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Bio-Diversity of *Lactobacillus* Cultures Associated with the Traditional Ethnic Fermented Foods of West Garo Hills, Meghalaya, India

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A B S T R A C T

To assess the nature of microbes and their source during spontaneous fermentation is generally studied to explore the microbial diversity of these foods. At present in West Garo Hills of Meghalaya state, these products are prepared for local consumption at house-hold level without much consideration to GMP and HACCP guidelines. Modern science (for quality control, packaging, etc.) and collaborative research should join hands for the commercialization of novel value added products derived from indigenous food items, converting this local market to a global industry thus providing means of employment and economy to the tribal communities. In this era, lactic acid bacteria has drawn maximum attention in food and nutrition science due to their nutraceutical potential producing certain biologically active peptides, along with other functional and probiotic attributes and most importantly having GRAS status. In the present investigation, isolates were screened on MRS medium, *Lactobacillus* strains were identified as a predominant species in the ethnic fermented foods by phenotypic (Gram staining, catalase activity and API test) and genotypic by molecular characterization (PCR). Phylogenetic tree of the most closely related *Lactobacillus* species has been constructed by using MAFFT sequence alignment tool. Sequences are deposited in GeneBank and NCBI bearing their specific accession number.

Introduction

West Garo Hills, Meghalaya (North East region of India) is characterized by a diverse population of tribal people (Garos, Khasis, Jaintias etc) with different ethnic background. Most of the people of this region are tribal and bear their own methods of fermenting food materials for the purpose of preservation and taste enhancement. All the fermented products are region specific and have their own unique substrates and preparation methods (Das and Deka, 2012). Materials such as soybeans, fish, curd, beverages and locally available vegetables are commonly fermented by most of the tribes. Traditional fermentation is a form of food processing, where microbes, for example, lactic acid bacteria (LAB) are utilized. The bacteria use food as a substrate for their propagation. Over the years, it became part of the cultural and traditional norm among the indigenous

communities (Chelule and Laleye, 2010). The rural folk have come to prefer fermented over the unfermented foods because of their pleasant taste, texture and colour. This popularity has made fermented foods one of the main dietary components of the developing world (Aderiye *et al.,* 2003; Mosha *et al.,* 2004; Nout *et al.,* 1999). The traditional way of carrying out fermentation at the household-scale is still followed using relatively simple processing facilities. These products often contain mixed microbial populations because of the lack of sterility and the use of natural fermentation (Nout *et al.,* 1999). The people of this particular state have a very rich reserve of traditional knowledge owing to their livelihood in the hilly terrains. This area is inhabited largely by tribal people who make up 75% of the population of the region (Agrahar-Murungkar and Subbulakshmi, 2006). These people possess great knowledge of the environment and depend on the forests, plants and plant products for food and other purposes (Jaiswal *et al.,* 2010).

Being one of the oldest tribes of India as well as of North East, the people of Meghalaya have a different taste and recipe which is a special feature of the region. The recipe known as the *Nakhem* or *Hindal* of Meghalaya is a delectable soup which is very popular among them. The *Nakham* is basically a special dry fish which is used to make the soup. The fresh fish is dried under the sun or over the fire first. Once the fish gets completely dried, it is mixed up with alkali, ginger, onion and salt, placed inside bamboo tube, the mouth of the tube covered with plantain leaves and made tight with thin bamboo slivers. The most preferred fish curry *T'pai bamsi* is prepared by rice powder, alkali and pulse (*Phaseolus radiatus*) along with *Hindal*. Sometime, small snails (*Neguria*) are also added to make the dish tastier. Lots of chilly and other spices are used to enhance the taste of the soup (Gitanjali and Mandal, 2015).

Alcoholic rice beverages are an integral part of life of several aboriginal communities and are known in different names in different places. It is known as *sake* in Japan, *lao-chaoin* China, *tape ketan* in Indonesia, *khao-mak* in Thailand (Devi *et al.,* 2015). In India, an alcoholic beverage called *sura* distilled from rice was in use between 3000 and 2000 B.C. Similarly, in the state of Meghalaya in India, the Garo tribal community has a distinct food culture or dietary culture which is paramount to their diet is *chu-a/s* which is an alcoholic rice beverage that symbolizes the heritage and socio cultural aspects of their ethnicity.

Fermented rice, *wanti* is prepared by adding water to cooked rice and incubating the mixture overnight. The water is drained off and used to cook vegetables or mixed with buttermilk and salt for direct consumption. Rice is mixed with dahi and salt. Strains of *Pediococcus*, *Lactobacillus* and *Streptococcus* have been isolated previously from fermented rice. The pH is decreased from 6.1 to 5.7 in 16 hrs. There is no change in volume, amino nitrogen or free sugar (Steinkraus, 1996).

Traditionally, *Dahi* is a naturally fermented milk product obtained from boiled cow or buffalo milk and soured using lactic cultures as *Lactococcus lactis* spp., *Lactococcus lactis* spp. *cremoris*, and *L. diacetylactis* it used separately or in combination. It is used in daily diet as a potential source of B-complex vitamins, folic acid, and riboflavin (Sarkar *et al.,* 2015).

This study deals with the isolation and identification of the predominant species of *Lactobacillus* in naturally fermented foods of Meghalaya, India. Phenotypic and genotypic characterization was carried out respectively for confirmation of genus and species level of

Lactobacillus strains, along with DNA sequencing and analysis of phylogenetic studies by utilizing 16S rRNA gene (Fig. 3).

Materials and Methods

Sample collection and bacterial growth enrichment

Homemade and commercial samples of fermented foods (Fig. 1) were collected from different region of Meghalaya state and included in this investigation (Table 1). The enrichment process was carried out by inoculating approximately 1 ml of a mix of the fermented food samples and poured into 50 ml sterile MRS broth (HiMedia, India) and incubated at 37˚C for (2-5) days (Abbas and Mahasneh, 2014). All samples were collected into sterile glass bottles and were kept in the laboratory at refrigeration temperature (4-6 ˚C) for further analysis.

Isolation and purification of *Lactobacillus* strains

Enriched fermented food samples were serially diluted in sterile normal saline. Aliquots of 100 µl from each dilution were then plated on MRS media supplemented with 0.01% bromocresol purple as a pH indicator. Plates were incubated at 37˚C for 24 hours. Presumptive *Lactobacillus* colonies with yellow halos were randomly picked up from the MRS plates and were further subcultured onto fresh plates of the same medium to ensure purity.

Identification of bacterial Strains

All isolates were tested for catalase activity, Gram reaction (Fig. 2) and cell morphology (Guessas and Khal, 2004). The identification of strains was performed according to their morphological, cultural and biochemical properties based on their specific characteristics as described in Bergy's manual (Buchanan and Gibbons, 1974). The strains were tested for the production of acids from carbohydrates and related compounds using API 50 CH kits (HiMedia, India) according to the manufacturer's instructions. Results were scored after incubation at 37˚C for 24 and 48 hours. These results were put on the apiweb™ identification software with database (V5.1) which uses the phenotypic data to predict a species identity. Interpretations of the fermentations profiles were facilitated by comparing all results obtained for the tested isolates with information from the computer aided database, apiweb™ (https://apiweb.biomerieux.com).

Confirmation of lactobacilli isolates by colony PCR

Molecular characterization of isolates was done by Polymerase Chain Reaction (PCR) using primers (27F and 1492R) (Table 2). Template was prepared by picking freshly grown colony and transferred to TE buffer and incubate at 80ºC for 15 minutes. This was further amplified by PCR and confirmed by running product on agarose gel (1%) in gel electrophoresis.

PCR mixture was initially heated at 94ºC for 5 minutes followed by cycles of denaturation at 94ºC for 1 min, annealing 56ºC for 1 min and extension was performed at 72ºC for 5 minutes.

Phylogenetic analysis

To determine the closest known relatives of the partial 16S rDNA sequences obtained, nucleotide database searches were performed in NCBI GenBank and later the sequences were analysed by multiple sequence alignment tools using the DNA alignment program MAFFT v6.864 to signify the evolutionary relatedness (Fig. 4) between the

strains by UPGMA (Unweighted Pair Group Method with Arithmetic Mean)

Results and Discussion

This study was conducted to isolate *Lactobacillus* strains from various ethnic fermented foods (Fig. 1) (fermented fish, fermented rice and rice beverages, curd samples) from the various places of Meghalaya (North-eastern region of India) and explore their phenotypic and genotypic characteristics for further, development of value added products by identifying productive microbial strains.

Phenotypic characterization of *Lactobacillus strains*

A total of nine strains were isolated from the various fermented food samples obtained from different places of Meghalaya, India (Table 1). Nine catalase negative and Gram positive bacteria were isolated from traditional fermented foods and considered as presumptive LAB (Fig. 2). Further, biochemical tests of all the isolates were carried out by API CH 50 Microbial Identification kit (*bioMerieux, India)* through sugar fermentation pattern, ammonia production from arginine, gas production from glucose were carried out for the initial characterisation of lactic acid bacteria isolated from the samples.

K7, K8, K10, K11, K14 showed positive whereas K3A, K4E, K5, K6 showed negative test results for L-arabinose respectively. K3A, K4E, K5, K6, K7, K8, K10 and K11 showed positive whereas K14 showed negative results for D-ribose, D-galactose, D-fructose, D-mannose, D-trehalose, Esculin ferric citrate, D-maltose, D-saccharose respectively (Table 3). K8 and K11 showed positive whereas K3A, K4E, K5, K6, K7, K10 and K14 showed negative results for D-xylose respectively. K4E and K7 showed positive whereas K3A, K5, K6, K8, K10, K11and K14 showed negative test results for L-sorbose respectively. K6 and K7 showed positive whereas K3A, K4E, K5, K8, K10, K11 and K14 showed negative results for Rhamnose respectively. K4E showed positivity whereas as K3A, K5K6, K7, K8, K10, K11 and K14 showed negative results for Dulcitol respectively. K4E, K7, K8, K10 and K11 showed positive whereas K3A, K5, K6 and K14 showed negative results for D-mannitol, D-melezitose, Salicin, D-Celibiose and Arbutin respectively. K4E, K7, K8 and K10 showed positive whereas K3A, K5, K6, K11 and K14 showed negative results for D-sorbitol respectively. K8 showed positivity whereas K3A, K4E, K5, K6, K7, K10, K11 and K14 showed negative results for methyl –α-D-glucopyranoside. K7, K11, K14 showed positive whereas K3A, K4E, K5, K6, K8 and K10 showed negative results for methyl–α-D-mannopyranoside respectively.

K4E, K7, K8, K10, K11 and K14 showed positive whereas K3A, K5, K6 exhibited negative results for N-acetyl glucosamine respectively. K7, K8, K10, K11, K14 showed positive whereas, K3A, K4E, K5, K6 showed negative for amygdalin respectively. K10 showed positive whereas K3A, K4E, K5, K6, K7, K8, K11, K14 exhibited negative for raffinose respectively. K7, K10, K11 showed positive whereas K3A, K4E, K5, K6, K8, K14 showed negative for D-turanose respectively. K4E, K7, K8, K11 showed positive whereas K3A, K5, K6, K10, K14 showed negative results for D-tagatose respectively. K8 and K14 showed positive whereas K3A, K4E, K5, K6, K7, K10, K11 showed negative results for potassium gluconate respectively. All the nine isolates were able to utilize D-glucose and D-lactose and all the nine isolates were unable to utilize Glycerol, Erythritol, D-arabinose, L-xylose, Methyl-β-D-xylopyranoside, D-adonitol,

Inositol, Starch, Inulin, D-adonitol, L-fucose, D-fucose, D-arbitol, D-lyxose, Glycogen, Xylitol, and Potassium-5-ketogluconate thereby resulting to be negative respectively. Previously, Krischina *et al.,* (2014) carried out the phenotypic identification for *Lactobacillus* isolates from the Brazilian grape sourdough which was performed by using the kit API50 CHL. Dilek *et al.*, (2011) reported identification of *Lactobacillus* strains isolated from faecal specimens of babies and human milk colostrum by API 50 CHL system. Similarly, Suk *et al.,* (2012) investigated the isolation and characterization of lactic acid bacteria (LAB) from naturally fermented sauce-type *kimchi*. Hence, from the above biochemical tests it was assumed that all the eight isolates belonged to the group of heterofermentative (K3A, K4E, K5, K6, K7, K8, K10, K11) and one was categorized under homofermentative (K14) lactic acid bacteria. The tentative identification by using API 50 CH was in good concordance with those by the genetic identification further.

Molecular confirmation and 16S rDNA sequence analysis of *Lactobacillus* strains

Easiest and simplest way for identification of LAB is amplification of 16S rRNA, 16S-23S intergenic spacer region (ISR), or 23S rRNA universal gene by designing specific primers (Kim *et al.,* 2005). In the present study, primer 27F and 1492R were used for amplification conserved regions of 16S rRNA, resulted in product of 1.5kb fragments confirming that the isolate bacteria was from genus *Lactobacillus*. Rahayu *et al.,* (2009) used same primers set for amplification of bacterial 16S rRNA gene and reported the PCR product of 1.5kbs.

A similar study was executed by Crispim *et al.,* (2013) where the genomic diversity of *Lactobacillus* spp. from *puba*, a Brazilian fermented cassava food, was investigated by molecular typing with rep (repetitive sequence)-based PCR using the primer ERIC2. Adeymo *et al.,* (2014) similarly reported the characterisation of *Lactobacillus plantarum* using molecular methods by polymerase chain reaction (PCR) and amplification of 16S rDNA genes to confirm their identities from fermented cereals. Direct amplification of 16S-23S intergenic space regions (ISRs) or PCR with specific primer derived from L-ISR was reported to be useful for specific typing of *Lactobacillus sanfranciscensis* (Valcheva *et al.,* 2006).

The electrophenogram data for 16S rDNA sequence was validated using Chromas 2.33 software. Sequences obtained were matched with previously published bacterial 16S rDNA sequences available in the GenBank database using BLAST. The sequences determined in this study have been deposited in the NCBI GenBank database with accession numbers (Table 4).

Phylogenetic analysis

From the following phylogram, it can be stated that the four isolates of *Lactobacillus fermentum* are closely related due to the sequence similarity match as well the nodal distance. The other distantly related isolate is of *Lactobacillus rhamnosus* that connects the branch of the four closely related *Lactobacillus fermentum* strains.

Similar work has been reported by Claesson *et al.,* (2008) selecting 12 genomes of *Lactobacillus* strains which were further subjected to an array of whole-genome and single-marker phylogenetic approaches, to investigate the case for extracting subgeneric groups and to determine whether a single congruent phylogeny could be identified. Ennahar *et al.,* (2003) also reported the phylogenetic diversity of Lactic Acid Bacteria associated with paddy rice silage. The

evolutionary relatedness is also revealed by the similarity match of the three strains of *Lactobacillus plantarum*. *Lactobacillus helveticus* is however distantly related from the other strains of *Lactobacills spp.* as revealed by the dendogram and its distantly located branch. Each node with descendants represents the inferred most recent common ancestor of the descendants which in this case is *Lactobacillus.*

Table.1 List of selected isolates with their phenotypical characterization

Sl. No.	Fermented Food Sample	Traditional name of collected fermented food sample (s)	Place of purchased fermented food sample (s)	Isolate Code	Morphological characteristics	Gram's Reaction	Microscopic Examination
1	Fermented Fish	*Nakham*	Tura, West Garo Hills	K3A	Small, translucent	positive	Bacilli
2	Fermented Fish	*Nakham*	Zanjal, West Garo Hills	K4	Medium, flat, entire, translucent	positive	Bacilli
3	Fermented Fish	*Nakham*	Rajabala, West Garo Hills	K5	Small, entire, translucent	positive	Bacilli
4	Local curd sample	*Dahi*	Sohra, Shillong	K6	Small, entire, rough,translucent	positive	Bacilli
5	Local curd sample	*Dahi*	Asanang, Garo Hills	K7	Medium, entire, rough translucent	positive	Bacilli
6	Fermented Rice	*Wanti*	Achetra, West Garo Hills	K8	Small pinpoint	positive	Bacilli
7	Rice beer	*chu-a/s*	Achetra, West Garo Hills	K10	Pinpoint, transperant	positive	Bacilli
8	Rice beer	*chu-a/s*	Sahaki, West Garo Hills	K11	Pinpoint, transperant	positive	Bacilli
9	Fermented fish	*Hindal*	Asanang, Garo Hills	K14	Pinpoint, transperant	positive	Bacilli

Table.2 Oligonucleotide sequences for PCR amplification

Primers	
27 F	5' TACGGYTACCTTGTTACGACTT 3'
1492 R	5' AGAGTTTGATCAMTGGCTCAG 3'

Table.3 Biochemical tests of screened isolates through API 50CH kit

API 50 CH		1	2	3	4	5	6	7	8	9
0	Control	-	-	-	-	-	-	-	-	-
1	Glycerol	-	-	-	-	-	-	-	-	-
2	Erythritol	-	-	-	-	-	-	-	-	-
3	D-Arabinose	-	-	-	-	-	-	-	-	-
4	L-Arabinose	-	-	-	-	+	+	+	+	-
5	D-Ribose	+	+	+	+	+	+	+	+	-
6	D-Xylose	-	-	-	-	-	+	-	+	-
7	L-Xylose	-	-	-	-	-	-	-	-	-
8	D-Adonitol	-	-	-	-	-	-	-	-	-
9	Methylβ-D-Xylopyranoside	-	-	-	-	-	-	-	-	-
10	D-Galactose	+	+	+	+	+	+	+	+	-
11	D-Glucose	+	+	+	+	+	+	+	+	+
12	D-Fructose	+	+	+	+	+	+	+	+	-
13	D-Mannose	+	+	+	-	+	+	+	+	-
14	L-Sorbose	-	+	-	-	+	-	-	-	-
15	L-Rhomnose	-	-	-	-	+	+	-	-	-
16	Dulcitol	-	+	-	-	-	-	-	-	-
17	Inositol	-	-	-	-	-	-	-	-	-
18	D-Mannitol	-	+	-	-	+	+	+	+	-
19	D-Sorbitol	-	+	-	-	+	+	+	-	-
20	Methyl α-D-Mannopyranoside	-	-	-	-	+	-	+	+	-
21	Methyl α-D-Glucopyranoside	-	-	-	-	-	+	-	-	-
22	N-Acetyl Glucosamine	-	+	-	-	+	+	+	+	+
23	Amygdalin	-	-	-	-	+	+	+	+	-
24	Arbutin	-	+	-	-	+	+	+	+	-
25	Esculin Ferric citrate	+	+	+	+	+	+	+	+	-
26	Salicin	-	+	-	-	+	+	+	+	-
27	D-Celiobiose	-	+	-	-	+	+	+	+	-
28	D-Maltose	+	+	+	+	+	+	+	+	-
29	D-Lactose (bovine origin)	+	+	+	+	+	+	+	+	+
30	D-Melibiose	+	+	+	+	+	+	+	+	-
31	D-Saccharose (Sucrose)	+	+	+	+	+	+	+	+	-
32	D-Trehalose	+	+	+	+	+	+	+	+	-
33	Inulin	-	-	-	-	-	-	-	-	-
34	D-Melezitose	-	+	-	-	+	+	+	+	-
35	D-reffinose	+	+	+	+	+	+	-	+	+
36	Amidon(starch)	-	-	-	-	-	-	-	-	-
37	Glycogen	-	-	-	-	-	-	-	-	-

38	Xylitol	-	-	-	-	-	-	-	-	-
39	Gentiobiose	-	+	-	-	+	-	+	+	-
40	D-Turanose	-	-	-	-	+	-	+	+	-
41	D-Lyxose	-	-	-	-	-	-	-	-	-
42	D-Tagatose	-	+	-	-	+	+	-	+	-
43	D-Fucose	-	-	-	-	-	-	-	-	-
44	L-Fucose	-	-	-	-	-	-	-	-	-
45	D-Arbitol	-	-	-	-	-	-	-	-	-
46	L-Arbitol	-	+	-	-	-	+	-	-	-
47	Potassium Gluconate	+	+	+	+	+	-	+	+	-
48	Potassium2-KetoGluconate	-	-	-	-	-	-	-	-	-
49	Potassium 5-keto Gluconate	-	-	-	-	-	-	-	-	-
	Catalase Test	-	-	-	-	-	-	-	-	-
	(+) = positive; (-)= negative									

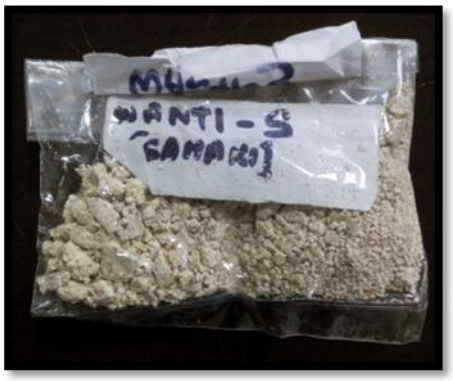
Table.4 NCBI GeneBank accession number of the identified *Lactobacillus* isolates

Isolates	Partially identified by BLAST	NCBI GeneBank accession no.
K3A	*Lactobacillus fermentum*	KU644575
K4E	*Lactobacillus rhamnosus*	KX950834
K5	*Lactobacillus fermentum*	KU213668
K6	*Lactobacillus fermentum*	KU644576
K7	*Lactobacillus fermentum*	KU213665
K8	*Lactobacillus plantarum*	KX519704
K10	*Lactobacillus plantarum*	KU644577
K11	*Lactobacillus plantarum*	KU213666
K14	*Lactobacillus helveticus*	KU644578

Fig.1 Ethnic fermented food samples from West Garo Hills, Meghalaya (India)

Fermented fish samples*(Nakhem)*

Rice beer*(chu-a/s)*



Fermented rice samples (*Wanti*)

Curd samples

Fig.2 Gram staining of the selected isolates

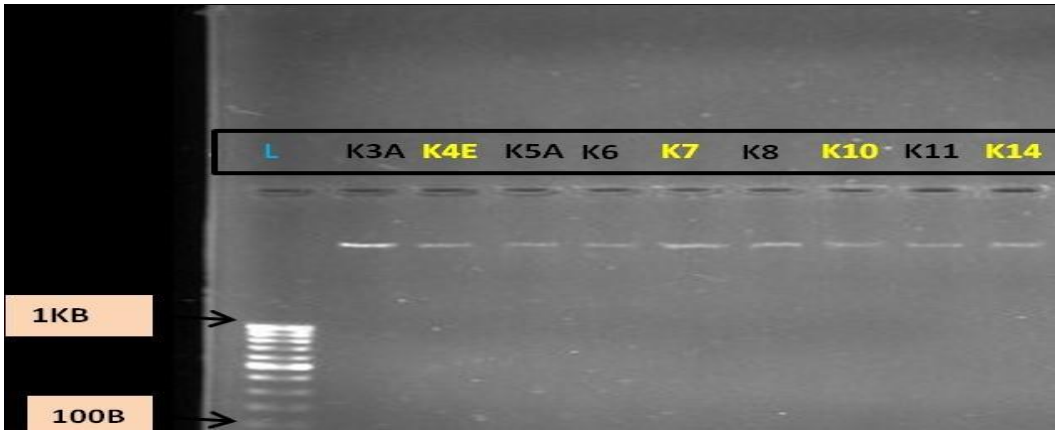


Fig.3 DNA isolation from the selected strains (L = Ladder)

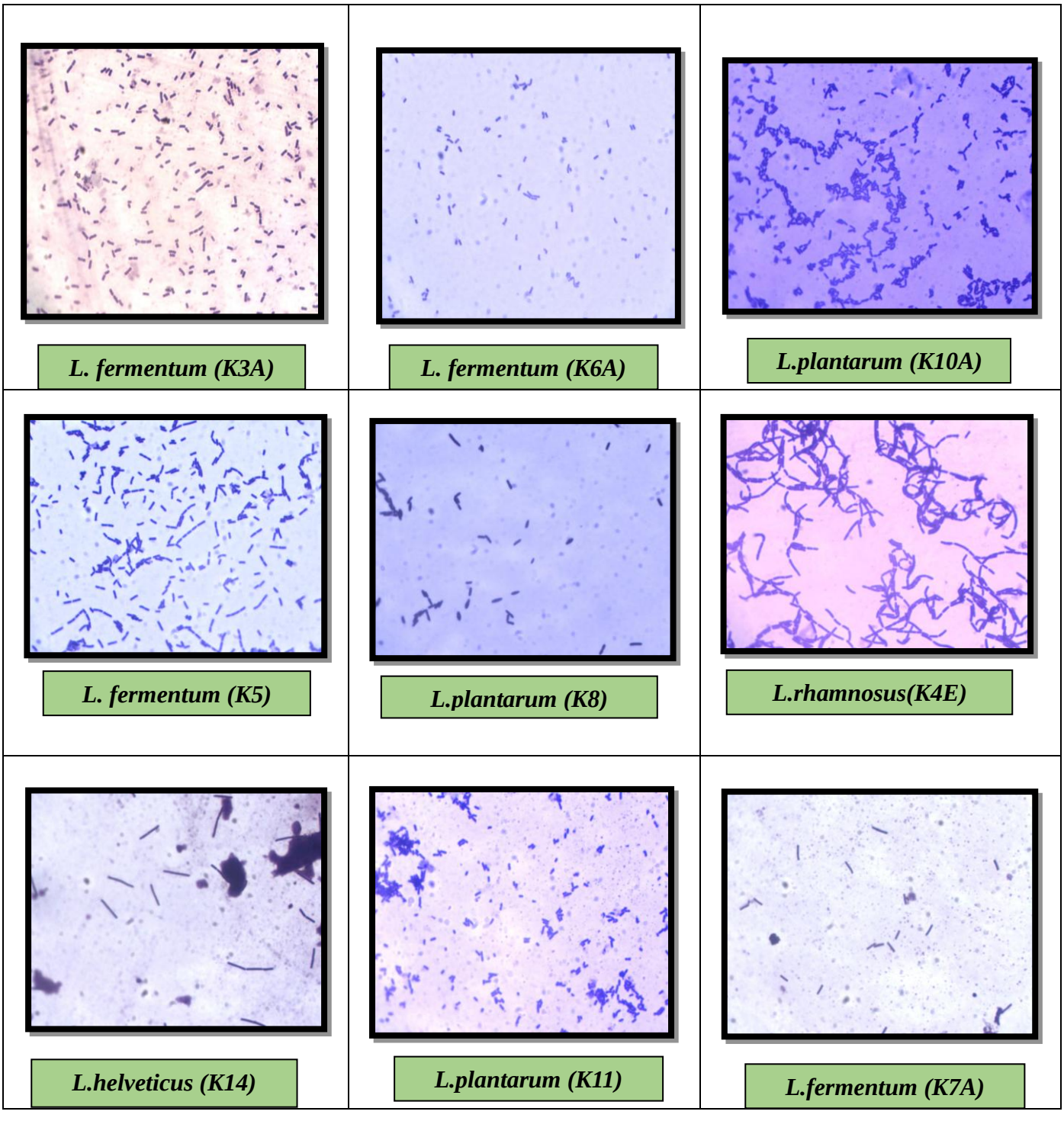
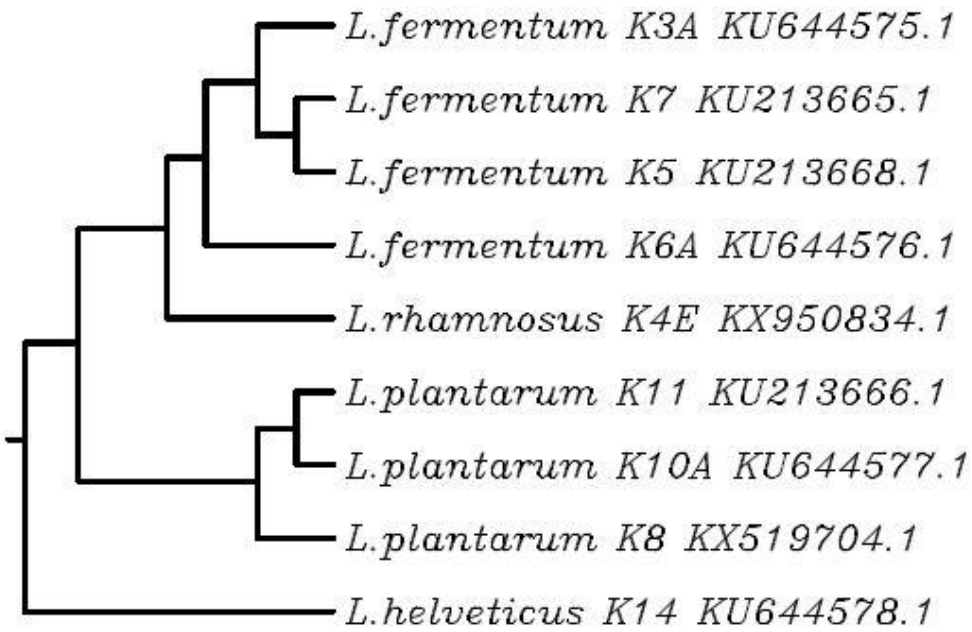


Fig.4 Rooted phylogenetic tree (UPGMA) for *Lactobacillus* isolates from fermented ethnic foods from Meghalaya



The present study concluded that *Lactobacillus* spp. were highly predominant in microflora of the various fermented foods from Meghalaya region. The tentative phenotypic identification of the nine isolates (K3A, K4E, K5, K6, K7, K8, K10, K11) were in good concordance with those by the genetic identification which derived that all the strains belonged to *Lactobacillus spp.* Further 16S rDNA sequence analysis of *Lactobacillus* strains confirmed the strains as *Lactobacillus fermentum* (K3A, K7, K5, and K6)*; Lactobacillus plantarum* (K8, K10 and K11)*; Lactobacillushelveticus* (K14) and *Lactobacillus rhamnosus* (K4E). The evolutionary relatedness were testified by connecting them through a phlyogram. Further, the strains can be checked for their specific probiotic attributes that could be exploited for the development of value added fermented foods in Meghalaya.

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References

Abbas, M.M., and Mahasneh, A.M. 2014. Isolation of *Lactobacillus* strains with probiotic potential from camel's milk. *Afr. J. Microbiol. Res.,* 8(15): 1645-1655.

Aderiye, B.I., and Laleye, S.A. 2003. Relevance of fermented food products in southwest Nigeria. *Plant Foods for Human Nutri.,* (Formerly Qualitas

Plantarum), 3: 1-16.

Adeyemo, S.M., and Onilude, A.A.2014. Molecular identification of *Lactobacillus plantarum* isolated from fermented cereals. *Int. J. Biotechnol. Mol. Biol. Res.,* 5(6): 59-67.

Agrahar-Murungkar, D., and Subbulakshmi, G. 2006. Preparation techniques and nutritive value of fermented foods from the Khasi tribes of Meghalaya. *Ecol. Food and Nutri.,* 45: 27–38.

Buchanan, R.E., and Gibbons, N.E. 1974. Bergy's Manual of Determentive Bacteriology,8th edn. (Williams and Wilkins, Baltimore), 1268.

Chelule, P.K., Mokoena, M.P., and Gqaleni, N. 2010. Advantages of traditional lactic acid bacteria fermentation of food in Africa. Current research, technology and education topics in applied microbiology and microbial biotechnology. A. Mendez–Vilas (Ed). *FORMATEX,* 1162-1167.

Claesson, M.J., Sinderen, D.V., and O'Toole, P.W. 2008. *Lactobacillus* phylogenomics – towards a reclassification of the genus. *Int. J. Systematic and Evol. Microbiol.,* 58: 2945–2954.

Crispim, S.M., Nascimento, A.M.A., Costa, P.S., Moreira, J.L.S, Nunes, A.C., Nicoli, J.R., Lima, F.L., Mota,V.T., and Nardi, R.M.D. 2013. Molecular identification of *Lactobacillus* spp. associated with *puba*, a Brazilian fermented cassava food. *Brazilian J. Microbiol.,* 44(1): 15-21.

Das, A.J., and Deka, S.C. 2012. Fermented foods and beverages of the North-East India. *Int. Food Res. J.,* 19(2): 377-392.

Devi, K.R., Deka, M. and Jeyaram, K. 2015. Bacterial dynamics during yearlong spontaneous fermentation for production of *Ngari*, a dry fermented fish product of Northeast India. *Int. J. Food Microbiol.,* 199: 62-71.

Dilek, O., and Hasibe, C.V. 2011. Identification of *Lactobacillus* strains isolated from faecal specimens of babies and human milk colostrum by API 50 CHL system. *J. Med. Genetics and Genomics*, 3(3): 46 – 49.

Ennahar, S., Cai, Y., and Fujita, Y. 2003. Phylogenetic Diversity of Lactic Acid Bacteria Associated with Paddy Rice Silage as Determined by 16S Ribosomal DNA Analysis. *Appl. Environ. Microbiol.,* 69(1): 444–451.

Gitanjali, B.S. and Mandal, S. 2015. Fermented products of India and its implication: A review. *Asian J. Dairy & Food Res.,* 35(1): 1-9.

Guessas, B. and Khal, M. 2004. Characterization of lactic acid bacteria isolated from Algerian arid zone raw goat milk. *Afr. J. Biotechnol.,* 3: 339-342.

Jaiswal, V. 2010. Culture and ethnobotany of Jaintia tribal community of Meghalaya, Northeast India – A mini review. *Indian J. Traditional Knowledge,* 9(1): 38-44.

Krischina, S.A., Jaciara, Z.M., Eunice, C.I., Andréia, Z.D., and Ernani, S.S.A. 2014. Isolation and characterization of lactic acid bacteria and yeasts from the Brazilian grape sourdough. *Brazilian J. Pharmaceutical Sci.,* 50(2): 321-327.

Mosha, T.C.E and Vicent, M.M. 2004. Nutritional value and acceptability of homemade maize/sorghum-based weaning mixtures supplemented with *rojo* bean flour, ground sardines and peanut paste. *Int. J. Food Sci. Nutri.,* 55(4): 301 – 315.

Nout, M.J.R., and Sarkar, P.K. 1999. Lactic acid food fermentation in tropical climates. *Antonie van Leeuwenhoek,* 76: 395-401.

Rahayu, T.H., Gandjar, I., Riani, E.,

Djunaidah, I.S., and Sjamsridzal, A. 2009. Identification and Phylogenetic Analysis of Bacterial Isolates from *Litopeanaeus vannamei* shrimp culture system and gut enviorment based on 16S rRNA gene sequence data. *Microbiol. Indones*, 3(2): 56-60.

Sarkar, P., Kumar, L.D.H., Dhumal, C., Panigrahi, S.S., and Choudhary, R. 2015. Traditional and ayurvedic foods of Indian origin. *J. Ethn. Foods*, 2: 97-109.

Steinkraus, K.H. 1996. Handbook of Indigenous Fermented Food. 2nd ed. Marcel Dekker, Inc., New York, NY.

Suk, H.J., Joung, W.P., Cho, I.J., Nam, K.L., In, C.Y., Byung, Y.K., Hye, K.K., and Young, T.H. 2012. Characterization of Lactic Acid Bacteria Isolated from Sauce-type Kimchi. *Prev. Nutr. Food Sci.*, 17: 217-222.

Valcheva, R., Kabadjova, P., Rachman, C., Ivanova, I., Onno, B., Pre´ vost, H., and Dousset, X. 2006. A rapid PCR procedure for the specific identification of *Lactobacillus sanfranciscensis*, based on the 16S-23S intergenic spacer regions. *J. Appl. Microbiol.*, 290-302.

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