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Original Article

Bacterial population diversity in *Sataw-Dong*, a traditional fermented stink bean, during fermentation using the combination of culture-dependent and culture independent methods through DGGE technique

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Abstract

Sataw-Dong is a pickled plant-based food in which stink beans (*Parkia speciose*) undergo spontaneous fermentation in brine and has its characteristic flavors and tastes. The changes in bacterial flora during *Sataw-Dong* fermentation was investigated using culture-dependent and culture-independent methods. Molecular approaches applying PCR-denaturant gel gradient electrophoresis (PCR-DGGE) was used as culture-independent. The bacterial profile targeting the V3 region of the 16S rRNA gene indicated lactic acid bacteria (LAB) belonged to *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus pentosus* and *Enterococcus feacium*. Results showed *L. plantarum* was the predominant species among LAB. Species of plant and environmental flora, such as *Cohnella collisoli*, *Erwinia billingiae*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Staphylococcus cohnii*, were detected at the early period of fermentation and thereafter disappeared due to the presence of dominant LAB. The combination of molecular techniques was greatly effective in accessing and profiling bacterial community for a successful application in selection of potential starter cultures.

Keywords: culture-dependent, culture-independent, PCR-DGGE, *Sataw-Dong*, V3 region

1. Introduction

The vegetable fermentation has been performed since ancient time because fresh vegetables have a very short shelf-life and the fermentation is able to deliver end-products appreciated for their typical sensory properties (Hutkins, 2006). *Parkia speciosa*, known as stink bean or *Sataw* in Thai, bears long and flat bean pods with green seeds. This plant is abundantly grown in the southeastern Asia, including Thailand, Malaysia, Indonesia and Philippines (Al Batran *et al.*, 2013). It is called stink bean due to its strong and pungent odor. *Sataw-Dong*, a traditional fermented stink bean, is a well-known pickle side dish that stink beans are spontaneously undergone by lactic fermentation for a period lasting from a few days to a few weeks. Furthermore, stink beans also have been reported to possess some folk medicinal properties, such as antiangiogenic, antioxidant and antihypertension (Aisha, Abu-Salah, Alrokayan, Ismail, & Abdulmajid, 2012).

Spontaneous vegetables fermentation is highly dependent on the naturally occurring lactic acid bacteria (LAB) present in raw materials, especially *Leuconostoc mesenteroides* and *Lactobacillus plantarum*, to develop organoleptic characteristics and improve hygienic stability of the final products. Several species of LAB have been isolated from various fermented vegetable products and therefore used as starter cultures**.** It is necessary to correctly profile LAB in fermented foods because of their specific characteristics

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leading to guarantee safety and the development of consistent aroma and flavor (Kesmen *et al.*, 2012).

Molecular approaches have been applied as an effective tool to identify the dominant flora in complex bacterial communities in fermented foods for over the past decades**.** In particular, PCR-based genomic fingerprinting techniques are believed to have the highest potential for more rapid and reliable detection, identification and classification of LAB (Ben Amor, Vaughan, & de Vos, 2007). Culture-dependent methods alone have not provided a completely accurate representation of complex communities occurring in natural food ecosystem according to the limitations of conventional microbiological methods that requires selective enrichment and sub-culturing (ben Omar & Ampe, 2000). Thus, the trend moving forward to methods that avoid the use of selective cultivation and isolation of bacteria from samples is justified, considering the biases related to traditional culture-dependent methods. Culture-independent method, termed denaturing gradient gel electrophoresis (DGGE), has potential for more rapid and reliable detection, identification and classification of LAB in many fermented products (Liu *et al.*, 2012).

The 16S rRNA gene seems to be the most widely used as a molecular marker for the determination of the phylogenetic relationships of bacteria. The hypervariable V3-region on the 16S rRNA gene is the most frequently used to study of an unknown and complex bacterial community**.** Moreover, the V3-region is proved to have a high grade of resolution, and it is regarded as a good choice when it comes to length and inter-species heterogeneity (Chen, Wang, & Chen, 2008). The use of combined culture-dependent and culture-independent methods have become the preferred approaches for determining and analyzing the species composition of targeted bacterial community in fermented foods (Silvestri *et al.*, 2007).

In order to further apply the use of autochthonous starter cultures that have potential for *Sataw-Dong* production, it is essential to understand the bacterial community and dominant bacteria occurring in this fermentation. Therefore, the aim of this study was to study the microbial profile during *Sataw-Dong* fermentation and to evaluate bacterial dynamic based on both culture-dependent and culture-independent molecular methods focusing on the use of the hypervariable V3-region on the 16S rDNA through DGGE technique.

2. Materials and Methods

2.1 *Sataw-Dong* samples and fermentation procedure

Sataw-Dong was prepared according to traditional recipe**.** The pods of stink bean were blanched, seeded out and washed with clean water. The beans were mixed with the brine, containing of table salt, 3-4%; sugar, 4-5%; and Malabar tamarind (*Garcinia cambogia*), 0.5%. The samples were transferred into containers with plastic lids. The containers were subjected to spontaneously fermentation at room temperature (30±2 °C). Triplicate samples of *Sataw-Dong* were subjected to microbiological and molecular analyses, at the following time points: 0, 1, 2, 4, 6, 8, and 10 days. Prior to analyses, the selected sample was blended and used as composite samples.

2.2 pH, total acidity and salt concentration analyses

The pH value of each sample was measured with pH meter (OHAUS, China). Total acidity as lactic acid in the brine was determined by titration with a standard solution of 0.1 N NaOH using phenolphthalein as an indicator (Association of Official Analytical Chemists [AOAC], 2000). The salt concentration of the brine was analyzed by titration with $AgNO_3$ and expressed as % (w/v) NaCl as described by Skoog *et al.* (1988).

2.3 Microbiological analyses

Twenty-five grams of each sample were mixed with 225 ml of sterile peptone water (0.1%, w/v) and homogenized using a stomacher (230 rpm, 5 min). Further appropriate dilutions were made, and the following analyses were carried out on duplicate agar plates by spread plate method: (a) total viable count (TVC) on plate count agar, PCA (LuQiao, China); (b) LAB on De Man Rogosa Sharpe (MRS) agar (LAB, UK); (c) Micrococcaceae on M17 agar (Merck, Germany); Enterobacteriaceae on violet red bile agar (Himedia, India); (d) staphylococci on Baird Parker medium (Himedia, India) with egg yolk tellurite emulsion added; (f) yeasts on potato dextrose agar (Himedia, India) supplemented with tartaric acid (1%, v/v)**.** All plates were incubated at 37 °C for 24-48 h, except yeasts at 30 °C for 48-72 hrs. After counting, means and standard deviations were calculated in term of colony forming unit per gram product (CFU/g). The plates were subsequently used for further bulk cell collection.

2.4 Harvesting for bulk cells

In order to investigate the cultivable bacteria of *Sataw-Dong*, bulk cells were individually harvested from TVC and MRS medium using the whole content of countable dilution. In particular, the colonies appeared on the surface of specific growth medium were suspended with sterile saline solution, then harvested with a sterile pipette and stored at -20 °C prior DNA extraction.

2.5 DNA extraction

Total genomic DNA was extracted according to the method described by Cocolin *et al.* (2004) with a minor modification. Brine was centrifuged at 8,000×*g* for 10 min. The pellets were washed twice with saline solution and resuspended in 50 µL of 20 mg/mL lysozyme (Fluka, USA). After incubation at 37 °C for 30 min, 30 µL of 25 mg/mL proteinase K (ARMESCO, USA) and 150 µL of proteinase K buffer were added. The mixture tubes were incubated at 65 °C for 90 min before the addition 400 µL of breaking buffer, then incubated at 65 °C for 15 min**.** The 400 µL of phenol:chloroform:isoamyl alcohol (25:24:1, pH 6.7) was added for DNA, RNA and protein extraction**.** The tubes were subjected to centrifugation (12,000×*g*, 10 min, 4 °C), the aqueous phase was taken into new clean tubes. The nucleic acid was precipitated with 1 mL of ice-cold absolute isopropanol. Genomic DNA was centrifuged (12,000×*g*, 10 min, 4 °C), washed with 70% (v/v**)** ice-cold ethanol. After centrifugation, DNA was dried at room temperature, thereafter

resuspended in 20 µL of RNase-Dnase-free sterile water, and treated with 5 µL of 10 mg/mL DNase-free Rnase (Vivantis, USA). After incubation for 5 min at 37 °C, genomic DNA was stored at -20 °C. For bulk cell DNA extraction, 500 µL of bulk cell mixture was used and followed the extraction as described above.

2.6 The amplification of the V3 region of 16S rRNA gene and DGGE analysis

The amplification of V3 region of the 16S rRNA gene was carried out using primer V3f (5´-CCTACGGG AGGCAGCAG-3´) and V3r (5´-ATTACCGCGGCTGCTGG-3´) and a GC-clamp was added to the forward primer (Muyzer, de Waal, & Uitterlinden, 1993). Amplification products were analyzed by electrophoresis in 2% (w/v) agarose gel. PCR products were analyzed by DGGE using Dcode apparatus (Cleaver Scientific, UK). Samples (30 µL) were run on 8% (w/v) polyacrylamide gels in 1× TAE buffer containing 30-55% linear denaturant gradient. The 100% denaturing solution contained 40% (v/v) formamide and 7.0 M urea. Electrophoresis was performed in 1×TAE buffer at constant temperature (60 °C) for 6 h at 120 V. Gels were immersed in 1×TAE buffer with 1× (final concentration) SYBR Gold (Invitrogen, USA) for 15 min, visualized and photographed under UV illumination using the Gel Documentation (UVI-TECH, England).

2.7 Identification of bands

The bands were excised from the gel with sterile scalpel and placed overnight at 4°C in sterile water to diffuse DNA out of the polyacrylamide matrix. The solution was used as template and re-amplified with primers without the GC clamp**.** For sequencing analysis, the PCR products were purified with the QIAquick PCR purification kit (QIAGEN, USA) and sequenced with a DNA sequencer (Ward Medic Ltd., Malaysia). Searches in GenBank with BLAST (Altschul *et al.*, 1997) were performed to determine the closest known relatives of the partial ribosomal DNA sequences obtained.

3. Results and Discussion

3.1 pH values, total acidity and salt concentration analysis

Changes in pH values, total acidity and salt concentration of *Sataw-Dong* are shown in Figure 1. The pH values rapidly decreased from 5.5 to 4.1 during at the beginning of fermentation and thereafter gradually declined to 3.5 at the end of fermentation. The rapid reduction of pH during the early stage of fermentation could be attributed in part to a low buffering capacity of the vegetable substrate (Palomino *et al.*, 2015). The decrease of pH values was corresponding with the increase of total acidity. The total acidity increased from 0.01 to 1.39% on each day until day 6 of fermentation, after which the rise almost remained stable, reaching the final acidity of 1.53%. The pH reduction occurred due to the organic acids (especially lactic acid) produced by homofermentative LAB via Embden-Meyerhof-Parnas pathway from hexose. The organic acids produced could affect the inhibition of food spoilage and/or pathogenic

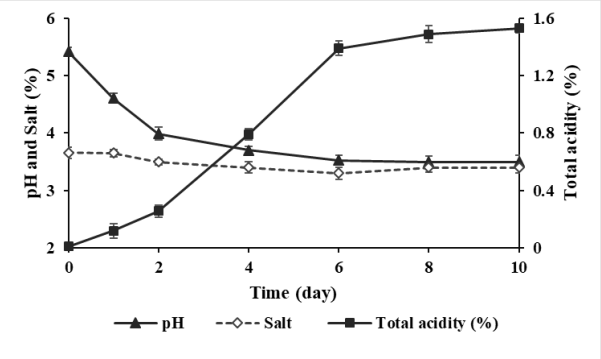

Figure 1. Changes in pH values, total acidity and salt concentration of *Sataw-Dong* during fermentation. The bar indicates standard deviation.

bacteria, the preservation of many fermented foods as well as the contribution of the taste, aroma and texture of the final products (Caplice & Fitzgerald, 1999).

The salt concentration in brine was determined throughout the fermentation. It was almost stable about a value of 3.5%. This result was in accordance with Bleve *et al.* (2014) who mentioned the stability of salt concentration in table olive brine during the whole period of fermentation**.** In addition, the brine with salt concentration less than 10% (w/v) allows spontaneous fermentation to take place and stimulate the growth of natural LAB derived from raw materials.

3.2 Microbiological analysis

Results of the changes in population profile concerning the viable counts of LAB, total viable count, Micrococcaceae, staphylococci, Enterobacteriaceae and yeasts during spontaneous fermentation of *Sataw-Dong* by culturing method are summarized in Figure 2. The initial LAB count was 6.1 log CFU/mL and gradually increased to reach the maximum level at 8.3 log CFU/mL after 6 days of fermentation. The slight decrease was thereafter observed until the end of fermentation. This slight decrease of LAB count is probably due to the depletion of fermentable carbohydrate available in an environment (Lorenzo & Franco, 2012). LAB are acid-tolerant species and they are the predominant microbiota in many fermentations of plant and

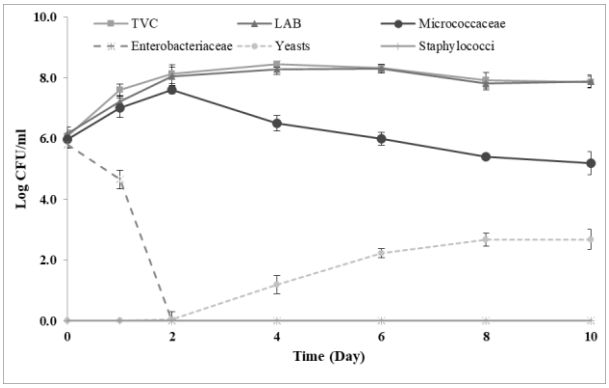

Figure 2. Distribution of microbiological population at different time during *Sataw-Dong* fermentation.

vegetable products, mainly because of their ability to convert fermentable carbohydrates into lactic acid and other organic acids depending on the metabolic pathway (Lorenzo & Franco, 2012). The TVC profile showed similar trend to the LAB count. Micrococcaceae reached maximum count after 2 days of 8 log CFU/mL and consequently decreased to 5.2 log CFU/mL at the end of fermentation, owing to the decrease in pH and limitation of nutrients (Visessanguan *et al.*, 2006). Relatively high viable count of LAB (ca. 6-7 log CFU/mL), initially acidified by the addition of Malabar tamarind, was observed at the beginning. This acidification has stimulated and favored the fermentation by LAB (Abriouel, Benomar, Lucas, & Gálvez, 2011). Yeasts were detected at day 3 and slightly increased to 2.7 log CFU/mL. Oxidative yeasts normally present in fermented vegetables in brine with some beneficial and adverse effects. The most problems of yeasts normally found in fermented vegetable are the formation of gas pockets, the softening of plant tissue, cloudy of brines, and production of off-flavor (Bleve *et al.*, 2014).

Regarding hygienic state of *Sataw-Dong*, Enterobacteriaceae were also detectable over 4 log CFU/mL at day 1 and then disappeared after day 2 of fermentation when the pH value was lower than 4.0. The disappearance of Enterobacteriaceae population was parallel with pH reduction**.** Surprisingly, staphylococci count was not detectable throughout the fermentation period. The depletion in Enterobacteriaceae and staphylococci suggested the poor competitiveness owing to the intensive growth of LAB associated with adverse condition when pH reaches low values (<4.3) of brine (Zhao *et al.*, 2011). In addition, the inhibitory effect of organic acids lies in the pH-lowering effect as well as the undissociated form of molecules**.** These molecules act on the bacterial cytoplasmatic membrane, interfering with maintenance of cell membrane potential, inhibiting active transport, reducing intracellular pH, and inhibiting a variety of metabolic functions (Caplice & Fitzgerald, 1999).

Therefore, microbiological quality of fermented products is quite important for food safety and its shelf-life**.** LAB have also been significantly found to produce organic acids, prevent any growth of spoilage and food-borne pathogens as well as enhance of a correct flavor and texture profile in final product (Bleve *et al.*, 2014).

3.3 Analysis of bacterial diversity in *Sataw-Dong*

The analysis results of bacterial diversity developed in *Sataw-Dong* sample during spontaneous fermentation revealed by DGGE approach were obtained by the amplification of the V3 region of the 16S rRNA gene, allowing molecular description of bacterial community. All of the bands on the DGGE profile were excised from the acrylamide gel and identified after sequencing by the BLAST search.

The DGGE profile of direct DNA extraction from *Sataw-Dong* sample as culture-independent is showed in Figure 3. The results of individual band sequencing and the GenBank accession numbers are summarized in Table 1. A high bacterial diversity at the beginning of fermentation was observed, this being evidenced by the presence of numerous bands (Figure 3). Only *Staphylococcus cohnii* (band 1), *S. hominis* (band 9), *Cohnella lupini* (band 11), *Cohnella collisoli* (band 12), *Erwinia billingiae* (band 16), *Enterobacter asburiae* (band 17), *Ent. cloacae* (band 18) , *Klebsiella pneumoniae* (band 19, 20) and *Kleb. veriicola* (band 21) were observed within the first day of fermentation. This data obtained revealed that the growth of bacteria, originated in stinky bean, was inhibited after the predominant LAB species increased. According to sequences affiliated with LAB, *Enterococcus faecium* (band 7), *Streptococcus thermophillus*

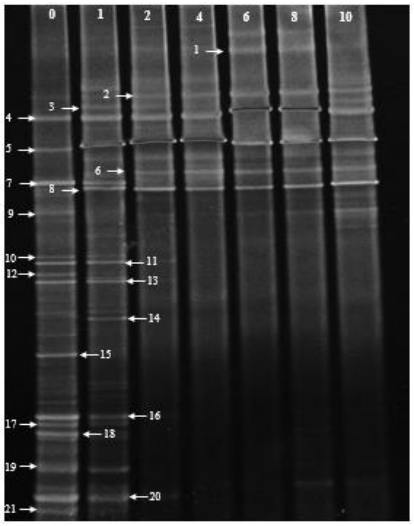

Figure 3. The DGGE profiles of the V3 region of the 16S rRNA gene obtained by PCR amplification of DNA direct extracted from *Sataw-Dong* during fermentation sampling at day 0, 1, 2, 4, 6, 8 and 10. Numbers Identification of each bands are presented in Table 1.

Table 1. Strains identification of the DGGE band sequences targeting the V3 region of the 16S rRNA gene of the total bacterial community DNA direct extracted from *Sataw-Dong*.

Isolate name[a]	Closest relative	% Identity	Accession NO.[b]
1	*Staphylococcus cohnii*	99	KP946712.1
2, 4, 5, 6	*Lactobacillus plantarum*	100	KP764192.1
3	*Lactobacillus fermentum*	100	HE858543.1
7	*Enterococcus faecium*	99	KT438165.1
8	*Lactobacillus pentosus*	100	AB362751.1
9	*Staphylococcus hominis*	100	KM392087.1
10	Uncultured bacteria	96	HG847996.1
11	*Cohnella lupini*	98	NR125663.1
12	*Cohnella collisoli*	98	KR011027.1
13	*Lactobacillus fermentum*	98	HE858541.1
14	*Streptococcus thermophilus*	99	FJ982787.1
15	Uncultured bacteria	95	KF344834.1
16	*Erwinia billingiae*	100	KF740580.1
17	*Enterobacter asburiae*	99	JF772078.1
18	*Enterobacter cloacae*	99	JX514420.1
19, 20	*Klebsiella pneumoniae*	99	CP022997.1
21	*Klebsiella veriicola*	99	KU312790.1

[a]Band are numbered according to Figure 3; [b] Accession number of the sequence of the closet relative found in NCBI database.

(band 14) and *Lactobacillus fermentum* (band 3, 13) were obtained only at time 0 and disappeared after day 1. On the contrary, *L. plantarum* (band 2, 4, 5, 6) and *L. pentosus* **(**band 8**)** were present and dominant throughout fermentation period showing different intensity (Figure 3). Two bands (labelled in Figure 3 numbered 10 and 15) affiliated with sequences corresponding to bacteria classified as "uncultured bacteria" in the BLAST database. The other less intense bands were identified as microorganism that were presented as natural contaminant of the vegetable and were not taken into this account.

The DGGE profile obtained from bulk cells harvested from medium plates are depicted in Figure 4. The individual DGGE obtained bands between PCA and MRS media were named A to R and a to g, respectively. The closest relatives DGGE band sequences are summarized in Table 2 and 3. In some cases retrieved sequences shared the same value of similarity with several closely related micro-organisms and in such cases only one example of each close relative was presented. The major LAB found in *Sataw-Dong* was corresponding to *L. plantarum* (band e, f, C, J), *L. paraplantarum* (band b), *L. fermentum* (band f, D), *L. plantarum* subsp. *plantarum* (c, d) and *L. pentosus* (band a, B) (Figure 4). Those LABs detected in both culture-dependent and culture-independent methods were found to play an important role in many kinds of vegetable fermentations (Kim & Chun 2005). Interestingly, *Staphylococcus haemolyticus* (band I), *S. hominis* (band F, H**)**, *Ent. cloaceae* (band O) were detected at the beginning of fermentation. However, they were subsequently faint owning to the increase of total acidity, which was similar to the result obtained above. Although, *Streptococcus thermophilus* (band M) and *St. salivarius* (band N, S) were also present at early state and they disappeared afterwards.

Naturally-fermented *Sataw-Dong* contains different groups of bacteria at the beginning of fermentation as revealed by DGGE analysis. Some Gram-negative species (*Entero-bacter* sp. and *Klebsiella* sp.) were also detected, especially at the beginning of fermentation (Abriouel *et al.*, 2011). The group of LAB was the main microorganisms active in *Sataw-Dong* fermentation and prevailed throughout the fermentation

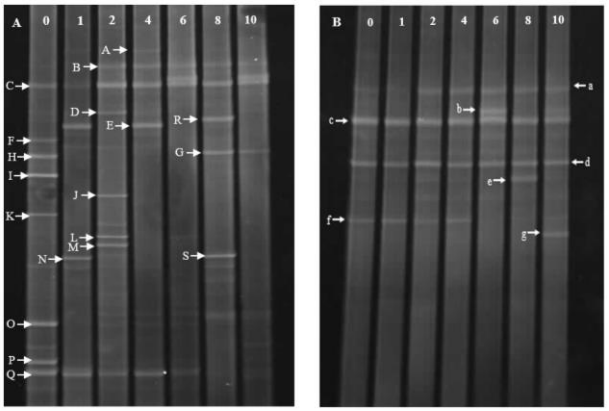

Figure 4. The DGGE profiles of the V3 region of the 16S rRNA gene obtained by PCR amplification of DNA extracted from bulk cells collected from PCA (A) and MRS (B) in *Sataw-Dong* during fermentation sampling at 0, 1, 2, 4, 6, 8, and 10 days. Bands indicated were excised for identification (Table 2 and 3).

Table 2. Strains identification of the DGGE band sequences targeting the V3 region of the 16S rRNA gene of the bacterial bulk cell collected from PCA medium.

Isolate name[a]	Closest relative	% Identity	Accession NO.[b]
A, E	*Staphylococcus cohnii*	99	KP946712.1
B	*Lactobacillus pentosus*	98	KT02593.1
C, J, K	*Lactobacillus plantarum*	100	KT025937.1
D	*Lactobacillus fermentum*	99	JN944734.1
F, H	*Staphylococcus hominis*	99	KM392087.1
G	Uncultured bacteria	95	FN784883.1
I	*Staphylococcus haemolyticus*	99	CP013911.1
L	Uncultured bacteria	99	KF098554.1
M	*Streptococcus thermophilus*	99	FJ982787.1
N, S	*Streptococcus salivarius*	98	LC061614.1
O	*Enterobacter cloacae*	99	KT260881.1
P, Q	*Klebsiella pneumoniae*	99	CP022997.1
R	*Klebsiella veriicola*	99	KU312790.1

[a] Band are numbered according to Figure 4A; [b] Accession number of the sequence of the closet relative found in NCBI database.

Table 3. Strains identification of the DGGE band sequences targeting the V3 region of the 16S rRNA gene of the bacterial bulk cell collected from MRS medium.

Isolate name[a]	Closest relative	% Identity	Accession NO.[b]
a	*Lactobacillus pentosus*	100	KT025934.1
b	*Lactobacillus paraplantarum*	100	KR006320.1
c, d	*Lactobacillus plantarum* subsp. *plantarum*	100	KT025937.1
e	*Lactobacillus plantarum*	99	KT025933.1
f	*Lactobacillus fermentum*	99	KU244508.1
g	*Lactobacillus brevis*	99	GU295950.1

[a] Bands are numbered according to Figure 4B; [b] Accession number of the sequence of the closet relative found in NCBI database.

period. Some representative LAB species in fermented vegetables (e.g., *L. plantarum, L. paraplantarum* and *L. pentosus*) are genotypically closely related which the sequencing of the 16S rDNA cannot be undistinguished between them (De Bellis, Valerio, Sisto, Lonigro, & Lavermicocca, 2010). Since *L. plantarum* was the most abundant specie regularly found in fermented vegetables and pickles (Gardner, Savard, Obermeier, Caldwell, & Champagne, 2001), these bands were considered to probably be *L. plantarum* (Figure 3, 4). This result corresponds to other studies demonstrating that *L. plantarum* was considerably associated with many vegetables fermentations in brine (olive, cucumber, cabbage and mustard green) (Beganović *et al.*, 2014; Wang *et al.*, 2014).

Although, various variable regions of the 16S rRNA gene have been performed for the DGGE analysis, the length and species-specific heterogeneity of the V3 region makes it one of the best choices (Ercolini, Hill, & Dodd, 2003). The detection of several DGGE patterns for a single species does not necessarily mean that different strains are present**.** This suggested the use of rRNA coding gene is the inherent sequence heterogeneity within same species, which is the result of multi-copies of gene (Fogel, Collins, Li, & Brunk,

1999). For instance, regarding to band no. 2, 5 and 6 (Figure 3), which showed the highest sequence similarity to *L. plantarum*, were found at different position of DGGE profile.

A number of faint bands could not be identified because of their lower content which might be related to the heterogeneous distribution of microorganism in the food matrix and ecosystem (Flórez & Mayo, 2006). The detection limit of DGGE analysis has been proposed to be approximately 3 log CFU/g (Cocolin, Manzano, Cantoni, & Comi, 2001). Moreover, the concentration and number of other microbial community and food matrix can influence the detection limit. These factors affect both the efficiency of DNA extraction and PCR amplification owing to the possibility of templates competition (Ercolini, 2004).

In Thailand, there are many kinds of fermented plant products; yet knowledge of their microbiology derives only from old results obtained by classical culturing techniques and traditional identification methods. In recent years, the priority of microbiological taxonomy has shifted from phenotypic characterization to phylogenetic analysis based on small sub-unit ribosomal RNA (16S rRNA) (Endo, Mizuno, & Okada, 2008).

As a result of this present study, a slight difference between both methods revealed that lower bacterial diversity occurred from culture-dependent method in comparison with culture-independent method. It suggested that the low diversity resulted from the uncultivated strains in sample ecosystems. In addition, our findings confirmed the use of combination approach are necessary for accurate and reliable to explore bacterial communities developing during the fermentation processes and revealed that *L. plantarum* was observed as the predominant bacterial specie associated in fermentation process.

Although DGGE approach provides a broad overview of bacterial community, interpretation of DGGE data should be accompanied with caution. This technique cannot differentiate dead and living cells, which would preclude the application of DGGE in microbial succession studies (Thanh, Mai, & Tuan, 2008). In this study, *Sataw-Dong* bacterial diversity and population dynamics occurring during fermentation are essential knowledge for better understanding in order to further develop of potential starter cultures for improving its organoleptic quality, its safety aspects as well as preventing fermentation failure on large-scale industrial production. In this way, the traditional processes should be replaced by the autochthonous LAB starter cultures with desirable properties to guarantee the safety and final quality of *Sataw-Dong* product.

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