarum Ek6	+	–	–
	Lb. plantarum Ek7	–	+	–
	Lb. brevis Ek8	–	–	–
	Lb. plantarum Ek10	–	+	–
	Lb. plantarum Ek11	+	+	+
	Lb. plantarum Ek12	+	+	–
	Lb. plantarum Ek15	–	–	–
	Lb. plantarum Ek18	–	–	–
	Lb. plantarum Ek19	–	–	–
Ekung	*Lb. plantarum* Ek20	–	+	+
	Lb. plantarum Ek21	–	–	–
	T. halophilus Ek 22	–	–	–
	Lb. plantarum Ek23	–	–	–
	Lb. plantarum Ek24	–	–	–
	Lb. plantarum Ek25	–	+	–
	T. halophilus Ek28	–	–	–
	Lb. plantarum Ek29	–	+	–
	Lb. casei Ek34	–	–	–
	Lb. casei Ek35	–	–	–
	Lb. plantarum Ek38	–	+	–
	Lb. plantarum Ek39	–	+	–
	Lb. plantarum Ek40	–	+	–
	Lb. plantarum Ep81	–	+	–
	Lb. plantarum Ep82	+	+	–
Eup	*Lb. plantarum* Ep84	–	+	–
	Lb. fermentum Ep85	+	+	–
	Lb. plantarum Ep86	+	+	–

Continued (Table 27)

Product	Strain	Phytic acid[a]	Raffinose[b]	Stachyose[c]
Eup	*Lb. plantarum* Ep87	+	+	–
	Lb. plantarum Ep89	–	+	–
	Lb. plantarum Ep90	–	+	–
	Lb. plantarum Ep91	+	+	–
	Lb. fermentum Ep92	+	+	+
	Lb. fermentum Ep93	+	+	–
	Lb. plantarum Ep94	–	+	–
	Lb. plantarum Ep95	–	+	–
	Lb. plantarum Ep96	–	+	–
	Lb. plantarum Ep97	+	+	–
	Lb. plantarum Ep98	–	+	–
	Lb. plantarum Ep99	+	+	–
	Lb. plantarum Ep101	+	+	–
	Lb. plantarum Ep102	+	+	–
	Lb. plantarum Ep103	+	+	–
	Lb. plantarum Ep106	+	+	–
	Lb. plantarum Ep110	+	+	+
	Lb. plantarum Ep111	+	+	–
	Lb. plantarum Ep112	–	+	–
	Lb. plantarum Ep115	–	+	+
Hirring	*Lb. plantarum* Hr41	+	+	+
	Lb. plantarum Hr42	+	+	+
	Lb. plantarum Hr45	–	–	–
	Lb. plantarum Hr47	–	–	–
	Lb. plantarum Hr48	+	+	–
	Lb. plantarum Hr49	+	–	–
	Lb. plantarum Hr50	+	+	+
	Lb. plantarum Hr55	+	–	–
	Lc. lactis Hr56	+	+	–
	Lb. plantarum Hr57	+	–	–
	Lb. plantarum Hr58	+	–	–
	Lb. plantarum Hr59	+	+	–

Continued (Table 27)

Product	Strain	Phytic acid[a]	Raffinose[b]	Stachyose[c]
Hirring	*Lb. plantarum* Hr60	+	–	–
	Lc. lactis Hr62	+	+	–
	Lb. plantarum Hr64	+	–	–
	Lb. plantarum Hr65	–	–	–
	Lb. plantarum Hr66	+	+	–
	Lb. plantarum Hr67	+	–	–
	Lb. plantarum Hr68	+	+	+
	Lb. plantarum Hr70	+	+	–
	Lb. plantarum Hr71	–	–	–
	Lb. plantarum Hr74	–	+	–
	Lb. plantarum Hr75	–	–	–
	Lb. plantarum Hr76	–	–	–
	Lb. plantarum Hr77	–	+	–
	Lb. plantarum Hr80	–	–	–
Mesu	*Lb. plantarum* MeL2	+	+	–
	Lb. brevis MeB4	+	–	–
	Lb. plantarum MeR1	+	+	–
	Lb. plantarum MeB3	+	–	–
	Lb. brevis MeR6	+	–	–
	Lb. brevis MeN3	+	–	–
	Lb. brevis MeTR	–	–	–
	Lb. brevis MeN7	–	–	–
	Lb. brevis MeN5	+	–	–
	Lb. plantarum MeL3	+	+	–
	Lb. plantarum MeBe3	–	+	–
	Lb. brevis MeN2	–	–	–
	Lb. plantarum MeNt1	–	+	–
	Lb. plantarum MeB1	–	+	–
	Lb. plantarum MeB2	–	–	–
	Lb. brevis MeNt2	–	–	–
	Lb. plantarum MeB5	–	+	–

Continued (Table 27)

Product	Strain	Phytic acid[a]	Raffinose[b]	Stachyose[c]
Mesu	*Lb. brevis* MeNt3	+	–	–
	Lb. plantarum MeN4	+	–	–
	Lb. plantarum MeN6	+	+	–
	Lb. plantarum MeR2	+	+	–
	Lb. plantarum MeT1	–	+	–
	Lb. plantarum MeT2	–	+	–
	Lb. plantarum MeT3	–	+	–
	Leuc. citreum MeN8	–	–	–
	Leuc. citreum MeN9	–	–	–
	Leuc. citreum MeK1	–	–	–
	Leuc. citreum MeK2	–	–	–
	Lb. brevis MeR7	–	–	–
	Lb. curvatus MeK3	–	–	–
	P. pentosaceus MeB:C3	+	–	–
	P. pentosaceus MeB:C4	+	–	–
	P. pentosaceus MeT5	–	–	–
	P. pentosaceus MeT6	–	–	–
	P. pentosaceus MeT7	–	–	–
Soibum	*Leuc. lactis* SbB1	+	+	+
	Leuc. fallax SbC1	+	–	–
	Leuc. mesenteroides SbC2	+	–	–
	Lb. plantarum SbB2	–	–	–
	Lb. plantarum SbB3	–	–	–
	E. durans SbB4	+	–	–
	Lb. brevis SbB5	+	–	–
	Lb. brevis SbB6	–	–	–

Continued (Table 27)

Product	Strain	Phytic acid[a]	Raffinose[b]	Stachyose[c]
Soidon	*Leuc. fallax* SdC1	+	–	–
	Leuc. lactis SdR2	+	–	–
	Lb. brevis SdR3	+	+	+
	Lb. brevis SdR4	–	+	–
	Lb. brevis SdR5	–	+	–
	Lb. lactis SdB1	+	+	–
	Leuc. lactis SdB2	–	+	–
	Leuc. lactis SdB3	+	+	–
	Lb. brevis SdB4	–	–	–
	Leuc. lactis SdB5	–	+	–
Soijim	*Leuc. fallax* SjC1	+	–	–
	Leuc. lactis SjR3	–	+	+
	Lb. brevis SjR7	+	–	–
	Lb. brevis SjB1	–	–	–
	Leuc. fallax SjB2	–	–	–
	Leuc. lactis SjB4	–	+	–
	Leuc. lactis SjB5	–	+	–
	Leuc. lactis SjB8	–	+	–

Data represents the means of 3 sets of experiment.

[a]In Ca-phytate agar plates.

[b]Fermentation in MRS broth without beef extract and glucose supplemented with 1% raffinose.

[c]Fermentation in MRS broth without beef extract and glucose supplemented with 1% stachyose.

+, indicates degradation of antinutritive factors.

–, indicates non-degradation of antinutritive factors.

Screening of biogenic amines-producing LAB

Strains of LAB isolated from fermented vegetables and fermented bamboo tender shoot products were screened for their ability to produce biogenic amines (Tables 28 and 29). Most of the strains did not produce biogenic amines with the method applied. Almost all the *Lb. plantarum,* except one *Lb. plantarum* strain MeL3 from mesu, were tested negative for amino acid decarboxylase activity. None of the 5 strains of enterococci and 5 strains of lactococci produced biogenic amines in the method applied. Biogenic amine mainly ornithine was detected in four strains of *Lb. brevis* SL:B1, SL:B6, SK:B1, SK:B2, SK:B3 (sinki), 2 strains of *Lb. brevis* MeB4 and MeN5 (mesu), one *Lb. brevis* strain SjR7 (soijim), *Lb. plantarum* strain MeL3 (mesu); *Leuc. lactis* strain SdR2, (soidon); showed decarboxylase activity on orthinine plates.

Table 28. Screening of biogenic amine producing LAB strains from fermented vegetable products

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
Goyang	*P. pentosaceus* Go1	–	–	–	–
	Lc. lactis Go5	–	–	–	–
	Lb. plantarum Go6	–	–	–	–
	Lc. lactis Go7	–	–	–	–
	Lc. lactis Go8	–	–	–	–
	E. faecium Go9	–	–	–	–
	Lb. brevis Go11	–	–	–	–
	Lb. plantarum Go12	–	–	–	–
	Lb. plantarum Go14	–	–	–	–
	Lb. plantarum Go16	–	–	–	–
	P. pentosaceus Go17	–	–	–	–
	E. faecium Go20	–	–	–	–
	Lb. plantarum Go22	–	–	–	–
	Lb. plantarum Go23	–	–	–	–
	Lb. brevis Go24	–	–	–	–
	Lb. plantarum Go25	–	–	–	–
	P. pentosaceus Go29	–	–	–	–
	Lb. plantarum Go30	–	–	–	–
	Lb. plantarum Go31	–	–	–	–
	E. faecium Go34	–	–	–	–
	Lb. plantarum Go37	–	–	–	–
	Lb. plantarum Go39	–	–	–	–
	Lb. plantarum Go40	–	–	–	–
	Lb. brevis Go41	–	–	–	–
	E. faecium Go43	–	–	–	–
	E. faecium Go45	–	–	–	–

Continued (Table 28)

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
Gundruk	*Lb. plantarum* GK:C1	–	–	–	–
	P. pentosaceus GK:C2	–	–	–	–
	P. pentosaceus GK:B3	–	–	–	–
	P. pentosaceus GK:B6	–	–	–	–
	P. pentosaceus GK:C3	–	–	–	–
	Lb. plantarum GLn:R1	–	–	–	–
	Lb. plantarum GLn:R5	–	–	–	–
	Lb. plantarum GLn:R6	–	–	–	–
	P. pentosaceus GLn:C1	–	–	–	–
	Lb. plantarum GLn:C2	–	–	–	–
	P. pentosaceus GLn:C3	–	–	–	–
	P. pentosaceus GLn:C5	–	–	–	–
	P. pentosaceus GLn:R2	–	–	–	–
	P. pentosaceus GLn:R3	–	–	–	–
	Lb. plantarum GSd:R1	–	–	–	–
	Lb. plantarum GSd:R4	–	–	–	–
	Lb. plantarum GSd:R5	–	–	–	–
	P. pentosaceus GSd:C1	–	–	–	–
	P. pentosaceus GSd:C2				
	Lb. plantarum GS:R1	–	–	–	–
	P. pentosaceus GS:B1	–	–	–	–
	P. pentosaceus GS:B2	–	–	–	–
	P. pentosaceus GS:B7	–	–	–	–
	P. pentosaceus GS:C1	–	–	–	–
	Lb. plantarum GLc:B1				
	P. pentosaceus GLc:C1	–	–	–	–
	P. pentosaceus GLc:C4				
Inziangsang	*Lb. brevis* IB1	–	–	–	–
	Lb. plantarum IB2	–	–	–	–
	Lb. plantarum IB3	–	–	–	–
	P. acidilactici IB5	–	–	–	–

Continued (Table 28)

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
Inziangsang	*Lb. brevis* IB9	–	–	–	–
	Lb. plantarum IB11	–	–	–	–
	Lb. plantarum IB12	–	–	–	–
	Lb. plantarum IB13	–	–	–	–
	P. acidilactici IB14	–	–	–	–
Khalpi	*Lb. plantarum* KG:B1	–	–	–	–
	Lb. brevis KG:B2	–	–	–	–
	Lb. plantarum KG:B3	–	–	–	–
	Lb. plantarum KG:B7	–	–	–	–
	Lb. brevis KG:B8	–	–	–	–
	Lb. plantarum KG:B9	–	–	–	–
	Lb. brevis KG:R1	–	–	–	–
	Lb. brevis KG:R2	–	–	–	–
	Lb. plantarum KB:B4	–	–	–	–
	Lb. brevis KB:B5	–	–	–	–
	Leuc. fallax KB:C1	–	–	–	–
	Lb. plantarum KB:B9	–	–	–	–
	Lb. brevis KK:B2	–	–	–	–
	Lb. plantarum KK:B3	–	–	–	–
	Lb. plantarum KK:B4	–	–	–	–
	Lb. plantarum KK:B5	–	–	–	–
	Lb. brevis KK:R1	–	–	–	–
	Lb. brevis KS:R1	–	–	–	–
	Lb. brevis KS:R2	–	–	–	–
	Lb. brevis KS:B1	–	–	–	–
	Lb. plantarum KS:B2	–	–	–	–
	Lb. plantarum KS:B9	–	–	–	–
	Leuc. fallax KS:C1	–	–	–	–
	Leuc. fallax KS:C2	–	–	–	–
	Lb. brevis KBe:B2	–	–	–	–

Continued (Table 28)

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
	Lb. brevis SL:B1	–	–	–	+
	Lb. brevis SL:B2	–	–	–	–
	Leuc. fallax SL:B3	–	–	–	–
	Leuc. fallax SL:B4	–	–	–	–
	Leuc. fallax SL:B5	–	–	–	–
	Lb. brevis SL:B6	–	–	–	+
	Lb. brevis SL:B7	–	–	–	–
	Lb. brevis SL:B8	–	–	–	–
	Lb. brevis SK:B1	–	–	–	+
	Lb. brevis SK:B2	–	–	–	+
	Lb. brevis SK:B3	–	–	–	+
	Leuc. fallax SK:C1	–	–	–	–
	Lb. plantarum SBe:B1	–	–	–	–
Sinki	*Lb. brevis* SBe:B2	–	–	–	–
	Lb. brevis SBe:B5	–	–	–	–
	Lb. brevis SBe:B6	–	–	–	–
	Lb. brevis SBe:B7	–	–	–	–
	Lb. brevis SS:B1	–	–	–	–
	Lb. brevis SS:B2	–	–	–	–
	Lb. casei SS:B4	–	–	–	–
	Lb. brevis SS:B5	–	–	–	–
	Lb. brevis SS:B6	–	–	–	–
	Lb. brevis SS:B7	–	–	–	–
	Lb. plantarum SG:B1	–	–	–	–
	Lb. plantarum SG:B4	–	–	–	–
	Lb. plantarum SG:B5	–	–	–	–
	Lb. plantarum SG:B7	–	–	–	–

Tyr = Tyrosine, tyramine precursor; Lys = Lysine, cadaverine precursor; His = Histidine, histamine precursor; Orn = Ornithine, putrescine precursor.

Table 29. Screening of biogenic amine producing LAB strains from fermented bamboo shoot products

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
Ekung	*Lb. plantarum* Ek1	–	–	–	–
	Lb. plantarum Ek4	–	–	–	–
	Lb. plantarum Ek5	–	–	–	–
	Lb. plantarum Ek6	–	–	–	–
	Lb. plantarum Ek7	–	–	–	–
	Lb. brevis Ek8	–	–	–	–
	Lb. plantarum Ek10	–	–	–	–
	Lb. plantarum Ek11	–	–	–	–
	Lb. plantarum Ek12	–	–	–	–
	Lb. plantarum Ek15	–	–	–	–
	Lb. plantarum Ek18	–	–	–	–
	Lb. plantarum Ek19	–	–	–	–
	Lb. plantarum Ek20	–	–	–	–
	Lb. plantarum Ek21	–	–	–	–
	T. halophilus Ek 22	–	–	–	–
	Lb. plantarum Ek23	–	–	–	–
	Lb. plantarum Ek24	–	–	–	–
	Lb. plantarum Ek25	–	–	–	–
	T. halophilus Ek28	–	–	–	–
	Lb. plantarum Ek29	–	–	–	–
	Lb. casei Ek34	–	–	–	–
	Lb. casei Ek35	–	–	–	–
	Lb. plantarum Ek38	–	–	–	–
	Lb. plantarum Ek39	–	–	–	–
	Lb. plantarum Ek40	–	–	–	–
Eup	*Lb. plantarum* Ep81	–	–	–	–
	Lb. plantarum Ep82	–	–	–	–
	Lb. plantarum Ep84	–	–	–	–
	Lb. fermentum Ep85	–	–	–	–

Continued (Table 29)

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
	Lb. plantarum Ep86	–	–	–	–
	Lb. plantarum Ep87	–	–	–	–
	Lb. plantarum Ep89	–	–	–	–
	Lb. plantarum Ep90	–	–	–	–
	Lb. plantarum Ep91	–	–	–	–
	Lb. fermentum Ep92	–	–	–	–
	Lb. fermentum Ep93	–	–	–	–
	Lb. plantarum Ep94	–	–	–	–
	Lb. plantarum Ep95	–	–	–	–
	Lb. plantarum Ep96	–	–	–	–
Eup	*Lb. plantarum* Ep97	–	–	–	–
	Lb. plantarum Ep98	–	–	–	–
	Lb. plantarum Ep99	–	–	–	–
	Lb. plantarum Ep101	–	–	–	–
	Lb. plantarum Ep102	–	–	–	–
	Lb. plantarum Ep103	–	–	–	–
	Lb. plantarum Ep106	–	–	–	–
	Lb. plantarum Ep110	–	–	–	–
	Lb. plantarum Ep111	–	–	–	–
	Lb. plantarum Ep112	–	–	–	–
	Lb. plantarum Ep115	–	–	–	–
	Lb. plantarum Hr41	–	–	–	–
	Lb. plantarum Hr42	–	–	–	–
	Lb. plantarum Hr45	–	–	–	–
	Lb. plantarum Hr47	–	–	–	–
Hirring	*Lb. plantarum* Hr48	–	–	–	–
	Lb. plantarum Hr49	–	–	–	–
	Lb. plantarum Hr50	–	–	–	–
	Lb. plantarum Hr55	–	–	–	–
	Lc. lactis Hr56	–	–	–	–

Continued (Table 29)

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
	Lb. plantarum Hr57	–	–	–	–
	Lb. plantarum Hr58	–	–	–	–
	Lb. plantarum Hr59	–	–	–	–
	Lb. plantarum Hr60	–	–	–	–
	Lc. lactis Hr62	–	–	–	–
	Lb. plantarum Hr64	–	–	–	–
	Lb. plantarum Hr65	–	–	–	–
	Lb. plantarum Hr66	–	–	–	–
Hirring	*Lb. plantarum* Hr67	–	–	–	–
	Lb.plantarum Hr68	–	–	–	–
	Lb. plantarum Hr70	–	–	–	–
	Lb. plantarum Hr71	–	–	–	–
	Lb. plantarum Hr74	–	–	–	–
	Lb. plantarum Hr75	–	–	–	–
	Lb. plantarum Hr76	–	–	–	–
	Lb. plantarum Hr77	–	–	–	–
	Lb. plantarum Hr80	–	–	–	–
	Lb. plantarum MeL2	–	–	–	–
	Lb. brevis MeB4	–	–	–	+
	Lb. plantarum MeR1	–	–	–	–
	Lb. plantarum MeB3	–	–	–	–
	Lb. brevis MeR6	–	–	–	–
	Lb. brevis MeN3	–	–	–	–
Mesu	*Lb. brevis* MeTR	–	–	–	–
	Lb. brevis MeN7	–	–	–	–
	Lb. brevis MeN5	–	–	–	+
	Lb. plantarum MeL3	–	–	–	+
	Lb. plantarum MeBe3	–	–	–	–
	Lb. brevis MeN2	–	–	–	–
	Lb. plantarum MeNt1	–	–	–	–

Continued (Table 29)

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
Mesu	*Lb. plantarum* MeB1	–	–	–	–
	Lb. plantarum MeB2	–	–	–	–
	Lb. brevis MeNt2	–	–	–	–
	Lb. plantarum MeB5	–	–	–	–
	Lb. brevis MeNt3	–	–	–	–
	Lb. plantarum MeN4	–	–	–	–
	Lb. plantarum MeN6	–	–	–	–
	Lb. plantarum MeR2	–	–	–	–
	Lb. plantarum MeT1	–	–	–	–
	Lb. plantarum MeT2	–	–	–	–
	Lb. plantarum MeT3	–	–	–	–
	Leuc. citreum MeN8	–	–	–	–
	Leuc. citreum MeN9	–	–	–	–
	Leuc. citreum MeK1	–	–	–	–
	Leuc. citreum MeK2	–	–	–	–
	Lb. brevis MeR7	–	–	–	–
	Lb. curvatus MeK3	–	–	–	–
	P. pentosaceus MeB:C3	–	–	–	–
	P. pentosaceus MeB:C4	–	–	–	–
	P. pentosaceus MeT5	–	–	–	–
	P. pentosaceus MeT6	–	–	–	–
	P. pentosaceus MeT7	–	–	–	–
Soibum	*Leuc. lactis* SbB1	–	–	–	–
	Leuc. fallax SbC1	–	–	–	–
	Leuc. mesenteroides SbC2	–	–	–	–
	Lb. plantarum SbB2	–	–	–	–
	Lb. plantarum SbB3	–	–	–	–
	E. durans SbB4	–	–	–	–
	Lb. brevis SbB5	–	–	–	–
	Lb. brevis SbB6	–	–	–	–

Continued (Table 29)

Product	Strain	Amino acids			
		Tyr	Lys	His	Orn
Soidon	*Leuc. fallax* SdC1	–	–	–	–
	Leuc. lactis SdR2	–	–	–	+
	Lb. brevis SdR3	–	–	–	–
	Lb. brevis SdR4	–	–	–	–
	Lb. brevis SdR5	–	–	–	–
	Leuc. lactis SdB1	–	–	–	–
	Leuc. lactis SdB2	–	–	–	–
	Leuc. lactis SdB3	–	–	–	–
	Lb. brevis SdB4	–	–	–	–
	Leuc. lactis SdB5	–	–	–	–
Soijim	*Leuc. fallax* SjC1	–	–	–	–
	Leuc. lactis SjR3	–	–	–	–
	Lb. brevis SjR7	–	–	–	+
	Lb. brevis SjB1	–	–	–	–
	Leuc. fallax SjB2	–	–	–	–
	Leuc. lactis SjB4	–	–	–	–
	Leuc. lactis SjB5	–	–	–	–
	Leuc. lactis SjB8	–	–	–	–

Tyr = Tyrosine, tyramine precursor; Lys = Lysine, cadaverine precursor; His = Histidine, histamine precursor; Orn = Ornithine, putrescine precursor.

Hydrophobicity of the LAB strains

Tables 30 and 31 show the percentage hydrophobicity of the LAB strains. Out of 251 strains, 216 had less than 30 % hydrophobicity; 32 strains had in between 30-70 %, and 3 strains had more than 70 % hydrophobicity. *Lb. brevis* KG:B2, isolated from khalpi showed highest degree of hydrophobicity of 81 % (Table 31). *Lb. plantarum* MeL3, isolated from mesu showed 75 % of degree of hydrophobicity and *Lb. brevis* MeTR from mesu showed 72 % of hydrophobicity. Out of 51 strains of *Lb. brevis*, only 2 strains (KG:B2 from khalpi and MeTR from mesu) showed than 70 % hydrophobicity.

Table 30. Percentage hydrophobicity of LAB strains from fermented vegetable products

Product	Strain	% Hydrophobicity
	P. pentosaceus Go1	1.9 ± 0.22
	Lc. lactis Go5	3.2 ± 0.71
	Lb. plantarum Go6	6.4 ± 2.32
	Lc. lactis Go7	8.0 ± 0.93
	Lc. lactis Go8	5.3 ± 0.40
	E. faecium Go9	2.5 ± 0.89
	Lb. brevis Go11	2.0 ± 0.84
	Lb. plantarum Go12	63.4 ± 7.36
	Lb. plantarum Go14	55.2 ± 5.22
	Lb. plantarum Go16	30.4 ± 4.32
	P. pentosaceus Go17	46.3 ± 3.72
Goyang	*E. faecium* Go20	3.2 ± 0.95
	Lb. plantarum Go22	1.1 ± 0.40
	Lb. plantarum Go23	6.3 ± 3.36
	Lb. brevis Go24	10.6 ± 3.53
	Lb. plantarum Go25	8.8 ± 3.03
	P. pentosaceus Go29	23.1 ± 4.17
	Lb. plantarum Go30	6.5 ± 1.23
	Lb. plantarum Go31	7.2 ± 1.25
	E. faecium Go34	4.2 ± 1.72
	Lb. plantarum Go37	20.2 ± 2.1
	Lb. plantarum Go39	24.5 ± 2.5
	Lb. plantarum Go40	4.7 ± 1.39

Continued (Table 30)

Product	Strain	% Hydrophobicity
Goyang	*Lb. brevis* Go41	1.1 ± 0.29
	E. faecium Go43	3.1 ± 0.43
	E. faecium Go45	1.9 ± 0.37
Gundruk	*Lb. plantarum* GK:C1	27.7 ± 5.81
	P. pentosaceus GK:C2	14.5 ± 2.42
	P. pentosaceus GK:B3	16. 3 ± 2.23
	P. pentosaceus GK:B6	13.2 ± 1.91
	P. pentosaceus GK:C3	15.3 ± 1.84
	Lb. plantarum GLn:R1	13.8 ± 1.93
	Lb. plantarum GLn:R5	5.7 ± 0.86
	Lb. plantarum GLn:R6	23.1 ± 1.66
	P. pentosaceus GLn:C1	13.5 ± 1.52
	Lb. plantarum GLn:C2	1.8 ± 0.25
	P. pentosaceus GLn:C3	1.4 ± 0.22
	P. pentosaceus GLn:C5	7.6 ± 0.81
	P. pentosaceus GLn:R2	2.5 ± 1.09
	P. pentosaceus GLn:R3	1.9 ± 0.54
	Lb. plantarum GSd:R1	3.0 ± 1.18
	Lb. plantarum GSd:R4	7.5 ± 1.23
	Lb. plantarum GSd:R5	26.5 ± 1.61
	P. pentosaceus GSd:C1	2.8 ± 0.38
	P. pentosaceus GSd:C2	5.6 ± 1.12
	Lb. plantarum GS:R1	4.3 ± 1.13
	P. pentosaceus GS:B1	12.5 ± 2.4
	P. pentosaceus GS:B2	16.2 ± 1.4
	P. pentosaceus GS:B7	8.6 ± 0.89
	P. pentosaceus GS:C1	11.7 ± 1.48
	Lb. plantarum GLc:B1	22.7 ± 3.45
	P. pentosaceus GLc:C1	8.1 ± 1.75
	P. pentosaceus GLc:C4	8.9 ± 1.14

Continued (Table 30)

Product	Strain	% Hydrophobicity
Inziangsang	*Lb. brevis* IB1	2.7 ± 0.39
	Lb. plantarum IB2	0.5 ± 0.08
	Lb. plantarum IB3	3.8 ± 0.53
	P. acidilactici IB5	1.3 ± 0.16
	Lb. brevis IB9	8.3 ± 0.34
	Lb. plantarum IB11	10.1 ± 0.76
	Lb. plantarum IB12	7.6 ± 0.56
	Lb. plantarum IB13	18.3 ± 0.76
	P. acidilactici IB14	3.2 ± 0.34
Khalpi	*Lb. plantarum* KG:B1	1.5 ± 0.22
	Lb. brevis KG:B2	80.9 ± 6.38
	Lb. plantarum KG:B3	5.6 ± 1.53
	Lb. plantarum KG:B7	6.5 ± 0.40
	Lb. brevis KG:B8	60.9 ± 0.27
	Lb. plantarum KG:B9	4.1 ± 0.82
	Lb. brevis KG:R1	42.1 ± 2.62
	Lb. brevis KG:R2	34.1 ± 4.51
	Lb. plantarum KB:B4	5.3 ± 1.45
	Lb. brevis KB:B5	63.7 ± 3.49
	Leuc. fallax KB:C1	6.5 ± 0.46
	Lb. plantarum KB:B9	16.4 ± 2.12
	Lb. brevis KK:B2	66.6 ± 1.26
	Lb. plantarum KK:B3	23.0 ± 0.45
	Lb. plantarum KK:B4	10.2 ± 0.24
	Lb. plantarum KK:B5	8.2 ± 0.23
	Lb. brevis KK:R1	34.8 ± 1.52
	Lb. brevis KS:R1	18.3 ± 1.29
	Lb. brevis KS:R2	28.2 ± 0.89
	Lb. brevis KS:B1	24. ± 0.56
	Lb. plantarum KS:B2	40.3 ± 0.66
	Lb. plantarum KS:B9	33.5 ± 0.65

Continued (Table 30)

Product	Strain	% Hydrophobicity
	Leuc. fallax KS:C1	5.1 ± 0.23
Khalpi	*Leuc. fallax* KS:C2	6.9 ± 0.34
	Lb. brevis KBe:B2	4.6 ± 0.18
	Lb. brevis SL:B1	3.1 ± 0.25
	Lb. brevis SL:B2	35.0 ± 3.03
	Leuc. fallax SL:B3	10.1 ± 0.87
	Leuc. fallax SL:B4	10.6 ± 1.88
	Leuc. fallax SL:B5	9.3 ± 0.86
	Lb. brevis SL:B6	2.3 ± 0.28
	Lb. brevis SL:B7	14.5 ± 3.05
	Lb. brevis SL:B8	27.6 ± 1.28
	Lb. brevis SK:B1	2.9 ± 1.02
	Lb. brevis SK:B2	1.4 ± 0.30
	Lb. brevis SK:B3	1.3 ± 0.27
	Leuc. fallax SK:C1	2.2 ± 0.56
	Lb. plantarum SBe:B1	2.2 ± 1.69
Sinki	*Lb. brevis* SBe:B2	16.6 ± 0.87
	Lb. brevis SBe:B5	12.5 ± 0.45
	Lb. brevis SBe:B6	14.2 ± 0.42
	Lb. brevis SBe:B7	17.3 ± 0.45
	Lb. brevis SS:B1	12.8 ± 1.12
	Lb. brevis SS:B2	20.5 ± 1.22
	Lb. casei SS:B4	8.1 ± 1.04
	Lb. brevis SS:B5	32.2 ± 0.86
	Lb. brevis SS:B6	14.2 ± 0.52
	Lb. brevis SS:B7	10.2 ± 0.33
	Lb. plantarum SG:B1	17.5 ± 0.66
	Lb. plantarum SG:B4	26.0 ± 0.45
	Lb. plantarum SG:B5	22.4 ± 0.37
	Lb. plantarum SG:B7	22.1 ± 0.65

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

Table 31. Percentage hydrophobicity of LAB strains from fermented bamboo shoot products

Product	Strain	% Hydrophobicity
Ekung	*Lb. plantarum* Ek1	4.4 ± 0.32
	Lb. plantarum Ek4	1.6 ± 0.45
	Lb. plantarum Ek5	2.5 ± 0.52
	Lb. plantarum Ek6	1.9 ± 0.64
	Lb. plantarum Ek7	2.1 ± 0.34
	Lb. brevis Ek8	2.3 ± 0.33
	Lb. plantarum Ek10	7.2 ± 0.24
	Lb. plantarum Ek11	9.2 ± 0.34
	Lb. plantarum Ek12	6.3 ± 0.58
	Lb. plantarum Ek15	16.2 ± 0.76
	Lb. plantarum Ek18	12.3 ± 0.34
	Lb. plantarum Ek19	11.2 ± 0.71
	Lb. plantarum Ek20	6.1 ± 0.32
	Lb. plantarum Ek21	2.5 ± 0.43
	T. halophilus Ek 22	23.7 ± 0.51
	Lb. plantarum Ek23	43.7 ± 5.80
	Lb. plantarum Ek24	23.1 ± 2.1
	Lb. plantarum Ek25	13.4 ± 0.34
	T. halophilus Ek28	67.7 ± 5.01
	Lb. plantarum Ek29	4.2 ± 0.13
	Lb. casei Ek34	20.7 ± 4.19
	Lb. casei Ek35	27.2 ± 3.22
	Lb. plantarum Ek38	8.9 ± 0.23
	Lb. plantarum Ek39	7.4 ± 0.24
	Lb. plantarum Ek40	6.4 ± 0.32

Continued (Table 31)

Product	Strain	% Hydrophobicity
	Lb. plantarum Ep81	58.3 ± 1.67
	Lb. plantarum Ep82	37.4 ± 1.23
	Lb. plantarum Ep84	25.2 ± 1.11
	Lb. fermentum Ep85	4.3 ± 0.23
	Lb. plantarum Ep86	14.2 ± 1.22
	Lb. plantarum Ep87	24.8 ± 0.67
	Lb. plantarum Ep89	31.6 ± 2.13
	Lb. plantarum Ep90	32.4 ± 1.92
	Lb. plantarum Ep91	1.3 ± 0.25
	Lb. fermentum Ep92	2.6 ± 1.93
	Lb. fermentum Ep93	4.2 ± 0.39
	Lb. plantarum Ep94	7.8 ± 0.78
Eup	*Lb. plantarum* Ep95	15.7 ± 1.94
	Lb. plantarum Ep96	14.2 ± 2.01
	Lb. plantarum Ep97	6.4 ± 0.66
	Lb. plantarum Ep98	4.3 ± 0.34
	Lb. plantarum Ep99	7.2 ± 0.36
	Lb. plantarum Ep101	18.3 ± 0.78
	Lb. plantarum Ep102	14.4 ± 0.96
	Lb. plantarum Ep103	23.9 ± 0.92
	Lb. plantarum Ep106	23.5 ± 1.26
	Lb. plantarum Ep110	12.7 ± 1.32
	Lb. plantarum Ep111	14.2 ± 0.72
	Lb. plantarum Ep112	23.6 ± 0.76
	Lb. plantarum Ep115	4.2 ± 0.22

Continued (Table 31)

Product	Strain	% Hydrophobicity
Hirring	*Lb. plantarum* Hr41	0.9 ± 0.04
	Lb. plantarum Hr42	1.5 ± 0.06
	Lb. plantarum Hr45	3.2 ± 0.21
	Lb. plantarum Hr47	2.2 ± 0.22
	Lb. plantarum Hr48	5.4 ± 0.21
	Lb. plantarum Hr49	8.2 ± 0.56
	Lb. plantarum Hr50	31.8 ± 0.97
	Lb. plantarum Hr55	12.3 ± 0.25
	Lc. lactis Hr56	59.5 ± 2.49
	Lb. plantarum Hr57	32.4 ± 0.81
	Lb. plantarum Hr58	15.3 ± 0.34
	Lb. plantarum Hr59	3.2 ± 0.05
	Lb. plantarum Hr60	6.2 ± 0.07
	Lc. lactis Hr62	7.8 ± 0.09
	Lb. plantarum Hr64	4.2 ± 0.08
	Lb. plantarum Hr65	5.3 ± 0.09
	Lb. plantarum Hr66	18.0 ± 0.16
	Lb. plantarum Hr67	3.7 ± 0.89
	Lb. plantarum Hr68	9.2 ± 0.18
	Lb. plantarum Hr70	7.4 ± 0.93
	Lb. plantarum Hr71	23.2 ± 0.44
	Lb. plantarum Hr74	5.3 ± 0.38
	Lb. plantarum Hr75	7.2 ± 0.92
	Lb. plantarum Hr76	12.7 ± 0.34
	Lb. plantarum Hr77	3.2 ± 0.77
	Lb. plantarum Hr80	43.5 ± 3.21

Continued (Table 31)

Product	Strain	% Hydrophobicity
	Lb. plantarum MeL2	47.6 ± 4.34
	Lb. brevis MeB4	9.5 ± 1.03
	Lb. plantarum MeR1	31.2 ± 0.20
	Lb. plantarum MeB3	5.3 ± 1.40
	Lb. brevis MeR6	47.4 ± 1.37
	Lb. brevis MeN3	38.2 ± 2.21
	Lb. brevis MeTR	71.9 ± 8.89
	Lb. brevis MeN7	65.2 ± 7.31
	Lb. brevis MeN5	3.6 ± 0.22
	Lb. plantarum MeL3	74.7 ± 4.13
	Lb. plantarum MeBe3	5.2 ± 0.12
Mesu	*Lb. brevis* MeN2	3.4 ± 0.10
	Lb. plantarum MeNt1	4.4 ± 0.10
	Lb. plantarum MeB1	6.3 ± 0.20
	Lb. plantarum MeB2	9.2 ± 0.32
	Lb. brevis MeNt2	3.2 ± 0.11
	Lb. plantarum MeB5	4.5 ± 0.12
	Lb. brevis MeNt3	5.2 ± 0.12
	Lb. plantarum MeN4	4.7 ± 0.23
	Lb. plantarum MeN6	8.4 ± 0.52
	Lb. plantarum MeR2	28.4 ± 1.41
	Lb. plantarum MeT1	3.6 ± 0.10
	Lb. plantarum MeT2	8.2 ± 0.32

Continued (Table 31)

Product	Strain	% Hydrophobicity
Mesu	*Lb. plantarum* MeT3	7.3 ± 0.12
	Leuc. citreum MeN8	3.2 ± 0.09
	Leuc. citreum MeN9	6.5 ± 0.09
	Leuc. citreum MeK1	5.5 ± 0.10
	Leuc. citreum MeK2	2.3 ± 0.08
	Lb. brevis MeR7	26.7 ± 0.23
	Lb. curvatus MeK3	1.4 ± 0.05
	P. pentosaceus MeB:C3	0.9 ± 0.09
	P. pentosaceus MeB:C4	1.9 ± 0.22
	P. pentosaceus MeT5	1.8 ± 0.12
	P. pentosaceus MeT6	2.2 ± 0.08
	P. pentosaceus MeT7	4.3 ± 0.09
Soibum	*Leuc. lactis* SbB1	6.6 ± 0.58
	Leuc. fallax SbC1	5.7 ± 2.56
	Leuc. mesenteroides SbC2	1.2 ± 0.08
	Lb. plantarum SbB2	6.3 ± 0.56
	Lb. plantarum SbB3	7.2 ± 0.24
	E. durans SbB4	6.1 ± 0.11
	Lb. brevis SbB5	3.5 ± 0.09
	Lb. brevis SbB6	4.2 ± 0.10
Soidon	*Leuc. fallax* SdC1	14.2 ± 4.39
	Leuc. lactis SdR2	57.0 ± 5.05
	Lb. brevis SdR3	8.8 ± 0.83
	Lb. brevis SdR4	4.3 ± 0.57
	Lb. brevis SdR5	25.2 ± 0.72

Continued (Table 31)

Product	Strain	% Hydrophobicity
Soidon	*Leuc. lactis* SdB1	5.2 ± 0.08
	Leuc. lactis SdB2	2.8 ± 0.08
	Leuc. lactis SdB3	2.1 ± 0.12
	Lb. brevis SdB4	4.2 ± 0.87
	Leuc. lactis SdB5	4.2 ± 0.43
Soijim	*Leuc. fallax* SjC1	3.6 ± 0.41
	Leuc. lactis SjR3	7.5 ± 1.04
	Lb. brevis SjR7	52.8 ± 8.41
	Lb. brevis SjB1	41.2 ± 4.6
	Leuc. fallax SjB2	3.2 ± 1.1
	Leuc. lactis SjB4	2.6 ± 0.09
	Leuc. lactis SjB5	3.7 ± 0.09
	Leuc. lactis SjB8	6.6 ± 0.12

Data represents the means (± SD) of 4 sets of experiment.

Fermentation dynamics *in situ*

Successional studies of LAB and other non-lactics, and their effect on pH and acidity of fermenting vegetables were studied. Two types of gundruk were prepared following the traditional method on the basis of raw materials; leaves of 'rayo-saag' and 'mula-saag' (leaves of radish), as mentioned in the materials and methods section. The temperature of fermenting 'rayo-saag' leaves remained around 18-20° C (Table 32). The pH dropped significantly ($p<0.05$) from 6.6 to 3.7 till the end of the fermentation. Increase in titratable acidity % (as lactic acid) was significant ($p<0.05$) during fermentation. Exponential increase in LAB population was significant ($p<0.05$) till 3rd day detecting at the level of almost 10^9 cfu/g (Fig 21). The LAB population after 3rd day, gradually decreased to a level of 10^7 cfu/g at the end. The population of yeast decreased significantly ($p<0.05$) from 10^3 cfu/g in raw leaves of 'rayo-saag' and disappeared after 3rd day. Significant ($p<0.05$) decrease in the beginning and disappearance of the population of *Staphylococcus aureus* and enterobacteriaceae was observed during fermentation. *Bacillus cereus* was not detected in raw 'rayo-saag' (Table 32).

As in 'rayo-saag' gundruk, the temperature of fermenting 'mula-saag' was around 18-20° C (Table 33). Significant ($p<0.05$) decrease in pH from 6.6 to 3.8 of fermenting radish leaves was observed during fermentation. Similarly, acidity also increased significantly ($p<0.05$) during fermentation. The load of LAB increased significantly ($p<0.05$) during

fermentation detecting at the level of 10^7 cfu/g at the end. Interestingly, maximum exponential growth of LAB was observed in 'rayo-saag' than in 'mula-saag' gundruk (Fig 21). During lactic fermentation, the population of the contaminating bacteria such as *Bacillus cereus, Staphylococcus aureus* and enterobacteriaceae, and also yeasts in raw radish leaves were disappeared till the final product is made (Fig 21).

Microbial changes in cucumber during khalpi fermentation was also studied (Table 34). Difference in temperature of the fermenting cucumbers during khalpi fermentation was not significant ($p<0.05$). The pH decreased significantly ($p<0.05$) from 5.6 to 3.2 and acidity % increased significantly ($p<0.05$) from 0.28 % to 1.24 at end of fermentation. Population of LAB increased significantly ($p<0.05$) from 10^3 cfu/g to 10^8 cfu/g till 36 hours, and then remained at the level of 10^7 cfu/g in the final product (Table 34). Microbial load of yeast in raw cucumber was 10^4 cfu/g, which disappeared after 48 hour. Load of *Staphylococcus aureus* and enterobacteriaceae reduced significantly ($p<0.05$) and disappeared during fermentation (Fig 22).

Table 32. Succession studies in 'rayo-saag' gundruk fermentation

Fermentation Time (Day)	Temperature (°C)	pH	Acidity (%)	Microbial load (log cfu/g)				
				LAB	Yeast	*Bacillus cereus*	*Staphylococcus aureus*	Enterobacteriaceae
0	18 ± 0.6^{a}	6.6 ± 0.1^{a}	0.05 ± 0.02^{m}	4.5 ± 0.2^{h}	3.1 ± 0.2^{a}	0	4.6 ± 0.1^{a}	4.7 ± 0.1^{a}
1	19 ± 1.0^{a}	5.9 ± 0.1^{b}	0.18 ± 0.07^{l}	7.2 ± 0.2^{g}	2.2 ± 0.1^{b}	0	4.5 ± 0.2^{b}	4.2 ± 0.3^{b}
2	19 ± 2.1^{a}	5.4 ± 0.1^{c}	0.30 ± 0.10^{k}	8.3 ± 0.1^{c}	$<DL^{c}$	0	4.2 ± 0.2^{c}	3.9 ± 0.1^{c}
3	20 ± 1.6^{a}	5.3 ± 0.1^{cd}	0.42 ± 0.09^{j}	8.9 ± 0.1^{a}	0	0	4.1 ± 0.1^{cd}	3.6 ± 0.2^{d}
4	20 ± 1.0^{a}	5.2 ± 0.1^{ce}	0.68 ± 0.06^{i}	8.9 ± 0.2^{a}	0	0	3.3 ± 0.2^{e}	3.0 ± 0.1^{e}
5	20 ± 1.5^{a}	5.0 ± 0.1^{ef}	0.70 ± 0.08^{h}	8.9 ± 0.1^{a}	0	0	3.0 ± 0.1^{f}	2.5 ± 0.1^{f}
6	20 ± 1.0^{a}	4.0 ± 0.1^{g}	0.75 ± 0.04^{g}	8.5 ± 0.2^{b}	0	0	2.3 ± 0.2^{g}	1.8 ± 0.1^{g}
7	20 ± 1.5^{a}	3.8 ± 0.1^{gij}	0.80 ± 0.10^{bf}	7.9 ± 0.1^{d}	0	0	$<DL^{h}$	1.2 ± 0.3^{h}
8	20 ± 1.0^{a}	3.8 ± 0.1^{gij}	0.90 ± 0.05^{be}	7.9 ± 0.1^{d}	0	0	0	$<DL^{i}$
9	20 ± 1.0^{a}	3.8 ± 0.1^{gij}	0.92 ± 0.05^{bd}	7.9 ± 0.2^{d}	0	0	0	0
10	20 ± 1.0^{a}	3.8 ± 0.1^{gij}	0.94 ± 0.06^{bc}	7.9 ± 0.1^{d}	0	0	0	0
11	20 ± 1.0^{a}	3.8 ± 0.1^{gij}	0.95 ± 0.05^{b}	7.8 ± 0.1^{de}	0	0	0	0
12	20 ± 0.6^{a}	3.7 ± 0.1^{j}	1.20 ± 0.09^{a}	7.8 ± 0.1^{de}	0	0	0	0
13	20 ± 0.6^{a}	3.7 ± 0.1^{j}	1.20 ± 0.08^{a}	7.8 ± 0.1^{de}	0	0	0	0
14	20 ± 1.0^{a}	3.7 ± 0.1^{j}	1.20 ± 0.08^{a}	7.8 ± 0.1^{de}	0	0	0	0
15	19 ± 1.0^{a}	3.7 ± 0.1^{j}	1.20 ± 0.09^{a}	7.6 ± 0.1^{f}	0	0	0	0
16	20 ± 1.5^{a}	3.7 ± 0.1^{j}	1.20 ± 0.09^{a}	7.6 ± 0.1^{f}	0	0	0	0

Data represents the means (±SD) of 3 sets of experiment. Values bearing different superscripts in each column differ significantly ($p<0.05$).

DL, Less than detection limit (10 cfu/g).

Mould was not detected.

Table 33. Succession studies in 'mula-saag' gundruk fermentation

Fermentation Time (Day)	Temperature (^{0}C)	pH	Acidity (%)	Microbial load (Log cfu/g)				
				LAB	Yeast	*Bacillus cereus*	*Staphylococcus aureus*	Enterobacteriaceae
0	18 ± 1.0^{a}	6.6 ± 0.1^{a}	0.03 ± 0.01^{ef}	4.2 ± 0.2^{c}	3.3 ± 0.1^{a}	1.5 ± 0.1^{a}	3.3 ± 0.3^{a}	4.6 ± 0.5^{a}
1	20 ± 1.0^{a}	5.1 ± 0.1^{b}	0.11 ± 0.06^{e}	6.5 ± 0.3^{b}	3.1 ± 0.2^{b}	<DL^{b}	2.3 ± 0.2^{b}	3.6 ± 0.3^{bc}
2	20 ± 2.0^{a}	4.7 ± 0.1^{c}	0.43 ± 0.04^{d}	8.0 ± 0.2^{a}	3.1 ± 0.1^{b}	0	2.0 ± 0.3^{c}	3.4 ± 0.3^{c}
3	20 ± 1.0^{a}	4.5 ± 0.1^{d}	0.64 ± 0.04^{c}	8.5 ± 0.2^{a}	2.0 ± 0.1^{c}	0	2.0 ± 0.1^{c}	2.8 ± 0.2^{de}
4	19 ± 1.0^{a}	4.2 ± 0.2^{e}	0.78 ± 0.09^{bc}	8.5 ± 0.3^{a}	<DL^{d}	0	1.8 ± 0.1^{d}	2.5 ± 0.3^{ef}
5	19 ± 1.0^{a}	4.0 ± 0.1^{f}	0.85 ± 0.08^{ac}	8.5 ± 0.1^{a}	0	0	1.5 ± 0.2^{e}	2.1 ± 0.2^{fg}
6	19 ± 1.0^{a}	3.9 ± 0.1^{fg}	0.90 ± 0.06^{ac}	8.4 ± 0.2^{a}	0	0	<DL^{f}	1.9 ± 0.1^{g}
7	19 ± 1.0^{a}	3.8 ± 0.1^{gh}	0.92 ± 0.08^{ab}	8.4 ± 0.2^{a}	0	0	0	1.7 ± 0.1^{gh}
8	19 ± 1.0^{a}	3.8 ± 0.1^{gh}	0.94 ± 0.04^{a}	8.3 ± 0.3^{a}	0	0	0	<DL^{i}
9	19 ± 1.0^{a}	3.8 ± 0.1^{gh}	0.94 ± 0.04^{a}	8.4 ± 0.2^{a}	0	0	0	0
10	20 ± 1.0^{a}	3.7 ± 0.1^{hi}	0.97 ± 0.13^{a}	8.4 ± 0.1^{a}	0	0	0	0
11	20 ± 1.0^{a}	3.7 ± 0.1^{hi}	0.97 ± 0.15^{a}	8.2 ± 0.3^{a}	0	0	0	0
12	20 ± 1.0^{a}	3.8 ± 0.1^{gh}	0.97 ± 0.13^{a}	8.1 ± 0.1^{a}	0	0	0	0
13	20 ± 1.0^{a}	3.8 ± 0.1^{gh}	0.96 ± 0.11^{a}	7.9 ± 0.1^{a}	0	0	0	0
14	20 ± 1.0^{a}	3.8 ± 0.1^{gh}	0.96 ± 0.11^{a}	7.8 ± 0.1^{a}	0	0	0	0
15	20 ± 1.0^{a}	3.8 ± 0.1^{gh}	0.96 ± 0.08^{a}	7.6 ± 0.1^{a}	0	0	0	0
16	20 ± 1.0^{a}	3.8 ± 0.1^{gh}	0.96 ± 0.04^{a}	7.4 ± 0.4^{a}	0	0	0	0

Data represents the means (±SD) of 3 sets of experiment. Values bearing different superscripts in each column differ significantly ($p<0.05$).

DL, Less than detection limit (10 cfu/g).

Mould was not detected.

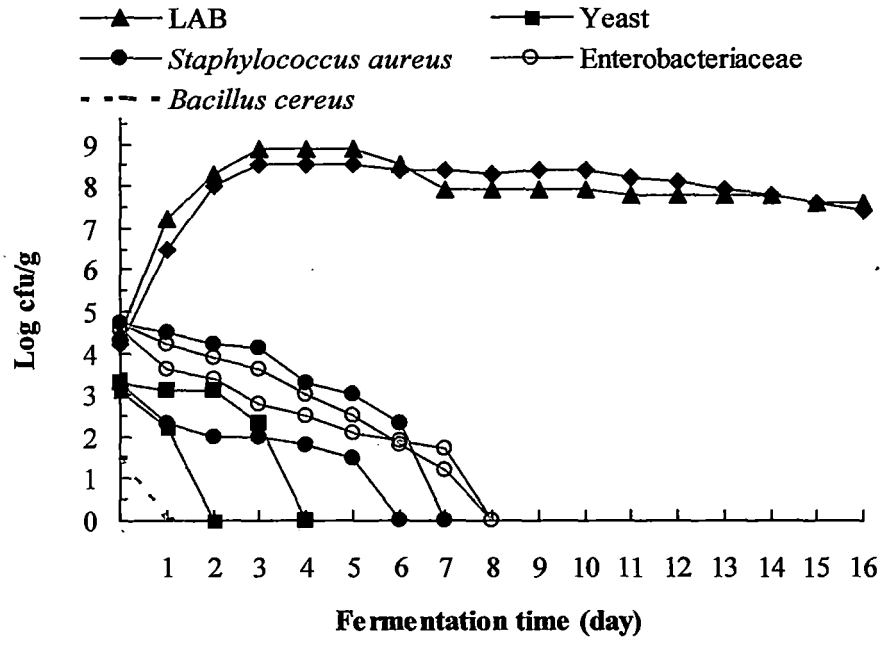


Fig 21. Changes in microbial load during gundruk fermentation. Red lines indicate 'rayo-saag' gundruk prepared from leaves of 'rayo-saag' and green lines indicate 'mula-saag' gundruk prepared from radish leaves.

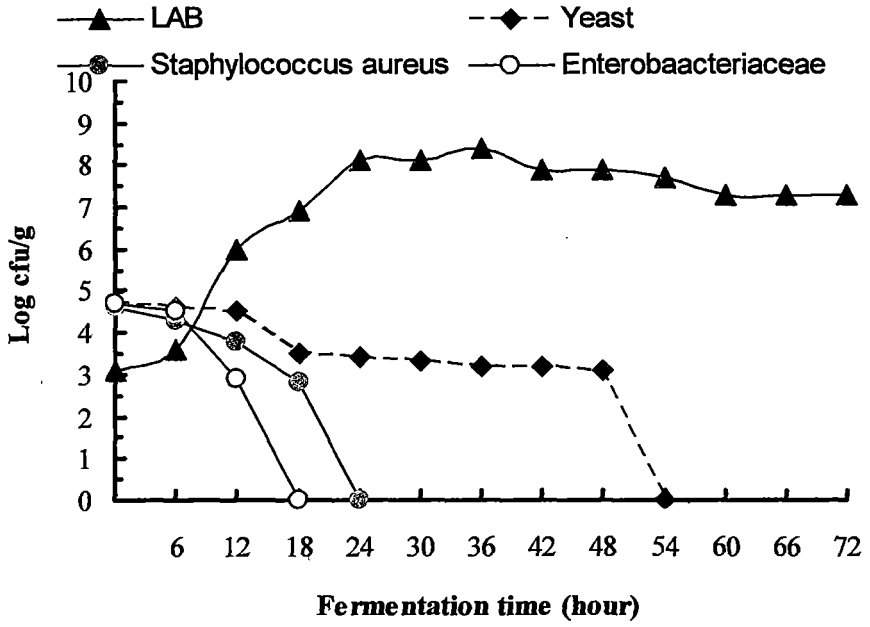


Fig 22. Changes in microbial load during khalpi fermentation.

Table 34. Succession studies in khalpi fermentation

Fermentation Time (Hour)	Temperature (^{0}C)	pH	Titratable Acidity (%)	Microbial load (log cfu/g)				
				LAB	Yeast	*Bacillus cereus*	*Staphylococcus aureus*	Enterobacteriaceae
0	23 ± 1.0^a	5.6 ± 0.2^a	0.28 ± 0.03^e	3.1 ± 0.1^i	4.7 ± 0.1^a	0	4.6 ± 0.1^a	4.7 ± 0.1^a
6	23 ± 1.0^a	5.5 ± 0.1^a	0.41 ± 0.04^d	3.6 ± 0.1^h	4.6 ± 0.1^a	0	4.3 ± 0.1^b	4.5 ± 0.1^a
12	24 ± 1.0^a	5.3 ± 0.1^b	0.57 ± 0.08^c	6.0 ± 0.1^g	4.5 ± 0.1^a	0	3.8 ± 0.1^c	2.9 ± 0.1^b
18	24 ± 1.0^a	5.1 ± 0.1^c	0.60 ± 0.07^c	6.9 ± 0.1^f	3.5 ± 0.1^b	0	2.8 ± 0.1^d	<DL^c
24	24 ± 1.0^a	4.4 ± 0.2^d	0.78 ± 0.06^b	8.1 ± 0.1^b	3.4 ± 0.2^b	0	<DL^e	0
30	24 ± 1.0^a	3.9 ± 0.2^e	0.80 ± 0.06^b	8.1 ± 0.1^b	3.3 ± 0.2^{bc}	0	0	0
36	24 ± 1.0^a	3.9 ± 0.1^e	0.88 ± 0.06^b	8.4 ± 0.1^a	3.2 ± 0.2^c	0	0	0
42	24 ± 1.0^a	3.8 ± 0.1^f	0.90 ± 0.04^b	7.9 ± 0.1^c	3.2 ± 0.1^c	0	0	0
48	24 ± 1.0^a	3.4 ± 0.1^g	0.95 ± 0.08^b	7.9 ± 0.1^c	3.1 ± 0.1^{cd}	0	0	0
54	23 ± 2.0^a	3.3 ± 0.1^h	1.20 ± 0.11^a	7.7 ± 0.1^d	<DL^e	0	0	0
60	23 ± 1.0^a	3.2 ± 0.1^i	1.23 ± 0.04^a	7.3 ± 0.1^e	0	0	0	0
66	24 ± 1.0^a	3.2 ± 0.1^i	1.24 ± 0.05^a	7.3 ± 0.1^e	0	0	0	0
72	24 ± 1.0^a	3.2 ± 0.1^i	1.24 ± 0.08^a	7.3 ± 0.1^e	0	0	0	0

Data represents the means (±SD) of 3 sets of experiment. Values bearing different superscripts in each column differ significantly ($p<0.05$).

DL, Less than detection limit (10 cfu/g).

Mould was not detected.

Gundruk and Khalpi preparation by selected starter cultures

On the basis of superior technological properties of LAB strains such as acidification ability, antagonistic properties, non-production of biogenic amines (absence of decarboxylase activity in BA medium), ability to degrade antinutritive factors such as phytic acid, raffinose and stachyose, and even high degree of hydrophobicity, *Lactobacillus plantarum* GLn:R1 and *Pediococcus pentosaceus* GLn:R2 were selected as a culture cultures for production of gundruk, as described in materials and methods. Wilted leaves of 'rayo-saag' (*Brassica campestris* var. *cumifolia*) were inoculated with a mixture of pure culture strains of *Lactobacillus plantarum* GLn:R1 and *Pediococcus pentosaceus* GLn:R2. Gundruk prepared by a starter culture was evaluated organoleptically. With respect to general acceptability, 6 day-old gundruk fermented at 20° C had the highest score with better aroma, acidic taste typical of gundruk and thus acceptable to consumers (Table 35). Significant ($p<0.05$) difference in aroma and taste, typical to gundruk, was found on 6 day-old gundruk.

Lactobacillus plantarum KG:B1, *Lactobacillus brevis* KG:B2 and *Leuconostoc fallax* KB:C1 previously isolated from khalpi, were selected, on the basis of superior technological properties such as acidifying capacity, antimicrobial activities, non-production of biogenic amines and their ability to degrade antinutritive factors of the raw materials. Khalpi produced with a mixed pure culture at 20° C for 72 hour had highest score

in aroma while those fermented at 25° C had highest score of aroma within 48 hour and at 30° C no significant ($p<0.05$) differences was observed, though the aroma was higher within 24 hour compared at other temperatures (Table 36). There was significant ($p<0.05$) increase in taste score with time at 20° C and had score of 4.5 at 72 hour while at 30° C it took just 48 hour to reach the same level. The highest score ($p<0.05$) in taste (strongly acidic) was observed at 72 hour fermented at 30° C. Decrease in pH and increase in acidity was faster at higher temperature. General acceptability score was highest in khalpi fermented at 20° C for 72 hour. Khalpi produced at 25° C was also more or less similar within 48 hour while at 30° C strongly acidic taste developed quickly and not preferred.

Table 35. Sensory evaluation of gundruk prepared from leaves of 'rayo-saag' using a mixed pure culture strains*

Fermentation period (day)	Attribute					pH	Titratable acidity %
	Aroma	Taste	Texture	Colour	General acceptability		
Fermentation at 20^{0}C							
3	3.8 ± 0.3^b	3.9 ± 0.3^b	3.6 ± 0.3^a	3.8 ± 0.3^a	3.8 ± 0.5^{ab}	4.3 ± 0.29^c	0.52 ± 0.09^b
6	4.6 ± 0.5^a	4.9 ± 0.3^a	3.1 ± 0.2^a	3.8 ± 0.5^a	4.4 ± 0.5^a	4.0 ± 0.13^d	0.75 ± 0.11^a
Fermentation at 25^{0}C							
3	3.0 ± 0.4^c	2.9 ± 0.5^c	3.5 ± 0.4^a	3.8 ± 0.5^a	2.8 ± 0.3^c	4.6 ± 0.20^{ab}	0.40 ± 0.06^{bd}
6	3.9 ± 0.4^b	3.5 ± 0.5^{bc}	3.4 ± 0.5^a	3.6 ± 0.5^a	3.5 ± 0.5^b	4.4 ± 0.20^{bc}	0.50 ± 0.10^{bc}
Fermentation at 30^{0}C							
3	1.4 ± 0.3^d	1.1 ± 0.3^e	3.6 ± 0.3^a	3.5 ± 0.4^a	1.3 ± 0.3^d	4.8 ± 0.12^a	0.37 ± 0.10^{cd}
6	1.3 ± 0.5^d	1.5 ± 0.8^{de}	3.9 ± 0.4^a	2.8 ± 0.9^b	1.3 ± 0.5^d	4.4 ± 0.20^{bc}	0.48 ± 0.08^{bd}

Market gundruk was used as control (score 3), score 1, bad; score 5, good.

Data represents the mean scores ± SD (n = 7). Values bearing different superscripts in each column differ significantly ($p<0.05$).

*Mixture of *Lb. plantarum* GLn:R1 and *P. pentosaceus* GLn:R2.

Table 36. Sensory evaluation of khalpi prepared from cucumber using a mixed pure culture strains*

Fermentation period (hour)	Attribute					pH	Titratable acidity %
	Aroma	Taste	Texture	Colour	General acceptability		
Fermentation at 20^0C							
24	1.5 ± 0.6^{c}	1.5 ± 0.6^{d}	2.1 ± 0.3^{fg}	3.8 ± 0.5^{a}	1.1 ± 0.3^{e}	4.3 ± 0.05^{a}	0.57 ± 0.01^{fg}
48	4.3 ± 0.5^{ad}	2.8 ± 0.5^{bc}	2.5 ± 0.6^{def}	3.5 ± 0.6^{a}	2.3 ± 0.5^{c}	4.1 ± 0.10^{b}	0.68 ± 0.01^{d}
72	4.8 ± 0.5^{a}	4.5 ± 0.6^{a}	3.5 ± 0.6^{b}	3.5 ± 0.6^{a}	4.8 ± 0.5^{a}	3.8 ± 0.02^{c}	0.95 ± 0.02^{a}
Fermentation at 25^0C							
24	1.5 ± 0.6^{c}	2.5 ± 0.6^{c}	2.1 ± 0.3^{fg}	3.8 ± 0.5^{a}	1.3 ± 0.5^{de}	4.3 ± 0.05^{a}	0.56 ± 0.01^{g}
48	4.3 ± 0.5^{ad}	4.3 ± 0.5^{a}	2.3 ± 0.5^{eg}	3.8 ± 0.5^{a}	4.5 ± 0.6^{ab}	3.8 ± 0.04^{c}	0.70 ± 0.04^{cd}
72	3.3 ± 0.5^{bd}	4.5 ± 0.6^{a}	3.1 ± 0.3^{bcde}	3.8 ± 0.5^{a}	3.5 ± 0.6^{b}	3.8 ± 0.04^{c}	0.78 ± 0.02^{b}
Fermentation at 30^0C							
24	3.8 ± 0.5^{ab}	2.8 ± 0.5^{b}	2.1 ± 0.3^{fg}	3.5 ± 0.6^{a}	2.3 ± 0.5^{c}	4.1 ± 0.03^{b}	0.58 ± 0.02^{efg}
48	3.8 ± 0.5^{ab}	4.5 ± 0.6^{a}	3.5 ± 0.6^{b}	3.5 ± 0.6^{a}	3.5 ± 0.6^{b}	3.6 ± 0.0^{d}	0.94 ± 0.02^{a}
72	3.8 ± 0.5^{ab}	4.8 ± 0.5^{a}	4.5 ± 0.6^{a}	3.8 ± 0.5^{a}	2.3 ± 0.5^{cd}	3.6 ± 0.03^{d}	0.97 ± 0.01^{a}

Market khalpi was used as control (score 3), score 1, bad; score 5, good.

Data represents the mean scores ± SD (n = 7). Values bearing different superscripts in each column differ significantly ($p<0.05$).

*Mixture of *Lb. plantarum* KG:B1, *Lb. brevis* KG:B2 and *Leuc. fallax* KB:C1.

Proximate composition

The proximate composition of freshly prepared wet samples of goyang, khalpi, ekung, hirring, mesu, soibum and soidon and sun-dried market samples of gundruk, sinki, inziangsang and eup was analysed to know nutritive value (Table 37 and 38). Food value and limited mineral contents (calcium, sodium and potassium) were also determined. In all samples food value ranged from 321.9-363.1 kcal/100g dry matter. Calcium, sodium and potassium contents were high in fermented leafy vegetables than fermented bamboo products (Table 37).

Table 37. Proximate composition of fermented vegetables products collected from different markets of North East India

Parameter	Product				
	Goyang[1] (n = 20)	Gundruk[2] (n = 25)	Inziangsang[3] (n = 11)	Khalpi[4] (n = 25)	Sinki[5] (n = 21)
Titratable acidity % (as lactic acid)	0.13 ± 0.04	0.49 ± 0.12	0.50 ± 0.07	0.95 ± 0.09	0.65 ± 0.1
Moisture %	92.5 ± 3.0	15.0 ± 1.8	17.6 ± 0.8	91.4 ± 1.0	22.8 ± 1.2
Ash (% DM)	12.9 ± 1.7	22.2 ± 1.4	16.9 ± 0.3	14.2 ± 2.2	15.6 ± 0.9
Fat (% DM)	2.1 ± 0.1	2.1 ± 0.2	3.2 ± 0.6	2.6 ± 0.5	1.4 ± 0.2
Protein (% DM)	35.9 ± 1.5	37.4 ± 0.9	38.7 ± 0.8	12.3 ± 1.6	14.9 ± 0.7
Carbohydrate (% DM)	48.9 ± 2.8	38.3 ± 1.9	41.2 ± 2.2	70.9 ± 3.6	68.0 ± 2.1
Food value (Kcal/100 g DM)	357.2 ± 9.9	321.9 ± 3.5	348.4 ± 2	356.2 ± 1.5	344.2 ± 1.6
Minerals (mg/100g)					
Ca	92.2 ± 2.0	234.6 ± 5.2	240.4 ± 3.6	6.4 ± 0.3	223.9 ± 0.7
Na	6.7 ± 1.1	142.2 ± 2.0	133.7 ± 4.2	2.2 ± 0.2	737.3 ± 2.6
K	268.4 ± 6.6	677.6 ± 6.8	658.4 ± 6.7	125.1 ± 2.3	2320.4 ± 4.8

n, total number of samples (n) collected from each source is given in parenthesis.

DM, dry matter.

Data represents the means (± SD) of triplicate of each sample.

[1]Samples collected from Toong (5), Tshangu (7), Chipsu (8).

[2]Samples collected from Khamdong (5), Lingmoo (5), Samdong (5), Sumbuk (5) and Lungchok (5).

[3]Samples collected from Bishnupur (6) and Imphal (5).

[4]Samples collected from Bermiok (5), Geyzing (5), Bhusuk (5), Khamdong (5) and Sumbuk (5).

[5]Samples collected from Luing (6), Khamdong (6), Bermiok (3), Geyzing (3) and Sumbuk (3).

Table 38. Proximate composition of fermented bamboo shoot products collected from different places in North East India

Parameter	Product					
	Ekung[1] (n = 16)	Eup[2] (n = 13)	Hirring[3] (n = 15)	Mesu[4] (n = 46)	Soibum[5] (n = 12)	Soidon[6] (n = 11)
Titratable acidity % (as lactic acid)	0.94 ± 0.1	0.80 ± 0.2	0.81 ± 0.2	0.88 ± 0.1	0.98 ± 0.3	0.96 ± 0.1
Moisture %	94.7 ± 0.7	36.8 ± 0.2	88.8 ± 0.5	89.9 ± 0.2	92.0 ± 0.4	92.2 ± 0.5
Ash (% DM)	14.0 ± 1.3	18.2 ± 1.8	15.0 ± 1.2	15.0 ± 1.3	13.3 ± 0.2	13.1 ± 4.0
Fat (% DM)	3.8 ± 1.7	3.1 ± 0.1	2.7 ± 1.1	2.6 ± 0.8	3.2 ± 0.2	3.1 ± 0.2
Protein (% DM)	30.1 ± 1.7	33.6 ± 4.0	33.0 ± 4.4	27.0 ± 2.7	36.3 ± 0.3	37.2 ± 0.3
Carbo-hydrate (% DM)	52.1 ± 6.2	45.1 ± 4.2	49.3 ± 8.8	55.6 ± 4.8	47.2 ± 0.4	46.6 ± 0.4
Food value (Kcal /100g DM)	363.0 ± 5.8	342.7 ± 6.3	353.5 ± 6.5	352.4 ± 6.3	362.8 ± 4.5	363.1 ± 4.6
Minerals (mg/100g)						
Ca	35.4 ± 6.5	76.9 ± 9.3	19.3 ± 6.1	7.9 ± 0.4	16.0 ± 0.7	18.5 ± 0.9
Na	10.9 ± 3.2	3.4 ± 0.7	3.4 ± 0.8	2.8 ± 1.1	2.9 ± 0.9	3.7 ± 0.7
K	168.6 ± 7.2	1815.6 ±7.5	272.4 ± 6.8	282.6 ± 3.2	212.1 ± 0.9	245.5 ± 0.8

n, total number of samples (n) collected from each source is given in parenthesis.

DM, dry matter.

Data represents the means (± SD) of triplicate of each sample.

[1]Samples collected from Itanagar (5), Naharlogun (6) and Ziro (5).

[2]Samples collected from Itanagar (6), Naharlogun (3) and Ziro (4).

[3]Samples collected from Itanagar (4), Naharlogun (5) and Ziro (6).

[4]Samples collected from Navey (5), Naitam (4), Biring (6), Lingtam (7), Ragdong (6), Tintek (8), Ranka (5), Bermiok (5).

[5]Samples collected from Bishnupur (6) and Imphal (6).

[6]Samples collected from Bishnupur (7) and Imphal (4).

DISCUSSION

Traditional Preservation

Indigenous knowledge of the ethnic people living in the North East states of India on production of fermented vegetable products including fermented bamboo shoots was worth documenting, both as low-cost functional foods, and for socio-cultural reasons. Gundruk and sinki are important fermented non-salted vegetable foods in the local diet of the Sikkim Himalayas, and are prepared at households. Gundruk and sinki production practiced by the ethnic people of Sikkim is a unique type of biopreservation of foods through lactic acid fermentation. It is usually prepared during winter when perishable leafy vegetables are plenty in the sub-Himalayan regions. Invention of biopreservation methods by the ethnic people of the Himalayan regions through pit fermentation or lactic acid fermentation is significant due to transforming of the availability of raw materials at a particular season to those of deficit. Moreover, there is a remarkable step to store the perishable vegetable in absence of cold-storage or refrigeration, where majority of rural people cannot afford canned or frozen foods.

Sun drying of freshly prepared gundruk, sinki and inziangsang is a traditional preserving method by which the shelf life of the products is prolonged. Dried products are preserved for several months without refrigeration and consumed during long monsoon season when fresh vegetables are scarce. Dry gundruk, sinki and inziangsang are comparatively lighter than the weight of fresh substrates and can therefore, be carried easily while traveling. People might have invented such preservation technique to feed themselves while traveling for long

distances, and also to store for future use whenever vegetables were in plenty. Carrying gundruk, sinki, inziangsang and other fermented products such as dried kinema and hawaijar (fermented soybeans), hard chhurpi (yak milk cheese-like product), sidra, sukuti, karoti (dried and smoked fish), ngari and tungtap (fermented fish products), etc. is still common practice in North East India while traveling for long distances (Tamang, 2005b). Because of the acidic taste, gundruk, sinki and inziangsang are said to be good appetizers, and the ethnic people use these foods for remedies from indigestion. Goyang is a lesser-known fermented vegetable food of the Sherpas living in high altitude in the Sikkim Himalayas. Fermentation of cucumber is observed only in Sikkim among the North East states, where the lesser-known product called khalpi is consumed as pickle. All these ethnic fermented foods have important bearing in the dietary habits of the people of North East India.

Young succulent shoots of the bamboo species are mostly used as an edible delicacy by different tribes of North East India (Sharma, 1989), and some as fermented products (Tamang, 2001). Several authors while reporting on the detailed taxonomical and ecological aspects of bamboos of North East India, did mention the use of bamboos as foods, such as soidon and soibum of Manipur (Giri and Janmejay, 1987), ekung and eup of Arunachal Pradesh (Basar and Bisht, 2002), kharisa and bah gazar of Assam (Singh, 2002), syrwa of Meghalaya (Bhatt *et al.*, 2003), zusem and zutsuk of Nagaland (Bhatt *et al.*, 2005) and rep of Mizoram (Bhatt *et al.*, 2003). However, most of these traditional fermented bamboo shoot products of North East India have not been studied in regards to

microbiology except mesu (Tamang, 1992; Tamang and Sarkar, 1996) and soibum (Giri and Janmejay 1987, 1994, 2000), and technological properties of functional LAB present in these products. Due to our convenience and other logistic reasons, we collected among the fermented bamboo shoots, only samples of ekung, eup and hirring from Arunachal Pradesh; soibum, soidon and soijim from Manipur, and of course, mesu from Sikkim for our study. It is worth to notice that no preservative is added during storage in all above-mentioned fermented vegetable foods.

Microorganisms

The microbial population of goyang, gundruk, inziangsang, khalpi, sinki, ekung, eup, hirring, mesu, soibum, soidon and soijim samples revealed that lactic acid bacteria (LAB), comprising lactobacilli, pediococci, leuconostocs, lactococci (in goyang and hirring samples only) and enterococcus (in soibum samples only) were the predominant microorganisms present in viable numbers above 10^7 cfu/g. 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). Taxonomically diverse species of LAB have been identified from goyang, gundruk, inziangsang, khalpi, sinki, ekung, eup, hirring, mesu, soibum, soidon and soijim samples of North East India. They represented the six genera of LAB - *Lactobacillus, Leuconostoc, Lactococcus, Pediococcus, Enterococcus* and *Tetragenococcus*. Classification of LAB into different genera is largely

based on morphology, gas production from glucose (Kandler, 1983), mode of glucose fermentation, and growth at different temperatures (Mundt, 1986).

On the basis of a combination of phenotypic properties and molecular techniques, strains of LAB isolated from gundruk, inziangsang, khalpi and sinki were identified as *Lb. brevis*, *Lb. plantarum, P. pentosaceus* and *P. acidilactici.* During the identification procedure, the phenotypic characterisation of the LAB strains was followed by the RAPD-PCR technique. The fingerprints obtained from this technique were used to identify clusters of closely related or even identical strains allowing selecting representative strains of these groups for further identification (Schillinger *et al.*, 2003). A rep-PCR using $(GTG)_5$ primer was an appropriate tool to separate *Lb. plantarum* from the phenotypically almost indistinguishable *Lb. pentosus* and *Lb. paraplantarum* and a species-specific PCR for *Lb. brevis* allowed the identification of the heterofermentative lactobacilli (Kostinek *et al.*, 2005).

On the basis of the RAPD profiles, the dedrogram shown in Fig 13, the pediococci strains from gundruk and inziangsang were separated into *P. pentosaceus*, and *P. acidilactici.* Randomly amplified polymorphic DNA (RAPD) is an accurate technique for separation of *P. pentosaceus and P. acidilactici*, isolated from Ethiopian fermented foods (Nigatu *et al.*, 1998).

Lb. plantarum. Lb. brevis, *Lactococcus* (*Lc.*) *lactis, P. pentosaceus* and *Enterococcus faecium*, isolated from goyang of Sikkim, and *Leuc. fallax* (khalpi and sinki) were identified on the basis of detailed phonotypic

characters including the API sugar profile data. *Lb. brevis*, *Lb. plantarum, P. pentosaceus,* and *P. acidilactici* have been reported to be involved in many lactic acid fermented vegetable foods including sauerkraut and cucumbers (Fleming *et al.*, 1985; Font de Valdez *et al.*, 1990; Tanasupawat *et al.*, 1993; Randazzo *et al.*, 2004). Enteroccoci play beneficial role in production of many fermented foods (Bouton *et al.*, 1998; Cintas *et al.*, 2000). *E. faecium* appears to pose a low risk for use in foods, because these strains generally harbour fewer recognised virulence determinants than *E. faecalis* (Franz *et al.*, 2003). Finding of *Lc. lactis* in goyang supports that this species is also commonly isolated from plant materials (Sandine *et al.*, 1972), though, the most recognised habitat for the lactococci is milk products (Teuber *et al.*, 1991).

Gundruk samples of Nepal contained *Lb. plantarum, Lb. casei* subsp. *casei, Lb. casei* subsp. *pseudoplantarum, Lb. cellobiosus* (= *Lb. fermentum*) and *P. pentosaceus* (Karki, 1986). Gundruk is also an important fermented vegetable food of Nepal (Dietz, 1984). However, there is no earlier report on microbial composition of gundruk samples prepared in Sikkim. *Leuc. mesenteroides, P. pentosaceus, Lb. brevis* and *Lb. plantarum* are known to contribute the complex sauerkraut fermentation process (Pederson and Albury, 1969). Several species of LAB were taxonomically identified from kimchi of Korea, which included *Leuc. mesenteroides, Lc. lactis, Lb. plantarum, Lb. brevis*, *E. faecalis, P. pentosaceus* and *Weissella kimchi* (Mheen and Kwon, 1984; Choi *et al.*, 2002; Kim and Chun, 2005). Recovery of lactobacilli in sinki and the confirmation of their identity by molecular tools confirmed the earlier

findings of Tamang and Sarkar (1993). There are not many reports on the occurrence of *Leuc. fallax* in foods. Strains of these species have been isolated from sauerkraut (Schillinger *et al.*, 1989), fermented rice cake (puto) in the Philippines (Kelly *et al.*, 1995) and from plant exudates of *Gerbera jamesonii* (Middelhoven and Klijn, 1997). *Leuc. fallax* has recently been recognised as one of predominating organisms in the early heterofermentative stage of sauerkraut fermentation (Barrangou *et al.*, 2002).

Strains of LAB isolated from mesu, soidon, soibum and soijim were identified using phenotypic tests and genotypic methods such as rep-PCR and RAPD-PCR, as *Lb. brevis*, *Lb. plantarum, Lb. curvatus* and *P. pentosaceus.* For the other LAB strains *Leuc. mesenteroides, Leuc. fallax*, *Leuc. lactis, Leuc. citreum* and *Enterococcus durans*.and also strains from ekung, eup and hirring of Arunachal Pradesh, phenotypic charaterisation including DAP, lactate configuration and API system was performed for the identification to species level. The typical enterococci (*Enterococcus durans)* can be easily distinguished from other Gram-positive, catalase-negative, homofermentative cocci such as streptococci and lactococci by their ability to grow both at 10 and 45° C, in 6.5 % NaCl, and at pH 9.6 (Franz *et al.*, 2003). The lactic floral composition in ekung, eup and hirring was *Lb. plantarum, Lb. casei, Lc. lactis,* and *Tetragenococcus halophilus* (only in ekung). *Lb. casei* has been found the dominant species in naturally fermented Sicilian green olives (Randazzo *et al.*, 2004). Recovery of *Lb. plantarum, Lb. brevis* and *P. pentosaceus* in mesu samples collected from Sikkim confirmed the earlier report of Tamang and

Sarkar (1996). *Leuc. citreum* is reported in traditional French cheese (Cibik *et al.*, 2000). Findings of *Lb. plantarum, Lb. brevis, Leuc. fallax, Leuc. mesenteroides* and *Enterococcus durans* in soibum of Manipur has updated the earlier findings of Giri and Janmejay (1994), the identification of which was based on a limited phenotypic characters (Giri and Janmejay, 1987). Microbial composition of soidon and soijim of Manipur was reported for the first time in this thesis. Similar fermented bamboo shoot products called naw-mai-dong or nor-mai-dorng of Thailand also contained lactobacilli, leuconostocs and pediococci (Dhavises, 1972; Phithakpol *et al.*, 1995). Though, *Enterococcus durans* has been isolated from Feta cheese (Igoumenidou *et al.*, 2005), there is no published report of its presence in any fermented vegetable product. Isolation, enrichment, purification, characterisation and proper identification and authentic nomenclature of functional microorganisms involved in fermented foods are important aspects of microbial systematic, which also ensure the quality control and normalised production of fermented foods (Tamang and Holzapfel, 1999). The isolated, identified and preserved microorganisms from lesser-known fermented vegetable products may contribute significant information on unknown microbial gene pool as genetic resources of North East India.

Pathogenic contaminants

Occurrence of bacterial contaminants mostly *Staphyloccus aureus* in few finish samples of goyang, gundruk and sinki, *Bacillus cereus* in few dry samples of sinki and enterobacteriaceae in goyang and gundruk

samples around 10^2 cfu/g was observed. These pathogens might have introduced during handling of raw leaves for preparation when pH was not low enough to inhibit their growth. Otherwise, no pathogenic bacteria such as *Listeria, Salmonella* and *Shigella* were detected in analysed samples, due to acidic nature of the products. Small number of *Bacillus cereus* in foods is not considered significant (Roberts *et al.*, 1996). Rapid growth of LAB could restrict the growth of other organisms simply by their physical occupation of available space and uptake of most readily assimilative nutrients (Adams and Nicolaides, 1997). Moreover, lactic acid produced by LAB may reduce pH to a level where pathogenic bacteria may be inhibited or destroyed (Holzapfel *et al.*, 1995; Tsai and Ingham, 1997).

Yeasts

Though the dominant microflora in fermented vegetable and bamboo products of North East India was lactic acid bacteria, a sizable number of yeasts mostly *Pichia, Candida, Saccharomyces* and *Rhodotorula* were also reported in some finish products of goyang, khalpi, sinki, ekung, hirring and mesu in numbers ranging between 10^4 and 10^6 cfu/g. These yeasts might be spoilage in the products or might have appeared during storage. The various genera and species of yeasts occur in kimchi (Song and Park, 1992). In cucumber pickles, *Hansenula, Saccharomyces* and *Torulopsis* were reported (Etchells *et al.*, 1961). Some oxidative yeasts belonging to *Candida, Pichia, Debaryomyces, Saccharomyces* and *Hansenula* were associated with gassy fermentation and softening of olives (Vaughn *et al.*, 1972). It was suggested that

acidification with lactic acid alone to pH 3.7 is insufficient to prevent the growth of spoilage yeast in fermented vegetables and that the presence of other organic acids is desirable (Savard *et al.*, 2002).

Technological properties

Acidification is an important technological property in relevance of selection for starter culture among the LAB (De Vuyst, 2000). LAB strains isolated from fermented vegetable and bamboo shoot products were screened for their acidifying capacity, and found that most of the LAB strains acidified with lowering of pH to 4.0 (Table 18 and 19). These strains although 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. The casein degradation initiated with milk clotting peptidases and proteinases, which produce peptides and amino acids (Mäyra-Mäkinen and Bigret, 1998). Justifying milk coagulation by the LAB strains isolated from fermented vegetables, the enzymatic activity of the LAB (Table 24 and 25) clearly shows the absence of proteinase but showed high activity of peptidases, which, may be responsible for coagulation of skim milk in the applied method. 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).

Strains of different species of *Lactobacillus, Pediococcus and Leuconostoc*, isolated from all fermented vegetable and bamboo shoot products, showed anitimicrobial activities against a number of potentially

pathogenic Gram-negative and Gram-positive bacteria (Table 20 and 21). This reveals that antimicrobial properties of functional LAB can reduce the number of other undesired microorganisms in vegetable products and simultaneously perform an essential role in the preservation of a food product for human consumption, by fermentation. In addition, LAB compete with other microbes by screening antagonistic compounds and modifying the micro-environment by their metabolism (Lindgren and Dobrogosz, 1990). However, only one strain of *Lb. plantarum* IB2 (inziangsang) was found to produce a bacteriocin against *Staphylococcus aureus* S1 (Table 22). This may be explained by the fact that also other factors are involved in antagonism, and by the influence of growth rate and competitiveness of a culture for bacteriocin production (Tagg, 1992; Yang and Ray, 1994). This is determined, amongst others, by its adaptation to a substrate and by a number of intrinsic and extrinsic factors including redox potential, water activity, pH and temperature (Holzapfel *et al.*, 1995; Ouwehand, 1998). A number of Gram-positive pathogenic bacteria including *Staphylococcus aureus* have been found sensitive to bacteriocin of *Lactobacillus* (Tichaezek *et al.*, 1992; Sudirman *et al.*, 1993; Niku-Paavola *et al.*, 1999). Our finding on bacteriocin activity of *Lb. plantarum* IB2 (inziangsang) against *Staphylococcus aureus* was justified by the recent report on the inhibitory effect of bacteriocin produced by *Lb. plantarum* in foods against *Staphylococcus aureus* and other Gram-negative bacteria (Jamuna *et al.*, 2005; Lash *et al.*, 2005). Crude bacteriocin activity of *Lb. plantarum* IB2 was quantified and calculated as 32 AU/ml. Quantification is necessary to observe the quotient between the

inhibition zone area and the sensitivity of the indicator bacterium used to compare different bacteriocins (Delgado *et al.*, 2005). Species of LAB strains isolated from several fermented vegetable products have the antimicrobial activities including bacteriocins and nisin production such as fermented olives (Cordeiro *et al.*, 2002; Rubia-Soria *et al.*, 2006), sauerkraut (Tolonen *et al.*, 2004), fermented carrots (Uhlman *et al.*, 1992), fermented cucumbers (Daeschel and Fleming, 1987) and cabbage juice (Kyung and Fleming, 1994).

The use of the API-zym technique has been reported (Arora *et al.*, 1990) as a rapid and simple means of evaluating and localising 19 different hydrolases of microorganisms associated with dairy fermentations. This method is also of 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 (Tamang *et al.*, 2000). Absence of proteinases (trypsin and chymotrypsin) and presence of strong peptidase (leucine-, valine-, and cystine-arylamidase) and esterase-lipase (C4 and C8) activities produced by the predominant LAB strains isolated from fermented vegetable products of North East India are possible traits of desirable quality for their use in production of typical flavour. Herreros *et al.* (2003) reported the highest aminopeptidase activity in *Lb. plantarum, Lc. casei* and *Leuc. mesenteroides* subsp. *dextranicum* isolated from Armada cheese.

Antinutritive factors such as phytic acids and oligosaccharides are of particular significance in unbalanced cereal-based diets (Holzapfel,

2002; Fredrikson *et al.*, 2002). Oligosaccharides such as raffinose, stachyose and verbascose cause flatulence, diarrhea and indigestion (Abdel Gawad, 1993; 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). High activity of phosphatase by LAB strains showed their role in phytic acid degradation in fermented vegetable products, as proved in Tables 26 and 27. It was also shown that strain of *Lb. plantrum*, *Lb. brevis* and *Leuc. lactis* had moderate to high α-galactosidase activity. This indicated their ability to hydrolyse oligosaccharides of raffinose family (Holzapfel, 2002). About 76.7 % of *Lb. plantarum* strains isolated from fermented vegetable and fermented bamboo shoot products of North East India degraded raffinose while none of the *P. pentosaceus* strains and *P. acidilactici* were able to degrade raffinose. This is in conformity with that report of Holzapfel (2002) where *Lb. plantarum* strains isolated from fermented Ghanaian maize products were able to ferment raffinose, while strains of *P. pentosaceus* and *P. acidilactici* were unable to do so. Several reports support our findings of phytate reduction in fermented vegetables by lactic fermentation, as shown in maize (Lopez *et al.*, 1983), idli, a fermented rice-black gram product (Reddy *et al.*, 1986); white sorghum (Svanberg and Sandberg, 1988), pearl millet (Mahajan and Chauhan, 1987; Khetarpaul and Chauhan, 1989) and non-tannin containing cereals (Svanberg *et al.*, 1993).

Biogenic amines are organic basic compounds which occur in different kinds of foods such as sauerkraut (Taylor *et al.*, 1978), fishery products, cheese, wine, beer, dry sausages and other fermented foods (ten Brink *et al.*, 1990; Halász *et al.*, 1994), fruits and vegetables (Suzzi and Gardini, 2003). 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). In foods, biogenic amines are mainly generated by decarboxylation of the corresponding amino acids through substrate specific enzymes of the microorganisms present in foods (ten Brink *et al.*, 1990; Straub *et al.*, 1995). The inability of most strains of LAB to produce biogenic amines in tested fermented vegetable products of North East India 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). Histaminine, precursor of biogenic amine has been recognised as the causative agent of scombroid poisoning (histamine intoxication), whereas tyramine has been related to food induced migraines and hypertensive (Taylor, 1986; Bover-cid and Holzapfel, 1999).

Bacterial adherence to hydrocarbons such as hexadecane, proved to be a simple and rapid method to determine cell surface hydrophobicity (Rosenberg *et al.*, 1980; van Loosdrecht *et al.*, 1987; Ding and Lämmler, 1992; Vinderola *et al.*, 2004). A few strains of LAB isolated from fermented vegetable products of North East India had more than 75 %

hydrophobicity (Table 30 and 31), indicating their hydrophilic nature. Percent of hydrophobicity greater than 70 % was arbitrarily classified as hydrophobic (Nostro *et al.*, 2004). The high degree of hydrophobicity of the LAB strains, isolated from fermented vegetable products of North East India, probably indicates the potential of adhesion to gut epithelial cells of human intestine, advocating their 'probiotic' character (Holzapfel *et al.*, 1998), provided these strains are consumed in a viable state. The ability to adhere to the intestinal mucosa is considered one of the main criteria in the selection of potential probiotic culture (Apostolou *et al.*, 2001; Shah, 2001; Holzapfel and Schillinger, 2002). Functional effects of probiotic bacteria include adherence to the intestinal cell wall for colonization in the gastro intestinal tract (GIT) with capacity to prevent pathogenic adherence or pathogen activation (Bernet *et al.*, 1993; Salminen *et al.*, 1996). The behaviour of LAB could be dependent on interfacial processes and thus on cell surfaces physicochemical properties and chemical composition (Gatti *et al.*, 1997; Boonaert and Rouxhet, 2000; Gómez-Zavaglia *et al.*, 2002).

Fermentation dynamics

During natural fermentation of both 'rayo-saag' gundruk and 'mula-saag' gundruk, indigenous lactic acid bacteria changed spontaneously and at the end of the process *Lactobacillus* species, mainly *Lb. plantarum* was involved (Table 32 and 33). Spontaneous change in LAB population during several vegetables fermentation involving lactobacilli was reported (Fernandez Gonzalez *et al.*, 1993; Harris, 1998). As expected in a typical

lactic fermentation (Vaughn, 1985; Lee, 1997; Lu *et al.*, 2001), the pH of the fermenting substrates decreased and the titratable acidity increased as the gundruk fermentation progressed due to growth of LAB which, converts fermentable sugars into lactic acid (Buono *et al.*, 1990). Some yeasts were detected in initial stage of both types of gundruk fermentation, and then disappeared. The population of pathogenic contaminants disappeared during fermentation because of dominance of LAB. By averting the invasion of these potential contaminants, lactic acid fermentation imparts attributes of robust stability and safety in the product like gundruk. There has been no report of any food poisoning or infectious disease infestation by consuming gundruk. The LAB produced sufficient acid for inhibition of pathogenic microorganisms in foods (Adam and Nicolaides, 1997). The LAB also preclude proliferation of contaminating acid-intolerant species of bacteria and fungi (Mensah *et al.*, 1990; Nout, 1991, 1994).

In situ fermentation of khalpi also revealed that significant decrease in pH accompanied by increase in titratable acidity with fermentation time (Table 34). After exponential increase, the population of LAB dropped slightly. Yeast, *Staphylococcus aureus* and enterobacteriaceae were associated with initial fermentation and finally disappeared during khalpi fermentation. Fresh vegetables, like cucumbers, contain a numerous and varied epiphytic microflora, including many spoilage microorganisms, and an extremely small population of LAB (Etchells *et al.*, 1961; Mundt and Hammer, 1968). Numerous chemical and physical factors influence the rate and extent of growth of various microorganisms, as well as their

sequence of appearance during the fermentation (Fleming and McFeeters, 1981). Acidity, pH and buffer capacity greatly influence establishment and extent of growth of LAB during cucumber fermentation (McDonald *et al.*, 1991).

Starter culture

Use of standard starter culture is not a practice in North East India except in alcoholic beverage production (Thapa and Tamang, 2004). An attempt was made to produce gundruk and khalpi under optimised condition using selected strains of LAB. Gundruk was selected since it is widely consumed in the various regions, while khalpi was selected since cucumber is available in plenty. It was found that gundruk produced under optimised conditions using a mixture of pure culture strain of *Lb. plantarum* GLn:R1 and *P. pentosaceus* GLn:R1, selected on the superior technological property as mentioned in the result section, at 20° C for 6 days, had highest score of general acceptability (Table 35). 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 (Table 36). Khalpi produced at 20° C for 72 hour had organoleptically scored the highest acceptability among the consumers; whereas khalpi made at 25° C scored the highest acceptability within 48 hour and acceptability decreased as sour taste develops further. Application of starter cultures may appear appropriate in gundruk and khalpi production at household level since it is cost-effective and may contribute to effective control and safeguarding of the fermentation process. Gundruk prepared by using a

starter culture had thus 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. Similarly, using a mixed pure culture strains in khalpi preparation takes less time, while in natural condition it takes more than 3 days for cucumber fermentation to complete.

The final product is not always consistent in natural fermentation; the use of a mixed lactic starter culture could provide more consistent fermentations and products of higher quality (Daeschel and Fleming, 1987; Gardner *et al.*, 2001). Modern starter cultures are selected, either as single or multiple strains, especially for their adaptation to a substrate or raw material, for example milk, meat, cereals, legumes, roots, and tubers (Holzapfel *et al.*, 2003). The authentic identity of indigenous LAB isolated from traditional fermented vegetable products and their detailed technological characters should be considered for development of starter culture. Though, optimised process condition is always superior and advantageous than the conventional method, however, introduction and replacement of natural and easily operated traditional technology may be difficult to change for the producers or rural populace. Authentic identity of functional microbes in fermented foods is necessary to develop the starter cultures isolated from conventionally prepared foods (Tamang and Holzapfel, 1999). Preservation and safeguarding of foods are still major objectives of fermentation (Holzapfel, 2002). Yet, other aspects such as wholesomeness, acceptability and overall quality have become increasingly important and valued features to account of the substrate,

technical properties of the strain, food safety requirements and quality expectations (Holzapfel *et al.*, 2003).

Proximate composition

Moisture content in gundruk, inziangsang, sinki and eup was low due to drying after fermentation; other products were freshly prepared with high moisture content. Samples of fermented vegetable collected from North East India had comparatively higher mineral content. Higher contents of mineral are observed in Indian vegetables (Gopalan *et al.*, 1995). Proximate and food value of fermented vegetable and bamboo shoot products of North East India are almost same as reported in other fermented vegetable products such as kimchi (Cheigh and Park, 1994), sauerkraut (Campbell-Platt, 1987), and fermented cucumber (Fleming, 1984).

Conclusion

Scientific knowledge on fermented vegetable and bamboo shoot products of North East India is sparse outside this region. Species of predominant lactic acid bacteria associated with biopreservation of perishable vegetables and some wild bamboo shoots were summarised as follows: *Lb. plantarum, Lb. brevis*, *Lc. lactis, E. faecium, P. pentosaceus* (goyang); *Lb. plantarum, P. pentosaceus* (gundruk); *Lb. plantarum, Lb. brevis, P. acidilactici* (inziangsang); *Lb. plantarum, Lb. brevis*, *Leuc. fallax* (khalpi); *Lb. plantarum, Lb. brevis, Lb. casei*, *Leuc. fallax* (sinki); *Lb. plantarum, Lb. brevis, Lb. casei*, *Tetragenococcus*

halophilus (ekung); *Lb. plantarum, Lb. fermentum* (eup); *Lb. plantarum, Lc. lactis* (hirring); *Lb. plantarum, Lb. brevis, Lb. curvatus, Leuc. citreum, P. pentosaceus* (mesu); *Lb. plantarum, Lb. brevis, Leuc. fallax, Leuc. lactis, Leuc. mesenteroides, E. durans* (soibum); *Lb. brevis, Leuc. fallax, Leuc. lactis* (soidon); *Lb. brevis, Leuc. fallax, Leuc. lactis* (soijim).

About 65.4 %, out of 547 LAB strains isolated from traditional fermented vegetable and bamboo shoots of North East India, belonged to *Lactobacillus*. Homofermenter lactobacilli were represented by 45.4 %, followed by heterofermenter lactobacilli representing 20 %; and leuconostoc 16.3%, pediococci 12.7%, lactococci 1.7% and *Tetragenococcus* 0.7 % represented remaining lactic flora.

This study showed that strains of LAB play an important and partly complex role in these traditional fermentation processes by their functional properties related to a specific and partly a wide enzyme spectrum, their acidifying capacity, degradation of antinutritive factors, by their antimicrobial activities against potential pathogens, probiotic properties (adherence potential indicated by a high degree of hydrophobicity), and even as non-producers of biogenic amines. Some of these LAB strains possess interesting protective and functional properties, which render them interesting candidates for use as starter culture for controlled and optimised production of fermented vegetable products typical of North East India. Gundruk and khalpi prepared by a mixed pure culture strains of LAB had many advantages over the conventionally prepared products.

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, hirring, 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, hirring, 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 hirring); *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 *Staphycoccus 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, hirring 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 anitimicrobial 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.

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