CHAPTER 5
DISCUSSION

The present study using polyphasic molecular approach of culture-independent supported culture-dependent analyses of time-series samples from various indigenous fermentations revealed for the first time that the natural bamboo shoot fermentation was linked with assemblage of LAB community structure which occurred through three-phase successional dynamics of autochthonous LAB belonging to Lactobacillaceae (Lactobacillus), Leuconostocaceae (Weissella, Leuconostoc) and Streptococcaceae (Lactococcus) of phylum Firmicutes. Normally, the raw material acts as the source for this autochthonous species in natural food fermentations. In the present study, this was evidenced from culture-dependent and culture-independent qPCR analyses which revealed the occurrence of LAB population in the fresh shoots though their population varied in different bamboo shoot fermentation (ranging from $10^3$ – $10^9$ CFU/g). In addition to non-LAB taxa belonging to Enterobacteriaceae of phylum Proteobacteria, Illumina data also showed incidence of fair abundance of the predominant fermenting LAB species belonging to the genera Lactobacillus, Weissella, Lactococcus and Leuconostoc in the fresh shoots before the fermentation started. As the fermentation progressed, the selective LAB replaced Proteobacteria and predominated the fermentations. This finding was consistent with the succession of LAB observed during conversion of dough to sourdough, where Gram-positive bacteria outgrew Gram-negative bacteria which originated from natural environment (Ercolini et al., 2013). Previous studies on fermentation of jiang-sun,
(FBS of Taiwan) and mesu (a FBS of Sikkim, India), also showed that *L. plantarum*, *L. brevis* and *L. lactis* were the main LAB species found in the bamboo shoots (Tamang and Sarkar, 1996; Chen et al., 2010). It has also been well-established in sourdough and cassava-dough fermentation that the raw flour acted as the source for the autochthonous species evolved during the fermentation and also played a crucial role in establishing the stable autochthonous LAB ecosystem of sourdough during short fermentation time (Brauman et al., 1996; De Vuyst et al., 2009; Siragusa et al., 2009; Ercolini et al., 2013). Differently, the presence of these predominant LAB species in the fresh raw materials could not be highlighted by the culture-dependent analysis, except in case of hikhu and sele. These observed difference between the two approaches could be explained by the fact that these LAB species can enter a VBNC state (Suzuki et al., 2006; Ganesan et al., 2007; Quiros et al., 2009; Ruggirello et al., 2014) in response to environmental stresses (such as high phenolic and cyanogenic glycoside content) associated with their existence in the fresh bamboo shoot (Bal et al., 2012; Nirmala et al., 2014) which made them undetectable in standard culture media. The existence of such unculturable microbiota in the fresh bamboo shoots was also confirmed during Illumina sequencing by the dominant detection of various fermenting but uncultured *L. acetotolerans, L. intestinalis, Lactobacillus* sp., *W. ghanensis*, *W. beninensis* and a microbial taxon assigned to "uncultured bacterium" category in all the bamboo shoot fermentations studied.

Though selective LAB species belonging to the genera *Lactobacillus, Weissella, Leuconostoc* and *Lactococcus* were the main microbiota playing major role in the natural bamboo shoot fermentations, each food fermentation harbored
distinct microbial community structure and composition with certain predominant phylotypes.

Short-duration *soidon* fermentation was associated with three-phase succession of LAB to attain a stable ecosystem within 7 days natural fermentation. Long-duration fermentations also highlighted three-phase succession of the selective LAB. *Andro*-type and *Kwatha*-type *soibum* fermentations showed quite similar microbial community structure. *L. plantarum*, *L. brevis* and *B. subtilis* were identified as the major dominant culturable bacterial phylotypes involved in the indigenous *soibum* fermentation, along with sub-dominant population of yeasts predominated by *M. guilliermondii*. The microbial succession during the fermentation was more prominent in Andro than Kwatha. Interestingly, the involvement of yeasts in natural bamboo shoot fermentations was observed only in the fermentations of *soibum* and *soidon* from Manipur, though both foods have different yeast species compositions. This was in consistent with the previous findings of the predominant presence of yeasts in *soibum* reported by Giri and Janmejay (2000). This indicated the possible role of geographical location in the selection and persistence of autochthonous fermenting microbiota in food ecosystems. Unlike *soibum* and *soidon*, the dominant culturable isolates involved in *hikhu* and *sele* fermentations belonged only to the *Lactobacillaceae*. However, combined culture-dependent and metagenomics-based analysis confirmed that *hikhu* and *sele* fermentations were driven by *Lactobacillaceae*, *Leuconostocaceae* and *Streptococcaceae*. The microbial community structure of finished products was significantly different from that associated with the fermentation stages. Association of these predominant LAB in natural food fermentation and three-
phase succession have been previously well described for fermented sourdoughs (Van der Meulen et al., 2007; Siragusa et al., 2009; Weckx et al., 2010; Ercolini et al., 2013), cassava (Brauman et al., 1996), and kimchi (Choi et al., 2003; Bae et al., 2005; Lee et al., 2005).

In any fermentation model, for the fermentation to start, the first and the foremost step is the adaptation of the naturally occurring or added microorganisms to the complex raw materials and the environmental conditions. This leads to the competitive selection of those populations which are more effectively adapted and responded to the in situ conditions, creating a dynamic change of microbial population until a stable ecosystem is achieved. Our current findings indicated that natural bamboo shoot fermentations are linked with a distinct and rapid succession of these selective LAB species by inhibiting Gram-negative non-LAB population present in the raw material. Succession of microbial populations could be correlated with the adaptation (substrate, temperature, pH), carbohydrate and amino acid metabolism, competition and stress tolerance (acidic, oxidative, osmotic) by the microorganisms during the dynamics process (Van der Meulen et al., 2007; Serrazanetti et al., 2013). The appearance and succession of LAB in bamboo shoot fermentation might be correlated with the presence of naturally occurring Gram-negative and aerobic bacteria and yeasts, which rapidly consumed the oxygen available before the starting of the fermentation, thereby promoting the rapid predominance of the facultatively anaerobic LAB. The growth inhibition and sudden disappearance of non-LAB population would have been due to the antimicrobial peptides produced by the members of Lactococcus, Leuconostoc and Weissella (De Vuyst and Vandamme 1994; Srionnual et al., 2007). Leuconostoc
spp., *Weissella* spp. and *Lactococcus* spp. have been recognized as the main organism responsible for initiation of short fermentation of vegetables (such as *sauerkraut, kimchi*) and cereals (sourdoughs) (Harris et al., 1992; Lee et al., 2005; Cho et al., 2006; Eom et al., 2007; Plengvidhya et al., 2007; Jung et al., 2011; Van der Meulen et al., 2007; Weckx et al., 2010). This may be because of the robustness to adapt at low temperature (Hamasaki et al., 2003; Eom et al., 2007; Ricciardi et al., 2009) (the temperature during the early phase of the *soidon* fermentation ranged from 19.6 ± 0.1 °C to 20.4 ± 0.06 °C), relatively less acidic environment (Cho et al., 2006; Ricciardi et al., 2009) and high salt concentration (Harris et al., 1992; Eom et al., 2007; Plengvidhya et al., 2007; Ricciardi et al., 2009) [bamboo shoot have high potassium content (1.3 - 3.5 %) (Waikhom et al., 2013)] and ability to resist the antimicrobial compounds of plant such as phenol (Serrazanetti et al., 2013). The domination of these heterofermentative LAB (*Weissella* and *Leuconostoc*) is a general feature of plant material fermentation (Daeschel et al., 1987; Brauman et al., 1996) due to the presence of a cohort of genes encoding enzymes and transporter proteins involved in metabolism and fermentation of complex carbohydrate substrates of plant origin (Kim et al., 2008; Siezen et al., 2010; Kim et al., 2011). It has also been shown that species such as *L. plantarum*, *L. lactis* and *Leuconostoc* sp. are linamarase-positive and could resist high cyanide content and dominate early fermentation of cyanogenic-cassava roots (Amoa-Awua et al., 1996; Brauman et al., 1996; Lei et al., 1999; Obilie et al., 2004), which may partly explain the early dominance of these organisms during the natural bamboo shoot fermentations. Dominance of high acid-producing *L. brevis* and *L. plantarum* during the final stage of *soidon* fermentation supported the
previous findings that these two species were dominantly present in the finished FBS products of NE India (Tamang and Sarkar 1996; Tamang et al., 2008; Tamang and Tamang 2009). The dominance of *L. brevis* and *L. plantarum* after initial fermentation by *Lactococcus*, *Leuconostoc* and *Weissella* has been observed in fermented cassava roots, *kimchi* and sourdough (Brauman et al., 1996; Choi et al., 2003; Van der Meulen et al., 2007). This was in contrast to the *sauerkraut* fermentation where an initial heterofermentative stage was supplanted by a homofermentative stage (Plengvidhya et al., 2007). Both culture-dependent and culture-independent analyses revealed that *L. lactis* was prevalent as subdominant species throughout *soidon* fermentation. The species is known to produce nisin, a bacteriocin that effectively inhibits growth of other lactobacilli (Choi and Park 2000; Fujita et al., 2007; Alegria et al., 2010). The co-existence of both *L. brevis* and *L. plantarum* with *L. lactis* could be explained by the ability of these lactobacilli to produce nisinases, enzymes which neutralize the antimicrobial activity of the peptide (Davidson and Harrison 2002; Takala and Saris 2007). Culture-dependent analysis showed that *L. citreum* was persistently present throughout *soidon* fermentation, though it was reported to be sensitive to low pH. This could be explained by its ability to produce insoluble dextran which may be responsible for tolerance to acidic environment (at pH < 4.0) of lactic acid fermentation (Choi et al., 2003; Eom et al., 2007). The dominant growth of both homo- and heterofermentative LAB produces organic acids, CO₂, ethanol, free sugars and other metabolites thereby rapidly reducing pH, increasing the acidity and creating an anaerobic environment favoring the growth of heterofermentative and high acid-producing LAB during the mid and late stages of fermentation. The
anaerobic condition, thus created, may favor stabilization of the texture and the formation of esters from acids and alcohols, contributing to the unique flavor and texture of mature FBSs. Though yeasts do not seem to play significant role during the microbial succession in bamboo shoot fermentation, their presence may influence the bioavailability of nutrients (minerals), flavor and preservation of mature FBSs (Brauman et al., 1996; Nuobariene et al., 2012; Weckx et al., 2010).

In the present study, ARDRA was very effective with high taxonomic resolution up to subspecies level, in grouping larger number of isolates in short time. Further, from their respective population, a reliable species level population dynamics was able to draw from the stage-wise collected samples. Culture-independent approaches also showed distinct population dynamics and changes in community structure which supported the observed multiphase microbial succession during the fermentation. However, some discrepancies were observed between these two approaches in the level of resolution of taxa detection and microbial diversity. Few examples are highlighted below. *L. plantarum*, which was predominantly cultivated in the late phase of *soidon* fermentation, was dominantly detected throughout the fermentation during PCR-DGGE analysis. This could be explained by the PCR sensitivity to lower template DNA, since very low population of *L. plantarum* was detected in the early to mid phases of fermentation by qPCR analysis. At the same time *L. acetotolerans, L. intestinalis, W. ghanensis, W. beninensis*, which were not at all isolated during culture-dependent analysis, were dominantly detected during the fermentations by PCR-DGGE and Illumina sequencing. *L. citreum*, the dominant cultivable species which is responsible for initiation of the fermentation, was not at all detected during PCR-DGGE analysis.
In silico analysis using ARB-SILVA database (http://www.arb-silva.de/search/testprime/) revealed that the currently used SSU rRNA gene V3 amplification primer pair 338f/518r had extremely low coverage (1.5 %) of *Leuconostoc* genus, while it covers more than 86 % of the members of the genera *Lactobacillus*, *Lactococcus* and *Weissella*. When investigating microbial ecology through culture-independent approaches, an efficient and reliable method for nucleic acid extraction is the most critical step. In our present study, an enzymatic and chemical lysis-based DNA extraction method was developed which efficiently extracted microbial DNA with high yield, purity, content and diversity. However, using DNA as the target had contributed to the overestimation of microbial species, particularly during PCR-DGGE, and failure to highlight distinct microbial succession and dynamics during the *soidon* fermentation. Such was also the case during Illumina data analysis, which dominantly detected uncultured *L. acetotolerans* throughout the fermentation. This could be because of DNA as the target, being more stable than RNA, persisted for long time allowing the detection of dead and VBNC cells as predominant population throughout the fermentation. Extraction of RNA from the complex food matrix may give information about the metabolically active bacterial species during fermentation.

We realized that subspecies level dynamics is important to understand the natural fermentation. But during culture-independent analysis the amplified SSU rRNA gene V3 and V4-V5 regions were not able to resolve the two subspecies of *L. lactis* (*L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* as differentiated by culture-dependent analysis) which were predominantly involved in the successional dynamics of *soidon* fermentation. Hence, *L. lactis* was detected dominantly
throughout the fermentation in PCR-DGGE and Illumina analysis, though *L. lactis* subsp. *cremoris* were isolated only during early to mid phases. Moreover, the dominant culturable species such as *L. zymae* and *W. cibaria* were not identified properly during PCR-DGGE as the corresponding DGGE bands showed the same sequence similarity with the closely related *W. confusa* and *L. spicheri* respectively. Recent research also concluded that the V3 region underestimated the bacterial community and V4, V5-V6 and V6-V7 regions estimated high species richness comparable to those generated from full length SSU rRNA gene sequences (Youssef *et al.*, 2009). Though PCR-DGGE analysis showed the indication that different strains of *W. cibaria*, *W. beninensis*, *L. zymae* and *L. fermentum* might probably be present as indicated by the consistent multiple DGGE banding patterns, it could not confirm the same because the pattern may also be due to the presence of multiple heterogeneous copies of rRNA genes indicating intra-species sequence divergence (Kim *et al.*, 2009; Kang *et al.*, 2010). Similar to previous findings (Lopez *et al.*, 2003), prominent bands corresponding to chloroplast and mitochondrial genome of bamboo were detected in all the stages of fermentation and in the raw bamboo shoot during PCR-DGGE analysis. Similar detection of chloroplast and mitochondrial specific amplicons of various plant origin was encountered during analysis of Illumina sequencing data from all the bamboo shoot fermentation studied. For example, from *soidon* samples analyzed by Illumina sequencing, of the 451 OTUs detected, the primer pair used for Illumina sequencing of V4-V5 region brought out 50 plant-specific OTUs (ranging from 9 – 23 between the samples) which on an average accounted for 23.71 % (1.08 – 59.67 %) of the total microbial composition of *soidon* fermentation. This showed that the
variable regions of SSU rRNA gene targeted for microbial ecology study and the primers are important factors to be considered for realistic ecological studies.

Taken together, although culture-independent analyses have become indispensable for in-depth microbial ecology study of fermented foods (Cocolin et al., 2013; Ercolini et al., 2013), our current findings indicated the importance of cultivation-based molecular approaches for explaining the microbial succession and population dynamics during food fermentation in which species, subspecies and strain-level population dynamics play highly significant role. In addition, the metabolic traits responsible for technological features of starter culture and health promoting properties of probiotics are recognized to be strain-specific rather than species-specific (Gänzle 2009; Turpin et al., 2010). In such ecosystem, mere identification of the microbial ecology with low taxonomic resolution (genus level by using PCR-DGGE and species level by Illumina sequencing) is insufficient for future applications on development of starter cultures and functional food products from the traditional fermented foods, which play important role in global enhancement of food security particularly in developing countries. More realistic view of microbial ecology of traditional food fermentation can be obtained by proper design of the study. We therefore suggest that for future studies, culture-independent supported culture-dependent approach should be followed. Cultivation-independent molecular analysis can be complemented to identify the missing predominant species during cultivation (such as *L. acetotolerans*, *L. intestinalis*, *Lactobacillus* sp., *W. ghanensis*, *W. beninensis*) for their selective isolation for efficient starter culture development. In the case of short food fermentation, frequent (hourly) sampling may give more realistic view of microbial
dynamics; a drastic change in microbial community profile during the first day of soidon fermentation in our present study was evidenced. During culture-independent analysis more focus can be given on careful optimization to reduce methodological biases, particularly nucleic acid extraction method (different extraction methods with different lysis principles are bringing out different community profile), target gene and its region (phylogenetically significant single copy house-keeping gene with regions of high taxonomic resolution), selection of primers (high coverage of wide range of microbial taxa with less cross-domain specificity) and PCR amplification conditions (not to overestimate the less prevalent organisms as predominant).

Unlike other FBS products reported in Taiwan (jiang-sun) (Chen et al., 2010), Thailand (naw-mai-dong) and Indonesia (asinan-rebung) (Tanasupawat and Komagata, 1995) which are being produced by fermentation in brine solution (2 - 3 %) for weeks to month, the FBSs of NE Indian origin are non-salted in nature. Consumers nowadays prefer low intake of dietary sodium to lower disease risk, which has led to research aimed at reducing the use of high NaCl concentration or partially replacing NaCl with KCl during fermentation without compromising the flavor, texture and stability of the final product (Viander et al., 2003; Mudgal et al., 2006). The present findings on the successional dynamics of the selective predominant LAB during natural bamboo shoot fermentation may aid in selection of superior starter strains and henceforth, development of fermentation technology for production of non-salted fermented vegetables which can even replaced the current starter cultures of well-established fermented vegetables such as kimchi and sauerkraut. In fact, L. citreum, L. plantarum, L. lactis and W. cibaria have been
successfully used as starter cultures in vegetable and cereal based fermented foods (Harris et al., 1992; Choi et al., 2003; Minervini et al., 2010; Wolter et al., 2014). Studies on microbial interaction and linking the microbial succession with the metabolite changes during bamboo shoot fermentation may bring out the specific role of the predominant species. In addition, studies on bacteriophage diversity and communities may also give useful insights in selecting phage-resistant starter culture strains, as the rapid lytic cycle of bacteriophage significantly affect the survival of the starter culture (Lu et al., 2003; Mudgal et al., 2006; Lu et al., 2010; Garneau and Moineau, 2011). Absence of E. coli, S. aureus, B. cereus, L. monocytogenes and Salmonella sp. during the indigenous fermentation in both classical plating and culture-independent molecular analysis, supported the microbial safety of the traditionally produced FBSs.

Strain level typing of L. plantarum and relating it with its phenotypic and functional attributes are in demand for selection of the best performing strains for various applications. Among the various typing methods, chromosomal restriction digestion pattern analysis is highly discriminative and reproducible (Pepe et al., 2004; López et al., 2008). In the present study, SfiI-REA-PFGE analysis showed very high genetic diversity among the L. plantarum population isolated from a single food niche of bamboo shoot fermentation during different stages of the indigenous fermentation. These strains were found to be associated with a particular type of bamboo shoot fermentation. Earlier studies showed a high ecological adaptability of L. plantarum, and their diversity was associated with different food niches such as fermented vegetables, fermented meat, fermented cereal and fermented dairy products, but not separated based on food or human
origin (Siezen et al., 2010). Food type specific association of these diverse strains evidences that selection pressure leads to rapid evolution in *L. plantarum* population to adapt and survive in a particular type of environmental niche. Though no association between strain diversity and stages of bamboo shoot fermentation was noticed, it indicated the involvement of multiple strains in the fermentation which is suggestive of a complex and dynamic nutritional requirement associated with bamboo shoot fermentation.

MLST based phylogenetic analysis could not establish the association of the *L. plantarum* population with food or human or geographical origin. Instead, the present findings evidenced that the *L. plantarum* population largely followed clonal structure with two universal clonal complexes of lineages (eBG-16 and eBG-24). The ancestral status of an ST is the one with the highest number SLVs in the lineage. Sequence types ST16 and ST24 seemed to be the founder genotypes of eBG-16 and eBG-24 respectively. Though the population size under each eBG was low, many redundant STs and higher SLVs were evidenced which is a classic feature of a clonal population (Diancourt et al., 2007; Tanganurat et al., 2009; Chaillou et al., 2013). Earlier studies also reported no association of highly diverse *L. plantarum* and *L. sakei* populations with human and food origin (Molenaar et al., 2005; Chaillou et al., 2013). In our present study, the two lineages are largely composed of fermented food strains while the strains of human origin (oral, gut, genital and fecal) are scattered over the two lineages which support the hypothesis that human related *L. plantarum* strains generally originate from the food eaten by the individual (Siezen et al., 2010).
Absence of homologous recombination indicated that the genetic diversity observed within the *L. plantarum* population was the sole outcome of the phylogenetic signal. Recombination often results in a highly diverse panmictic population structure (Suzzi, 2011). In contrast to our findings, panmictic population structure (presence of recombination) of *L. plantarum* was reported by de Las Rivas *et al.* (2006). This may be due to the choice of MLST loci analyzed and the choice of diverse representative strains studied after discriminating through chromosomal restriction digestion profiling. Though we had analyzed only 5 loci, they were sufficient enough to capture the high diversity and complexity of *L. plantarum* population. The nucleotide diversity of the housekeeping genes analyzed in the present study [0.011 (rpoA) to 0.028 (gyrB)] is much higher than the earlier reported diversity of 0.0004 (pgm) to 0.0072 (gdh) for *L. plantarum* population (de Las Rivas *et al.*, 2006). Similarly, the percentage of polymorphic sites, which ranges from 10.81 % (rpoA) to 24.48 % (gyrB), is also higher in comparison to 1.03 % (ddl) to 7.73 % (gdh) reported earlier. The observed diversity in the *L. plantarum* population is higher than other *Lactobacillus* spp., namely *L. casei* (0.0029 - 0.0109), *Lactobacillus delbrueckii* (0.0051 - 0.0096) and *L. sakei* (0.0044 - 0.0265), but the diversity is lower when compared with *E. coli* (0.015 - 0.038) (Diancourt *et al.*, 2007; Tanigawa and Watanabe, 2011; Chaillou *et al.*, 2013). Evolutionary distance can be estimated from the nucleotide diversity (synonymous changes per synonymous site) by using the substitution rate of $4.5 \times 10^{-9}$ per site per year for *E. coli* (Ochman *et al.*, 1999). The mean pairwise difference at synonymous site resulting from the concatenated sequence of the five loci from 36 *L. plantarum* strains was 0.3. From this, the average age of *L.*
plantarum was estimated to be 66.67 million years, which is higher than the predicted age of 12.95 million years for L. reuteri (Oh et al., 2010). This indicated the ancestral nature of L. plantarum among the Lactobacillus spp. studied. A large scale study involving sufficiently large number of strains of diverse origin with genetically diverse background (confirmed by chromosomal restriction digestion profiling) and the use of more number of housekeeping loci might give a better resolution (Chaillou et al., 2013) into the population structure and evolutionary history of L. plantarum.

Three L. plantarum strains (SD1S6L2, EGD-AQ4 and AY01) were clearly separated out from other strains during phylogenetic analysis. Among these, EGD-AQ4 and AY01 were distantly related to other L. plantarum strains available in NCBI whole genome database and showed similarity less than the cut off limit of 70 % at genome level which is normally used to claim for novel species or subspecies (Tanigawa and Watanabe, 2011). A putative subspecies Lactobacillus plantarum subsp. argentoratensis (Bringel et al., 2005) showed only 11 - 20 % dissimilarity with L. plantarum strain WCFS1 during comparative genome hybridization (Siezen et al., 2010). The divergent strain SD1S6L2 showed a rare phenotypic property of D-xylose fermentation, which was also observed for the strains isolated from Vietnamese fermented foods (Siezen et al., 2010). Similar observation of genetically distinct sub-population of L. plantarum strains was reported by Bringel et al (2005), which was claimed later as new subspecies (L. plantarum subsp. argentoratensis). Based on the above genotypic and phenotypic diversity, the strain SD1S6L2 which can utilize D-xylose is proposed here as Lactobacillus plantarum subsp. xylosus. Further comparative studies of the above
three strains and other xylose utilizing *L. plantarum* strains are proposed here for further confirmation of subspeciation. All these three deviant strains originated from Indo-Burma biodiversity hotspot in which SD1S6L2 and EGD-AQ4 were from bamboo shoot fermentation. Taken together, our present findings suggested for the evidence of possible subspeciation within the *L. plantarum* population. This is a microbial evidence of evolution in the Indo-Burma biodiversity hotspot. The *L. plantarum* strains of FBS origin with similar genotype grouping possessed quite comparable carbohydrate fermentation and hydrolytic enzyme production profiles. *L. plantarum* is the key initiator of bamboo shoot fermentation. Our earlier attempts to initiate bamboo shoot fermentation with other *Lactobacillus* spp. and *Bacillus* spp. were not successful. Production of strong β-glucosidase might play an important role in detoxifying the cyanogenic glycosoïdes present in the raw bamboo shoot (Lei et al., 1999), which would pave way for other bacteria to succeed the later stages of fermentation.

The lower $d_\text{S}/d_\text{S}$ ratios (much lower than 1) indicated that all the target genes were under purifying selection which is a typically expected natural variability for conserved housekeeping genes (de Las Rivas et al., 2006; Oh et al., 2010). High allelic variation without recombination supported that the nucleotide diversity reflected the phylogenetic variation. Excess of low frequency allelic variation without recombination and negative Tajima’s D values of the studied genes indicated a recent selection sweep in *L. plantarum* population. *L. plantarum* domestication for food fermentation followed by human colonization may be one of the recent selection pressures.