

# Culture-independent bacterial community analysis of the salty-fermented fish paste products of Thailand and Laos

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Received September 25, 2014; Accepted December 15, 2014; Published online in J-STAGE January 21, 2015

A bacterial community analysis, using a culture-independent method (polymerase chain reaction-denaturing gradient gel electrophoresis), detected 17 species of bacteria including species of the genera *Tetragenococcus*, *Lactobacillus*, *Pediococcus*, *Weissella Halanaerobium*, *Clostridium*, and *Sphingomonas* in a traditional salty-fermented fish paste known as *pla-ra* or *pa-daek* in Thailand and Laos, which is used as a storage-stable multi-purpose seasoning. The representative genus of lactic acid bacteria seemed to vary in the 10 products collected from Thailand and Laos. *Tetragenococci* were common in products from central Thailand and Vientiane in Laos which had salinities of not less than 11% and pH values ranging from 5.6 to 6.1. However, lactobacilli were common in products from northern Thailand which had the lowest salinities (8.3–8.6%) and pH values (4.5–4.8) of all the samples examined. Two *Lactobacillus* and one *Tetragenococcus* species were detected in one product from northeastern Thailand containing 10% salt. These results suggest that salinity in *pla-ra/pa-daek* is an important determinant of the representative genus of lactic acid bacteria such as, *Tetragenococcus* or *Lactobacillus*. Additionally, differences in the acidity between these two groups seemed to be related to the production of D-/L-lactic acid in the lactic acid bacteria in each product. This is the first study to report a correlation between bacterial community structure and taste components in *pla-ra/pa-daek* products from various regions. This scientific work on a traditional fermented food will be useful in helping local producers meet differing consumer preferences in various regions.

**Key words:** fermented fish, *pla-ra*, *pa-daek*, lactic acid bacteria, bacterial community, PCR-DGGE

## INTRODUCTION

The salty-fermented fish paste known as *pla-ra* or *pa-daek* in the Thai and Lao languages is popularly used as an all-purpose seasoning in Thai and Laotian cooking. It is produced using freshwater fish in central, northern, and northeastern Thailand, and Laos. For the preparation of *pla-ra/pa-daek*, the raw fish is de-scaled, eviscerated, and cleaned well with water, then mixed with salt and pickled with rice bran or roasted rice powder. The fermentation generally takes place at ambient temperatures in the

tropical climate for at least 6 months [1]. As the use of a starter culture is not common for *pla-ra/pa-daek* production, fermentation occurs spontaneously with microbes in the raw materials and the production environment. The fermented products are usually stored without refrigeration while being used and can last for a year or longer. It is empirically believed that a longer fermentation makes the product taste better. This shows the benefits of a traditional fermentation technique that furnishes a seasonally available indigenous food resource with additional values such as palatability and a long shelf life. Currently, eating uncooked freshwater fish products including *pla-ra/pa-daek* is not advisable because of the health risks from parasites [2, 3], therefore it is generally cooked before being eaten.

*Pla-ra/pa-daek* is often made and consumed at home, particularly in rural areas, or by relatively small-scale manufacturers for local consumption [1]. Consumers often think that the taste and flavor of *pla-ra/pa-daek*

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products vary from place to place. Factors producing such differences between products are of scientific interest to those who produce fermented products to meet consumer preferences as well as to those who wish to preserve the diversity of a regional food culture. Seventeen species of lactic acid bacteria, including species of the genera *Lactobacillus* (7 species), *Weissella* (3 species), *Pediococcus* (2 species), *Enterococcus* (2 species), *Tetragenococcus* (1 species), *Aerococcus* (1 species), and *Leuconostoc* (1 species) have been isolated by culture-based methods from Thai *pla-ra* products [4–7]. This suggests that these bacteria have an important role in determining the palatability and keeping quality. However, less attention has been paid to possible variations in bacterial community structures between varieties of *pla-ra/pa-daek* products from various regions, and how this is related to product qualities.

During the last decade, polymerase chain reaction-denaturant gradient gel electrophoresis (PCR-DGGE) has been extensively used as a culture-independent approach for studying microbial communities in a wide variety of fermented foods around the world [8]. Most recently, the time-dependent development of a bacterial community consisting of six species of lactic acid bacteria, including species of the genera *Lactococcus* (2 species), *Weissella* (2 species), *Pediococcus* (1 species) and *Enterococcus* (1 species), and other bacteria including species of the genera *Staphylococcus* (3 species), *Macroccoccus* (1 species), *Clostridium* (1 species), *Flavobacterium* (1 species) and *Plesiomonas* (1 species) were shown during the production of *pa-som*, a short-term (up to 4 days) fermented, freshwater fish product from Laos [9]. By using this technique, microbial communities in food samples of interest can be visualized and compared with each other in a single gel image and assessed at the species level regardless of the ability of each bacterium to be cultured in synthetic growth media.

In the present study, the bacterial communities in *pla-ra/pa-daek* products collected from central, northern, northeastern Thailand, and Vientiane in Laos were investigated using PCR-DGGE. Additionally, the relationship between the lactic acid bacteria community profiles and the product properties, salinity and pH, and content of D-/L-lactic acid was also examined and discussed.

## MATERIALS AND METHODS

### Sample collection

*Pla-ra* products made in the Suphanburi, Singburi, and Sukhothai provinces of central, northern, and

northeastern Thailand, were obtained from markets in Bangkok, Nakhon Ratchasima, and Khon Kaen in Thailand, respectively. *Pa-daek* products made in the Vientiane province of Laos were obtained from local markets in the Vientiane capital area. The information about production region, fish materials, and fermentation period for each product was provided by the sellers (Table 1). The scientific name of each fish was assigned, if available, by referring to FishBase (<http://www.fishbase.org/search.php>). The samples were collected from May 29th to June 4th in 2012. After purchase at the markets, the samples were cold-stored in a refrigerated container and refrigerators to minimize microbial activity. All the samples were shipped by air in the same refrigerated container to the laboratory in Tsukuba, Japan on June 7th, 2012, following the storage in a freezer at  $-20^{\circ}\text{C}$ . The samples were stored in the freezer until analysis.

### Bacterial community analysis

The bacterial 16S rRNA gene for DGGE analysis was amplified by nested PCR as described by Kim et al [10]. DNA for the PCR template was extracted from 1 g of each *pla-ra/pa-daek* sample using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The nearly complete 16S rRNA gene was first amplified using 5  $\mu\text{L}$  of DNA extract by PCR using Takara Ex Taq (Takara Bio Inc., Shiga, Japan) with the pair of primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGCTACCTTGTTACGACTT-3') in a 50- $\mu\text{L}$  reaction volume. The resulting product was diluted 100-fold, and 2  $\mu\text{L}$  of the diluent was subjected to a second PCR using the pair of primers 338fGC (5'-CGCCCGCCGCGCGCGGC G G G C G G G C G G G G G C A C G G G G G GACTCCTACGGGAGGCAGCAG-3') and 518r (5'-ATTACCGCGGCTGCTGG-3') to amplify the V3 region of the 16S rRNA gene. The PCR products (10  $\mu\text{L}$ ) were electrophoresed using a DCode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA) at a constant voltage of 50 V in 1 $\times$ Tris-acetate-EDTA buffer (40 mM Tris-acetate, 1 mM EDTA) for 18 h at 58 $^{\circ}\text{C}$  using a polyacrylamide gel consisting of 7% (w/v) acrylamide-bisacrylamide (37.5:1) with a denaturing gradient in the range of 40–70% denaturant (100% denaturation corresponds to 7 M urea and 40% (v/v) formamide). After electrophoresis, the gels were stained with GelRed (Biotium, Hayward, CA, USA) and the DNA fragments were visualized by 254-nm ultraviolet light and photographed. The brightness and contrast of the DGGE images were optimized by Photoscape v3.6.4 software (<http://www.photoscape.org/>). The reproducibility of the band patterns of PCR-

Table 1. Production region, fish material, and fermentation period of products used in this study

Sample number	Province	Region	Local name of fish materials used with academic names in parenthesis*	fermentation period
1	Suphanburi	Central Thailand	Pla chon ( <i>Channa striata</i> )	1 year
2	Singburi	Central Thailand	Pla ka di ( <i>Trichopodus trichopterus</i> ) and other small-sized fish	1 year
3	Sukhothai	Northern Thailand	Mixture of small-sized fish	1 year
4	Sukhothai	Northern Thailand	Mixture of small-sized fish	1 year
5	Sukhothai	Northern Thailand	Pla ka di ( <i>Trichopodus trichopterus</i> )	1 year
6	Khon Kaen	Northeastern Thailand	Mixture of small-sized fish	1 year
7	Vientiane	Laos	Mixture of small-sized fish	10 months
8	Vientiane	Laos	Pa keng ( <i>Cirrhinus molitorella</i> )	1 year
9	Vientiane	Laos	Mixture of small-sized fish	1 year
10	Vientiane	Laos	Mixture of small-sized fish	1 year

\* "small-sized" approximately 10 cm or smaller.

DGGE analysis observed in the present study was confirmed by a repeat experiment starting from PCR (data not shown). The DNA bands selected for nucleotide sequence analysis were excised from the DGGE gel under a 500-nm light-emitting diode. The DNA in each excised gel was eluted in sterilized water and subjected to PCR using the pair of primers, 338fGC and 518r. The purity of the PCR products was confirmed by DGGE as described above. The PCR products were purified using a FastGene Gel/PCR Extraction Kit (Nippon Genetics Co., Ltd., Tokyo, Japan). The nucleotide sequences of the purified PCR products were determined by the laboratory of Eurofins Genomics Co., Ltd. (Tokyo, Japan) using the primer, 338f (5'-ACTCCTACGGGAGGCAGCAG-3'). The nucleotide sequence homology search of each purified PCR product was performed using the blastn search tool against the 16S ribosomal RNA sequences database in the NCBI (National Center for Biotechnology Information (<http://www.ncbi.nlm.gov/>)).

#### Measurements of salt concentration, pH, and D-/L-lactic acid contents

One gram of each sample was mixed vigorously in 10 mL of sterilized water, followed by centrifugation at  $15,000 \times g$  for 10 min at 4°C. The supernatant was collected and used for the analysis. The salt concentration and pH were measured three times with LAQUA twin compact salt and pH meters, respectively (Horiba Ltd., Kyoto, Japan). The lactic acid content was measured three times using a D-/L-lactic acid enzymatic test kit (R-Biopharm AG, Darmstadt, Germany).

## RESULTS

#### Bacterial community analysis by PCR-DGGE

Because the mobility of each DNA band in the

DGGE gel is determined by the sequence-dependent partial denaturation of the double strand at the specific denaturant concentration in the gel, the DNA bands exhibiting identical mobility between samples in the gel can be deduced to have been derived from genetically identical or closely related bacterial species. Moreover, in the present study, some DNA bands in the same mobility group were additionally isolated and subjected to nucleotide sequence analysis to confirm their identities (Table 3). As shown in Fig. 1, and Tables 2 and 3, each product appeared to contain two to eight types of lactic acid bacteria. In particular, two types of *Tetragenococcus muriaticus* (bands "a" and "b") were detected in a product made in central Thailand (lane 1). In addition, band "a" was detected in products from Laos (lanes 7, 8, and 10). Band "b" was also detected in the other product from central Thailand (lane 2) as well as those from Laos (lanes 7 to 10). In the two bands of *Tetragenococcus halophilus* detected (bands "g" and "q"), band "g" was common in the same product areas (central Thailand and Laos) as the *T. muriaticus* species (band "b"), except for the sample 1. Band "g" was also detected in the product made in northeastern Thailand (lane 6). Band "q" appeared relatively weak and was specific to products from Laos (lanes 8 and 9). Bands "m" and "n" corresponded to *Weissella paramesenteroides* and *Pediococcus argentinicus*, respectively. It was obvious that *Lactobacillus* species such as *Lactobacillus futsaii* (band "o"), *Lactobacillus acidipiscis* (bands "h" and "i"), and *Lactobacillus rennini* (bands "j", "k", "l" and "p") were common to the products made in northern Thailand (lanes 3, 4, and 5). Variants of the *L. acidipiscis* and *L. rennini*, represented by bands "h" and "k", respectively, were also detected in the product made in northeastern Thailand (lane 6). Additionally, a product from Laos (lane 7) contained a *L. rennini* species (band "k").

Table 2. Homology search results for the DNA fragments from selected DGGE bands

Band	Highest match(es)	Accession number	Identity (%)
a	<i>Tetragenococcus muriaticus</i> strain NBRC 100499	NR113915.1	99
	<i>Tetragenococcus muriaticus</i> strain LMG 18498	NR116418.1	99
	<i>Tetragenococcus muriaticus</i> strain X-1	NR025887.1	99
b	<i>Tetragenococcus muriaticus</i> strain NBRC 100499	NR113915.1	100
	<i>Tetragenococcus muriaticus</i> strain LMG 18498	NR116418.1	100
	<i>Tetragenococcus muriaticus</i> strain X-1	NR025887.1	100
c	<i>Halanaerobium fermentans</i> strain R-9	NR024715.1	100
d	<i>Clostridium haemolyticum</i> strain JCM 1402	NR113381.1	100
	<i>Clostridium haemolyticum</i> strain DSM 5565	NR117767.1	100
	<i>Clostridium haemolyticum</i> strain ATCC 9650	NR024749.1	100
	<i>Clostridium haemolyticum</i> strain DSM 5565	NR119281.1	100
e*	<i>Sphingomonas roseiflava</i> strain MK341	NR117716.1	100
f	<i>Clostridium drakei</i> strain FP	NR114863.1	100
	<i>Clostridium drakei</i> strain SL1	NR044942.1	100
	<i>Clostridium carboxidivorans</i> strain P7	NR104768.1	100
	<i>Clostridium vincentii</i> strain DSM 10228	NR026336.1	100
	<i>Clostridium scatologenes</i> strain ATCC 25775	NR118727.1	100
	<i>Tetragenococcus halophilus</i> subsp. <i>flandriensis</i> strain T5	NR108208.1	100
h	<i>Lactobacillus acidipiscis</i> strain NBRC 102163	NR112693.1	99
	<i>Lactobacillus acidipiscis</i> strain FS60-1	NR024718.1	99
i	<i>Lactobacillus acidipiscis</i> strain NBRC 102163	NR112693.1	100
j	<i>Lactobacillus rennini</i> strain CECT 5922	NR042195.1	99
k	<i>Lactobacillus rennini</i> strain CECT 5922	NR042195.1	100
l	<i>Lactobacillus rennini</i> strain CECT 5922	NR042195.1	99
m	<i>Weissella paramesenteroides</i>	NR044759.1	100
	<i>Weissella paramesenteroides</i>	NR040815.1	100
	<i>Weissella hellenica</i> strain NBRC 15553	NR113775.1	100
	<i>Weissella hellenica</i> strain NCFB 2973	NR119224.1	100
n	<i>Pediococcus pentosaceus</i> ATCC 25745 strain	NR075052.1	100
	<i>Pediococcus pentosaceus</i> strain DSM 20336	NR042058.1	100
	<i>Pediococcus argentiniensis</i> strain CRL 776	NR042623.1	100
o	<i>Lactobacillus futsaii</i> strain YM 0097	NR117973.1	100
	<i>Lactobacillus farciminis</i> strain BCRC 14043	NR114398.1	100
	<i>Lactobacillus heilongjiangensis</i> strain S4-3	NR109370.1	100
	<i>Lactobacillus rennini</i> strain CECT 5922	NR042195.1	99
q	<i>Tetragenococcus halophilus</i> strain JCM 5888	NR115655.1	100

\*Another 33 *Sphingomonas*, 15 *Sphingobium*, 9 *Sphingopyxis*, 2 *Erythrobacter*, 1 *Altererythrobacter*, 1 *Novosphingobium* and 1 *Stakelama* species were matched with 100% identity (data not shown).

Besides the lactic acid bacteria mentioned above, two *Clostridium* species (bands “d” and “f”) were found only in a product from central Thailand (lane 1). A band corresponding to *Sphingobium* or a related species (band “e”) was detected in a product from central Thailand (lane 1) as well as from Laos (lanes 7 to 10). The nucleotide sequence of this DNA band matched to the 16S rRNA genes of more than 60 types of bacteria deposited in the database with 100% identity. Band “c”, corresponding to *Halanaerobium fermentans*, was common to products from central Thailand and Laos (lanes 1, 2, 8, and 10).

#### Salt concentration, pH, and D-/L-lactic acid content

The salinity, pH, and D-/L-lactic acid content of the *plara/pa-daek* samples are presented in Table 3. Among the samples examined, the products from northern Thailand (samples 3, 4 and 5) contained the lowest levels of salt (8.3–8.6%), while the products from the other regions contained more than 10% salt. The salinity in products from Laos (samples 7 to 10) was relatively higher (15–20%) than those from the other places. The pH value of the products from northern (samples 3, 4, and 5) and northeastern (sample 6) Thailand was less than 5.0, the former exhibiting the lowest pH levels (4.5–4.8). The products from the other regions exhibited pH values of



Table 3. Salinity, pH, and D-/L-lactic acid content

Sample number	Salinity (%)	pH	D-lactic acid	L-lactic acid	Total lactic acid	DGGE bands detected
1	12.3	5.7	0.02	0.86	0.88	a*, b*, c*, d*, e*, f*
2	11.3	5.6	0.18	0.29	0.47	b*, c*, g*
3	8.3	4.8	1.22	1.22	2.44	h, i, j, k*, l
4	8.4	4.6	1.58	1.49	3.08	h*, i, j, k, l, m*, n*, o*
5	8.6	4.5	1.13	1.46	2.58	h, i*, j*, k*, l*, m, o, p*
6	10.3	4.9	0.47	1.22	1.69	h*, g*, k
7	15.3	5.7	0.04	0.58	0.62	a*, b*, e, g*, k*
8	20.3	5.7	0.16	0.37	0.53	a*, b, c*, e, g*, q*
9	16.7	5.9	0.20	0.55	0.75	b, e*, g*, q
10	18.3	6.1	0.18	0.22	0.40	a, b, c, e, g

Values shown are the means of three measurements. pH was measured in one tenth diluted samples. D-/L-lactic acid contents are shown as g/100 g sample. The bands that were isolated and subjected to nucleotide sequence analysis are indicated by asterisks (\*).

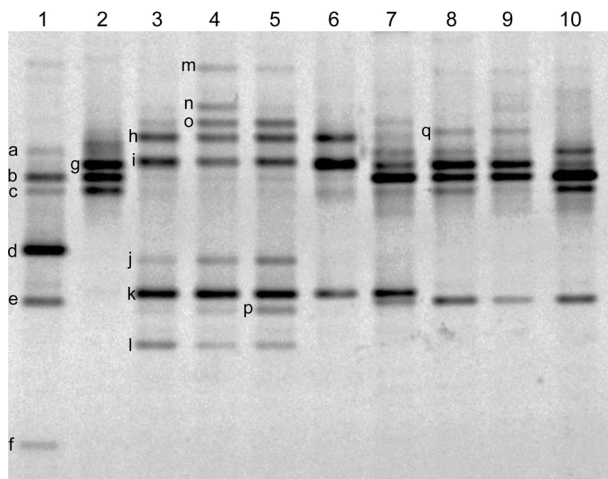


Fig. 1. PCR-DGGE profiles of the bacterial communities in *pla-ra* and *pa-daek*. The PCR products from samples 1 to 10 presented in Table 1 are shown in lanes 1 to 10, respectively. The representative DGGE band in each mobility group is indicated by lower-case letters. The nucleotide sequence of the bands indicated in Table 3 was subjected to a homology search against a 16S rRNA sequence database.

#### 5.6–6.1.

In the present study, the D-/L-lactic acid content in the samples was measured, because the D-/L ratio in each sample should be related to the characteristics of the lactic acid bacteria community. The highest levels of total lactic acid content (approximately 2.4–3.0 g/100 g of sample) were observed in the products from northern Thailand (samples 3, 4, and 5) and the contents of D- and L-lactic acids were nearly identical in each product. The product from northeastern Thailand (sample 6) contained L-lactic acid at a similar level to the northern Thailand products, but the D-lactic acid content was less than half. In samples from central Thailand (samples 1 and 2) and

Laos (samples 7 to 10), the total lactic acid contents varied from 0.40 to 0.88 g/100 g of sample. The D-lactic acid content in these products were noticeably lower than those in products from northern Thailand.

## DISCUSSION

In the present study, we first used a culture-independent PCR-DGGE technique to assess the bacteria community structure of *pla-ra/pa-daek*, which is one of the most frequently used fermented seasonings, particularly in northeastern Thailand and Laos. Through examining the differences in their 16S rRNA gene sequences, 13 species of lactic acid bacteria, including species of the genera *Tetragenococcus*, *Pediococcus*, *Weissella*, and *Lactobacillus* were detected in the 10 *pla-ra/pa-daek* products collected, showing the variety and importance of lactic acid fermentation for the production of *pla-ra/pa-daek*. Other than lactic acid bacteria, 4 species of bacteria including the species of genera *Halanaerobium*, *Clostridium*, and *Sphingomonas* were detected. The *H. fermentas*, and *Sphingomonas* or related species were commonly detected in the products exhibiting relatively high salinity. On the other hand, clostridia were detected in only one sample in the present study, suggesting this species might not be common in *pla-ra/pa-daek* products, although further comprehensive and quantitative analysis will be necessary to fully confirm this. Compared with previous culture-based isolations from Thai *pla-ra*, in this study, *T. muritaticus* and *L. rennini* were newly found by PCR-DGGE analysis in multiple samples, showing the benefit of this culture-independent method in the exploration of the diversity of lactic acid bacteria profiles and the comparison of *pla-ra/pa-daek* products collected from various regions. Additionally, two to four sequence variations were found in the DGGE bands

identified as *T. muriaticus* (Fig. 1, bands “a” and “b”), *T. halophilus* (bands “g” and “q”), *L. acidipiscis* (bands “h” and “l”), and *L. rennini* (bands “j”, “k”, “l”, and “p”). Such variations might suggest that the lactic acid bacteria profiles in *pla-ra/pa-daek* could be characterized at the strain level and conserved between products in some cases, although further assessment at the genomic level is necessary to fully confirm the species diversity.

It is noteworthy that the *pla-ra/pa-daek* samples examined in the present study could be classified into two groups by the representative genus of lactic acid bacteria, such as *Lactobacillus*, or *Tetragenococcus*, regardless of the fish material used. A salt concentration of approximately 10% might be the dividing point between these two groups, because both *Lactobacillus* and *Tetragenococcus* species were detected in the product from northeastern Thailand containing 10% salt. Additionally, the lactic acid bacteria profile appeared to determine the D-/L-lactic acid ratio in each product: lactobacilli are known to produce D- and/or L-lactic acid, while tetragenococci produce only the L-form [11]. The small amount of D-lactic acid detected in the samples where tetragenococci were mainly detected by PCR-DGGE might have been produced by bacteria other than lactic acid bacteria. Alternatively, the population of such D-lactic acid-producing bacteria in these products might be below the detection limit of the PCR-DGGE used in this study, or detectable only at the beginning and/or the middle phase of fermentation.

Tetragenococci appeared to be the representative lactic acid bacteria in products containing more than 11% salt. In such cases, *T. muriaticus* and *halophilus* coexisted except in the product from central Thailand (Fig. 1, lane 1). Both species are known to be associated with habitats rich in salt and protein, and play an important role in the halophilic fermentation process [11]. It has been reported that the growth rate and lactic acid production of *T. muriaticus* in high salinity (23% NaCl) conditions were higher than those in *T. halophilus*, but they were severely affected by low pH (5.8) conditions compared with *T. halophilus* [12]. Thus, the coexistence of these two halophilic species might be a reasonable assumption for starting and continuing the lactic acid fermentation during the *pla-ra/pa-daek* production in adapting to the high salinity as well as to changing pH conditions over time. A strictly anaerobic and moderately halophilic bacterium, *H. fermentas*, previously reported to coexist with *T. muriaticus* and *T. halophilus* in salt-preserved food, such as fermented puffer fish ovaries [13–15], was also detected in the present study. The possible contribution of this bacterium to the production of salty-

fermented foods is therefore of interest. *Sphingomonas* or related species was also commonly detected in samples exhibiting relatively high salinity. Because the nucleotide sequence of the corresponding band (e) matched the 16S rRNA genes of more than 60 types of bacteria, isolation of this bacterium and its detailed characterization will be required to understand the role of this bacterium in the fermentation.

Although the salinity of the products from northern Thailand was lower than those from the other regions, the acidic conditions (pH 4.5–4.8) presumably created by the markedly higher levels of D- and L-lactic acid may be advantageous for preventing the growth of food-spoilage bacteria. The growth of lactobacilli in these samples might occur selectively depending on the fermentation methods, particularly under the favorable conditions of relatively low salinity. Indeed, according to previous reports, the type strains of lactobacilli, such as *L. acidipiscis* FS60-1<sup>T</sup> [16], *L. rennini* CECT 5922<sup>T</sup> [17], and *L. futsaii* YM0097<sup>T</sup> [18], are viable in the ranges of salinity (8.3–8.6%) and pH (4.5–4.8) observed in the products from northern Thailand.

For the manufacture of cheddar cheese, it has been proposed that the glutamate dehydrogenase activity of lactobacilli should be considered for use as adjuncts in the ripening process, because the  $\alpha$ -keto-glutarate formed by glutamate dehydrogenase activity would enhance the amino acid catabolism of the lactobacilli, thereby generating amino acid-related aroma compounds which would improve flavor development during maturation [19]. The occurrence and degree of glutamate dehydrogenase activity has been shown to be dependent on the strain of lactobacilli [20]. Its viability during cheese ripening is also important for the intensification and acceleration of aroma and flavor formation [20]. Thus, it should be interesting to assess the possible involvement of glutamate dehydrogenase activity of varieties of lactic acid bacteria, such as lactobacilli and tetragenococci in the formation of aroma compounds in *pla-ra/pa-daek* products, as it might be related to the regional characteristics of flavor and taste.

Since ancient times, fermentation methods for *pla-ra/pa-daek* have been developed to preserve seasonally available freshwater fish resources in the production areas without any microbial knowledge. Fermentation can give value to small-sized or juvenile fish that are often worth little in a local fresh fish market. A precise elucidation of such traditional techniques using recent molecular microbiology and analytical techniques should be of use in helping local producers to improve and/or maintain their product quality. For example, product

improvement could be achieved by monitoring the initial salinity, or possibly using the representative lactic acid bacteria as a fermentation starter so that the characteristic lactic acid bacteria communities develop steadily, leading to the creation of tastes and flavors that can meet the differing consumer preferences of various regions. Further microbial profiling of various *pla-ra/pa-daek* products in Thailand and Laos, using a combination of culture-independent analysis and a statistical approach, could reveal the regional characteristics and their link to product quality as in the cases of the production of traditional water-buffalo mozzarella cheese [21], and the fermented dairy products of Mongolia [22].

The results of the present study are encouraging for the use of PCR-DGGE analysis as a monitoring technique for the time-dependent development of bacteria community structure during *pla-ra/pa-daek* fermentation with the different initial salinities used in various regions. This should offer local producers and consumers important scientific evidence for understanding the traditionally based production method and fermentation period. It will also be useful for improving the quality of the traditional fermented products and increasing manufacturing productivity. A laboratory fermentation trial based on local traditional recipes is now in progress.

#### ACKNOWLEDGEMENTS

We would like to thank Dr. Gassinee Trakoontivakorn and Ms. Plernchai Tangkanakul of IFRPD (Kasetsart University, Bangkok, Thailand), Mr. Bounsong Vongvichith and Ms. Vonhnida Bounlaphan of the Living Aquatic Resources Research Center (LARReC) (Vientiane, Lao PDR) and Dr. Shinsuke Morioka of JIRCAS (Tsukuba, Japan) for their support in collecting the *pla-ra* and *pa-daek* samples.

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