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Determination of Fish Origin by Using 16S rDNA Fingerprinting of Microbial Communities by PCR-DGGE: An Application on Fish from Different Tropical Origins

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1. Introduction

Food safety is now a compulsory issue for food imported to European Union. Bovine Spongiform Encephalitis, pathogens and avian influenza remain in the memories of European consumers. With similar scares occurring globally, the need for vigilance and strict monitoring is necessary. EU regulation 178/2002 imposed traceability to all food imported to EU. For a long time, food industry had simple traceability systems, but with the increasing implementation of current Good Manufacturing Practice, traceability systems have become more important in the production chain.

There are only a few analytical techniques that permit to trace food. In view of the difficulties of installing these documentary systems in developing country, and to follow the product during processing, we propose to identify and validate some pertinent biological markers which come from the environment of the fish to assure traceability of aquaculture product during international trade.

We proposed to trace the origin of fish by analysing in a global way the bacterial communities on the fish samples. The predominant bacterial flora would permit the determination of the capture area, production process or sanitary or hygienic conditions during post harvest operations (Montet et al., 2004; Le Nguyen et al., 2007, Montet, 2008).

Aquatic micro-organisms are known to be closely associated with the physiological status of fish. Numerous studies of the microbiota in fish captured from various geographical locations have been done (Grisez et al., 1997; Spanggaard et al., 2000; Al-Harbi and Uddin, 2003; Leasing, 2005). The bacterial communities of fish could be influence by water composition, temperature, weather conditions and farmer practices.

Separation of PCR products in DGGE is based on the decrease of the electrophoretic mobility of partially melted doubled-stranded DNA molecules in polyacrylamide gels containing a linear gradient of DNA denaturants like formamide and urea at 60°C. Molecules with different sequences will have a different melting behaviour and will stop migrating at different position in the gel (Muyzer et al., 1993; Leising, 2005). PCR-DGGE has been already used to investigate several patterns of distribution of fish bacterial assemblages (Murray et al., 1996; Øvreas et al., 1997; Moeseneder et al., 1999; Riemann et al., 1999; Maiworé et al., 2009a, 2009b; Tatsadjieu et al., 2010) and was used by our team to study the bacteria on fresh water fish for their traceability (Le Nguyen et al., 2007 ; Montet et al., 2008).

A specific advantage of this technique is that it permits the analysis of both cultivable and non cultivable, anaerobic and aerobic bacteria and provides a rapid method to observe the changes in community structure in response to different environmental factors (Yang et al., 2001).

The purpose of our study is to apply the PCR-DGGE method for analyzing the bacteria in fish in order to create a technique to link bacterial communities to the geographical origin and avoid the individual analysis of each bacterial strain. The acquired band patterns for the bacterial species of different fish from Viet Nam were compared and analysed statistically to determine the fish origin. We give also an example of the following of the ecology of bacteria in a tropical and traditional fish fermentation from Ivory Coast, the Adjuevan.

2. Materials and methods

2.1 Fish sampling

Pangasius fish (*Pangasius hypophthalmus*) were collected in a unique pond in five aquaculture farms of five different districts from the South Vietnam namely Chau Phu, An Phu, Phu Tan, Chau Doc, Tan Chau of An Giang Province (Fig. 1). The samples were collected in two seasons in Vietnam: the rainy season (October 2005) and the dry season (February 2006). In each farm of each district, the samples were taken from the same pond and aseptically transferred to storage bags. The samples were maintained on crushed ice and transported to the laboratory. Then the skin, gills and intestines were aseptically removed from each fish specimen and put in separate sealed plastic bags, then kept frozen at -20°C prior to analysis.

Tilapia fish (*Oreochromis niloticus*) were collected in freshwater aquariums at 27°C at Cemagref-Cirad at Montpellier (France) in April and June 2010. Sample weights were 500 ± 10 g. After sampling with sterile gloves, fish were placed individually in sterile plastic bags and then transported in cooled containers refrigerated by crush ice to the laboratory (500m). Fermentation immediately followed the fish arrival.

2.2 Production of fermented fish “Adjuevan”

Adjuevan is a salted and fermented fish traditionally produced in the west coast of Ivory Coast at ambient temperature (28-30°C) following two traditional methods. First method of production took place in jars covered with plastics and stones for 5 days and second method

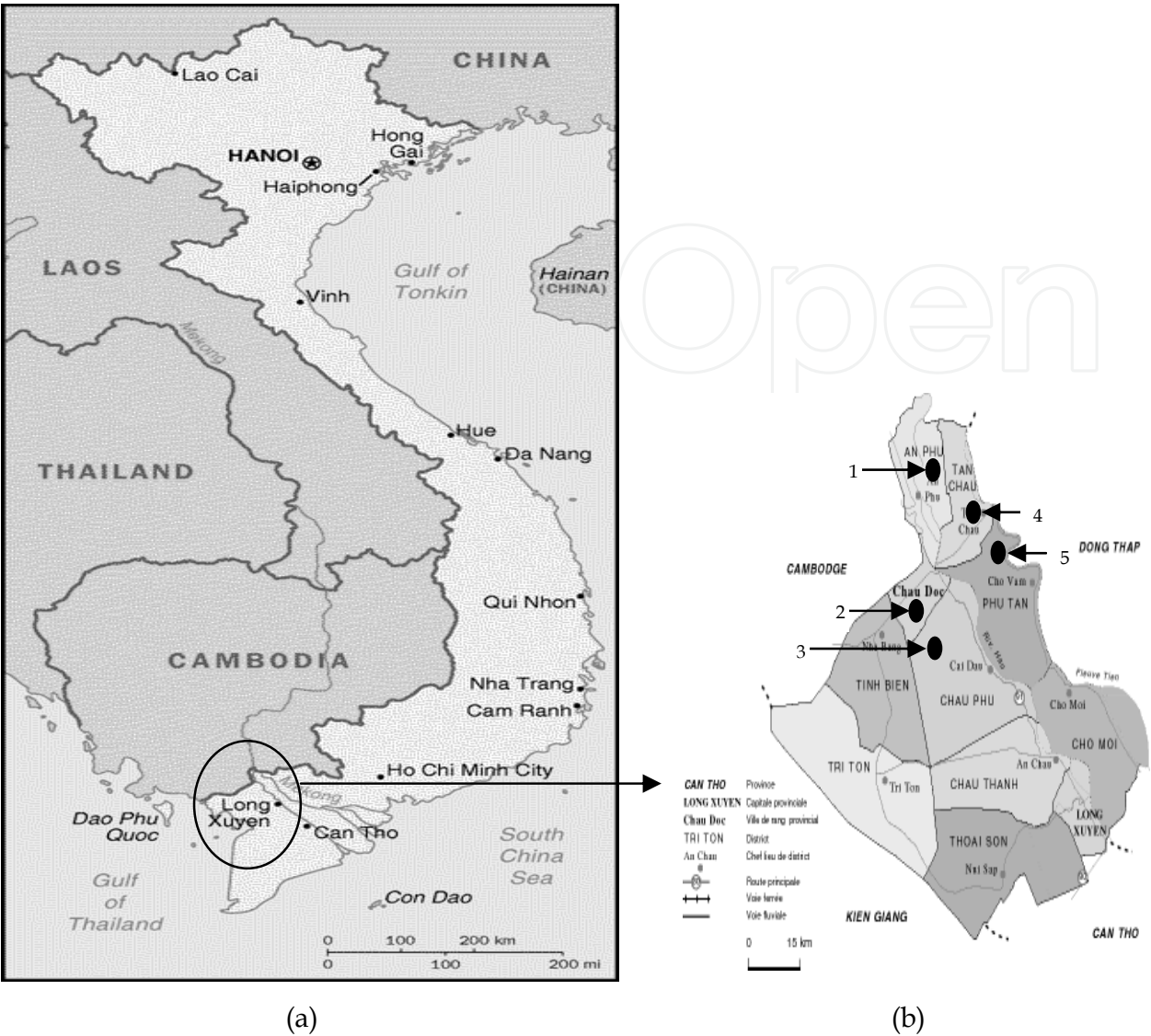


Fig. 1. (a). The map of Viet nam (b) The expansion of An giang province with five different sampling locations: 1) An Phu; 2) Chau Doc; 3) Chau Phu; 4) Tan Chau; 5) Phu Tan.

followed the same fermentation process and then fish were dried on racks or nets for at least 10 days. We studied the changes in physicochemical characteristics and the dynamic of bacterial flora by PCR-DGGE on artificial Adjuevan made in our laboratory at 30°C, with different percentages of salts of 10%, 15% 20%, 25%, and 30% following the both methods.

Fermentation methods are described in Figure 2. About 1.5 kg of fish was gutted, washed and salt was added at the following concentrations 10%, 15%, 20%, 25%, 30% (w/w) after 24 h of maturation (stored at room temperature). For method 1, fish was salted, then wrapped in sterile plastic containers and arranged in different coolers. They are left to ferment for 5 days at ambient temperature (30°C) followed by drying in a ventilated dryer (at 30°C with minimal ventilation) for 24 h. For method 2, fish after been salted, was deposited on a sterile plastic surface then placed in sterile trays. Fermentation was done simultaneously as well as drying for 5 days at 30°C followed by ventilation for 24 h (Fig. 2). Fermentation experiments were done in triplicate.

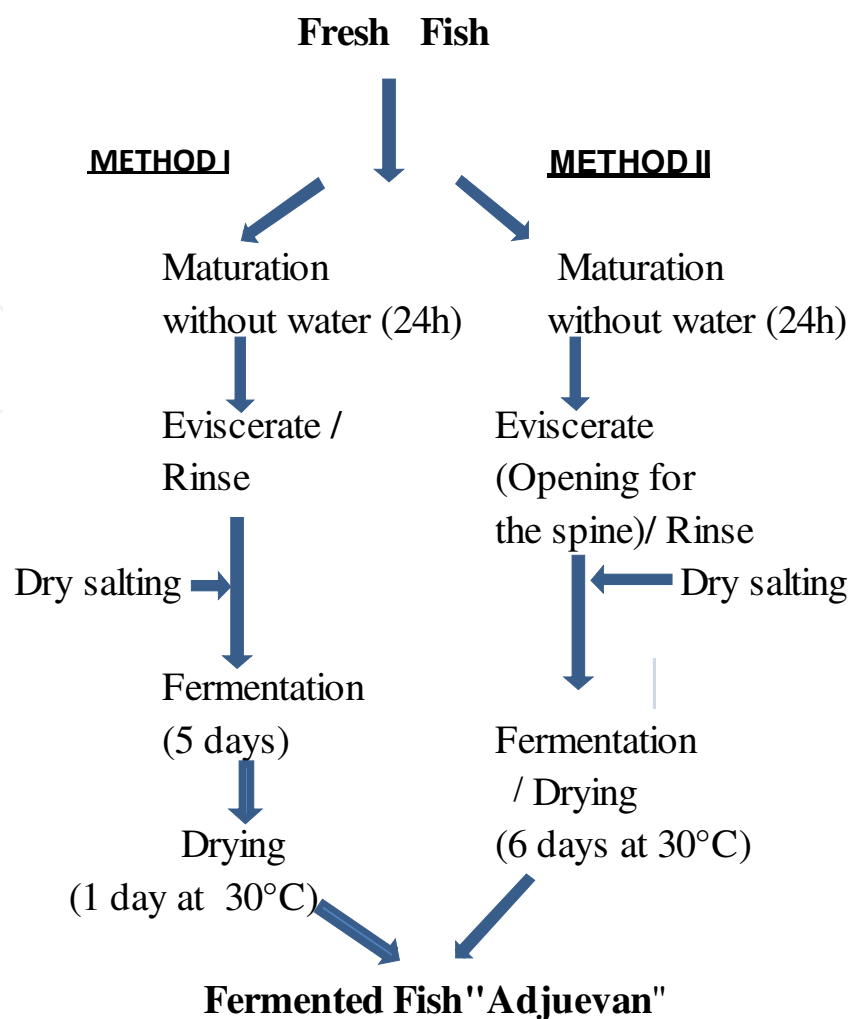


Fig. 2. Fermentation methods for Adjuvants at laboratory level

Fish samples were collected during fermentation primarily on fresh fish, then after 24 h followed by maturation and 1, 2, 3, 4 and 5 days during fermentation. They were put in aseptic sterile tubes, stored in a cooler filled with ice and transported immediately to laboratory for physicochemical analysis.

2.3 Total DNA extraction

DNA extraction was based on the methods of Ampe et al. (1999) and Leesing (2005) but modified and optimised. Around 2g each of gills, skin and intestine were homogenized for 3 min with vortexing after addition of 6 mL sterile peptone water (pH 7.0, Dickinson, France). Four 1.5-mL tubes containing the resulting suspension were then centrifuged at 10,000g for 10 min. 100 µL of lysis buffer TE (10 mM Tris-HCl; 1 mM EDTA; pH 8.0, Promega, France) and 100 µL of lysozyme solution (25 µg.µL⁻¹, Eurobio, France) and 50 µL of proteinase K solution (10 µg.µL⁻¹, Eurobio, France) were added to each pellet. Samples were vortexed for 1 min and incubated at 42°C for 30 min. Then 50µL of 20% SDS (Sodium Dodecyl Sulphate, Sigma, France) were added to each tube, and the tubes were incubated at 42°C for 10 min. 300 µL of MATAB (Mixed Alkyltrimethyl Ammonium Bromide, Sigma France) were added to each tube, and the tubes were incubated at 65°C

for 10 min. The lysates were then purified by repeated extraction with 700 μ L of phenol-chloroform-isoamyl alcohol (25:24:1, Carlo Erba, France), and the residual phenol was removed by extraction with an equal volume of chloroform-isoamyl alcohol (24:1). The DNA was precipitated with isopropanol, washed with 70% ethanol and then air dried at room temperature. Finally, the DNA was resuspended in 100 μ L of ultra pure water and stored at -20°C until analysis.

2.4 PCR-denaturing gradient gel electrophoresis (DGGE) analysis

The V3 variable region of bacterial 16S rDNA from fish was amplified using primers gc338f (5'-CGCCCGCCGCGCGCGGGCGGGGCGGGGGCACGGGGGGACTCCTACGGGAG GCAGCAG, Sigma, France) and 518r (5'-ATTACCGCGGCTGCTGG, Sigma, France) (Øvreas et al., 1997; Ampe et al., 1999; Leasing, 2005). A 40-bp GC-clamp (Sigma, France) was added to the forward primer in order to insure that the fragment of DNA will remain partially double-stranded and that the region screened is in the lowest melting domain (Sheffield et al., 1989). Each mixture (final volume 50 μ L) contained about 100ng of template DNA, all the primers at 0.2 μ M, all the deoxyribonucleotide triphosphate (dNTPs) at 200 μ M, 1.5mM MgCl₂, 5 μ L of 10x of reaction *Tag* buffer (MgCl₂ free) (Promega, France) and 5U of Taq polymerase (Promega, France). In order to increase the specificity of amplification and to reduce the formation of spurious by-products, a "touchdown" PCR was performed according to the protocol of Diez et al. (2001). An initial denaturation at 94°C for 1 min and 10 touchdown cycles of denaturation at 94°C for 1 min, then annealing at 65°C (with the temperature decreasing 1°C per cycle) for 1 min, and extension at 72°C for 3 min, followed 20 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 3 min. During the last cycle, the extension step was increased to 10 min. Aliquots (5 μ L) of PCR products were analysed first by conventional electrophoresis in 2% (w/v) agarose gel with TAE 1X buffer (40 mM Tris-HCl pH 7.4, 20 mM sodium acetate, 1.0 mM Na₂-EDTA), stained with ethidium bromide (Sigma, France) 0.5 μ g/mL in TAE 1X and quantified by using a standard (DNA mass ladder 100 bp, Promega, France).

The PCR products were analyzed by Denaturing Gradient Gel Electrophoresis (DGGE) by using a Bio-Rad DCode™ universal mutation detection system (Bio-Rad Laboratories, Hercules, USA) and the procedure first described by Muyzer et al. (1993) and improved by Leasing (2005, 2011). Samples containing approximately equal amounts of PCR amplicons were loaded into 8% (wt/v) polyacrylamide gels (acrylamide/NN'-methylene bisacrylamide, 37.5:1, Promega, France) in 1X TAE buffer (40 mM Tris-HCl pH 7.4, 20 mM sodium acetate, 1.0mM Na₂-EDTA). All electrophoresis experiments were performed at 60°C using a denaturing gradient ranging from 30 to 60% (100% corresponded to 7M urea and 40% [v/v] formamide, Promega, France). The gels were electrophoresed at 20 V for 10 min and then at 180 V for 12h.

After electrophoresis, the gels were stained for 30 min with ethidium bromide and rinsed for 20 min in distilled water and then photographed on a UV transilluminator with the Gel Smart 7.3 system (Clara Vision, Les Ulis, France).

2.5 Image and statistical analysis

Individual lanes of the gel images were straightened and aligned using ImageQuant TL software version 2003 (Amesham Biosciences, USA). Banding patterns were standardized

with the two reference patterns included in all gels which are the patterns of *Escherichia coli* DNA and *Lactobacillus plantarum* DNA. This software permitted to identify the bands and their relative position compared with the standard patterns.

In DGGE analysis, the generated banding pattern is considered as an “image” of all of the major bacterial species in the population. An individual discrete band refers to a unique “sequence type” or phylotype (Muyzer et al., 1995; van Hannen et al., 1999), which is treated as a discrete bacterial population. It is expected that PCR fragments generated from a single population will display an identical electrophoretic mobility in the analysis. This was confirmed by Kowalchuk et al. (1997) who showed that co-migrating bands generally corresponded to identical sequence.

The DGGE fingerprints were manually scored by the presence and absence of co-migrating bands, independent of intensity. Pairwise community similarities were quantified using the Dice similarity coefficient (S_D) (Heyndrickx et al., 1996).

$$S_D = 2 N_c / N_a + N_b$$

Where N_a represented the number of bands detected in the sample A, N_b represented the number of bands detected in the sample B, and N_c represented the numbers of bands common to both sample. Similarity index were expressed within a range of 0 (completely dissimilar) to 1.0 (perfect similarity). Dendograms were constructed using the Statistica version 6 software (StatSoft, France). Similarities in community structure were determined using the cluster analysis by the single linkage method with the Euclidean distance measure. Significant differences of bacterial communities of fish between seasons were determined by factorial correspondence analysis using the first 2 variances which described most of the variation in the data set.

3. Results

3.1 DGGE pattern of different locations in Viet Nam within the same sampling period

Fish were collected during the rainy season (October 2005) in 5 different districts of An Giang province, Viet Nam. PCR-DGGE fingerprinting of 5 replicates for each location revealed the presence of 8 to 12 bands of bacteria in the fish (Fig. 3). Some of the bands were common to all the different regions. The pattern obtained for the bacterial community for 5 replicates of the same pond of a unique farm in each district was totally similar among the same season (Fig. 4). We observed also high similarities on bacteria patterns for the samples from the same districts, as well as the neighbouring district where the water was supplied by the same branch of the Mekong River. Statistical analysis of the DGGE gel patterns for the 5 replicates of fish samples from 5 different districts of An Giang province harvested in the rainy season (25 samples), showed the community similarity among the different geographical locations where the fish samples were collected (Fig. 3). Two main clusters were observed at 70% similarities level (Fig. 3): 1. The first cluster included the samples from Chau Doc and Chau Phu districts; 2. The second cluster comprised the samples from An Phu, Tan Chau and Phu Tan districts. The bacterial communities of Chau Doc and Chau Phu districts were closely related, as well as the bacterial communities from An Phu, Tan Chau and Phu Tan districts.

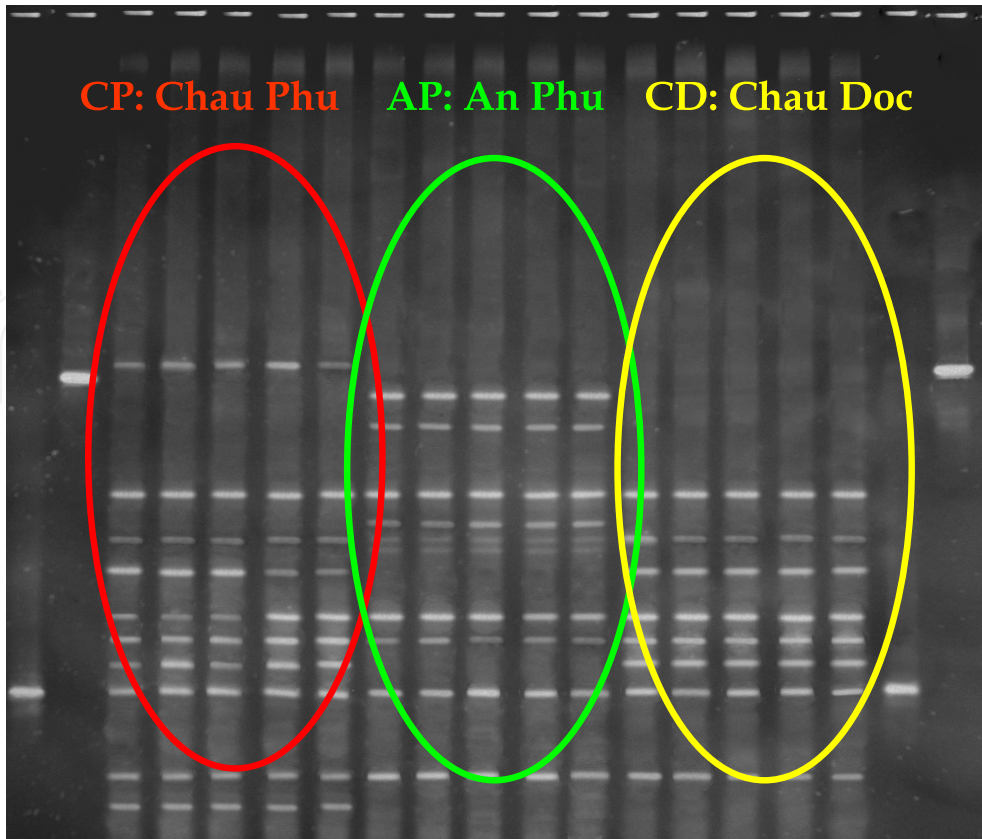


Fig. 3. PCR-DGGE 16S rDNA banding profiles of fish bacteria from 3 districts of An Giang province (five fish from the same pond in the same farm in each district), Viet Nam in rainy season : CP: Chau Phu district; AP: An Phu district; CD: Chau Doc district;

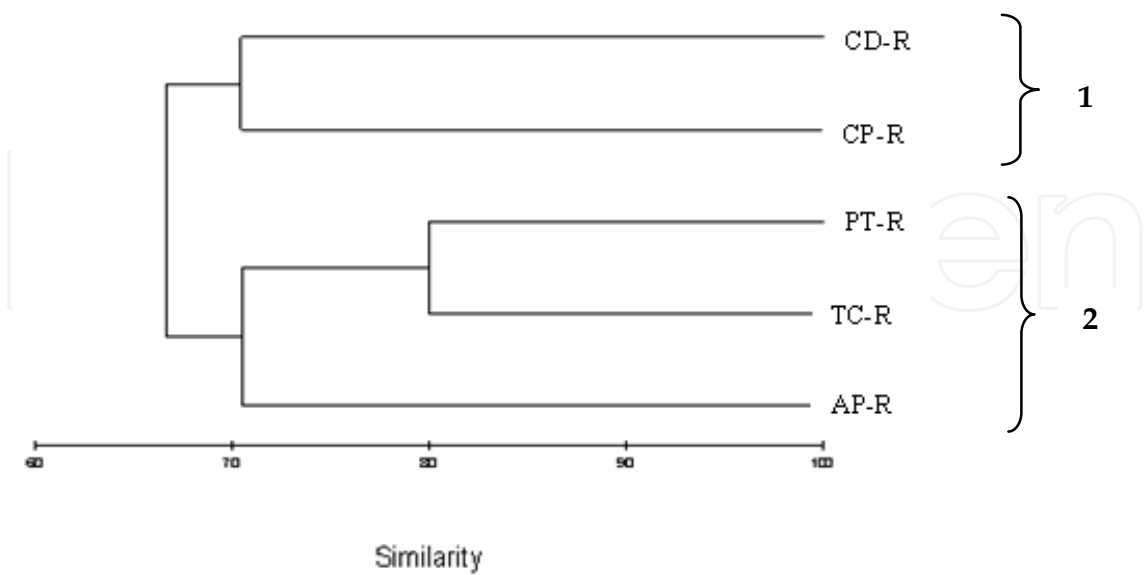


Fig. 4. Cluster analysis of 16S rDNA banding profiles for fish bacterial communities from 5 districts of An Giang province, Viet Nam in rainy season (R) 2006: CP: Chau Phu district; AP: An Phu district; CD: Chau Doc district; TC: Tan Chau district; PT: Phu Tan district.

3.2 DGGE pattern of fish within the same location in Viet Nam at two sampling seasons

Samples were taken at 8 months interval from the same ponds as previous experiments in the dry (February 2006) and rainy season (October 2005). Only four districts were studied for the dry season due to the closing down of Phu Tan pond. Differences on the DGGE band patterns of the same ponds at two different seasons can be clearly noted (Fig. 5). However, some dominant DNA bands could be observed that remained the same during the two seasons. Cluster analysis by Statistica software also showed that the bacterial communities of the same ponds were quite similar for the two seasons. Two main clusters were also observed at 75% similarities levels (Fig. 5): 1. The first cluster included samples from An Phu and Tan Chau districts; 2. The second cluster comprised all the samples from Chau Doc and Chau Phu districts. It was also shown that throughout the 2 seasons for the same location, the number of main bands observed on the gel was slightly different. For example, for the farm in An Phu district, 10 bands were observed in the dry season and 9 bands in the rainy season but only 7 bands are common and the calculation by Statistica showed that there was 75 % similarity between the two seasons. The similarities were higher for the other farms (88% for Than Chau, 84% for Chau Phu and 86 % for Chau Doc).

In addition, similarities of the bacterial communities from the 4 different geographical locations for two seasons were compared at 8 months interval by Factorial Correspondence Analysis (FCA). The first two variances described 76 % of the variation in the data set (Fig. 6). Four different groups of 4 different districts were observed regardless the seasons.

3.3 Microbiological changes during “Adjuvian” fermentation in Ivory Coast

Fish samples were collected between April and June 2010 at the pilot plant of Cemagref-Cirad. There were used to produce fermented fish following the two methods of Adjuvian fermentation used in Ivory Coast. PCR-DGGE analysis was done on samples taken successively from the first day to the fifth day of fermentation.

3.3.1 Microbiological changes following method 1

DGGE analyses conducted on fish collected after 24 h of maturation and before salting, gave 3 to 5 common bands of bacteria that were identified by sequencing as *Pseudomonas putida*, *Pseudomonas fluorescens*, *Aeromonas* spp., *Staphylococcus* spp. A similar DGGE profile was obtained at the second day of fermentation. But more bands appeared, from 9 to 21 bands composed of 94 % of positive gram bacteria dominated by *Staphylococcus* spp. (55%), *Bacillus* spp. (24 %), *Micrococcus* spp. (13%) and 5% of aerobic mesophilic bacteria. Three percent of unidentified bacteria were revealed by PCR-DGGE in the third day of fermentation. *Staphylococcus xylosum*, *Staphylococcus auricularis*, *Staphylococcus piscifermentans*, *Staphylococcus saprophyticus*, *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus licheniformis* and *Streptococcus* spp. were the genera identified (Fig.7). *Staphylococcus saprophyticus*, *Bacillus licheniformis*, and *Micrococcus luteus* were present in all samples. Some bacteria were common to some samples. However a loss of about 40% of bands of aerobic mesophilic bacteria and some genera of *Micrococcus* was observed at the fifth day of fermentation.

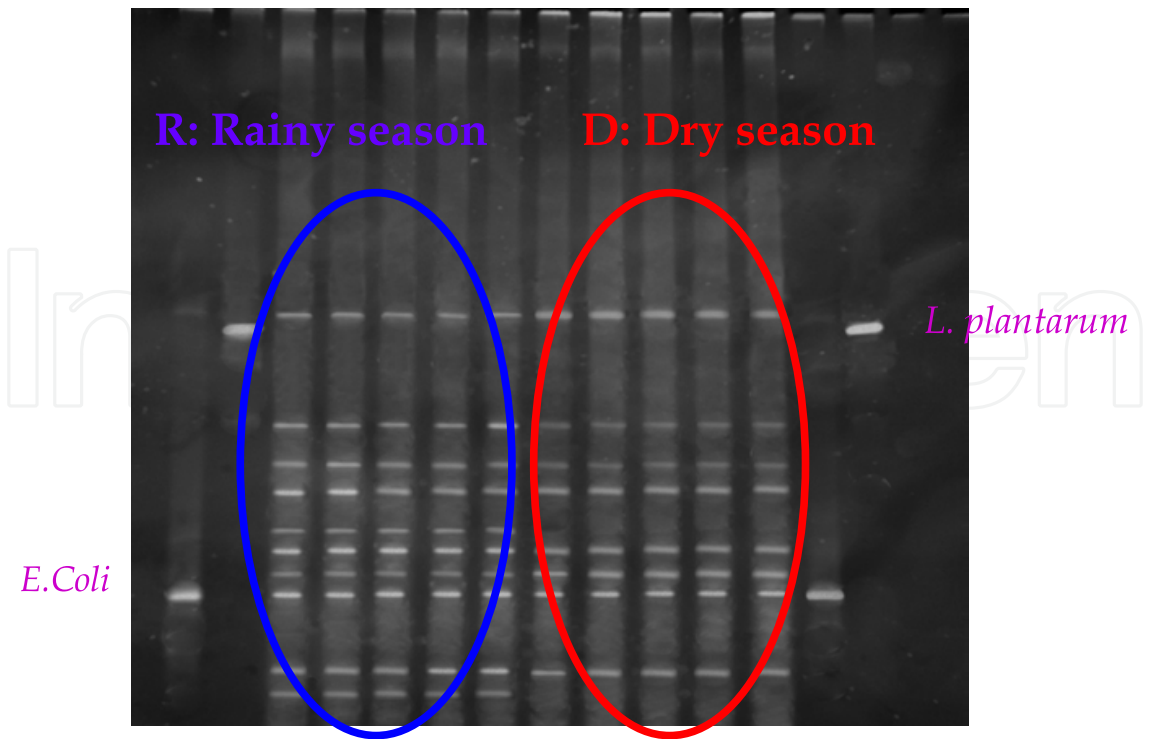


Fig. 5. PCR-DGGE 16S rDNA banding profiles of fish bacteria from one district of An Giang province, Viet Nam in the dry season (D) and rainy season (R)

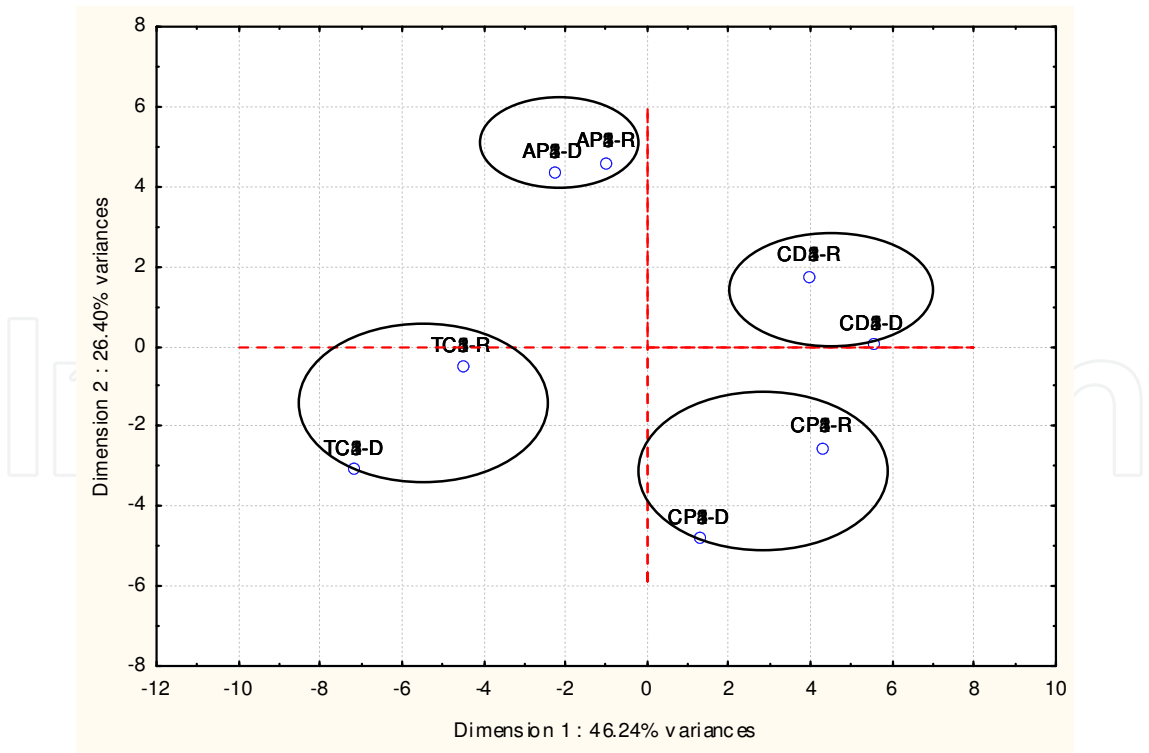
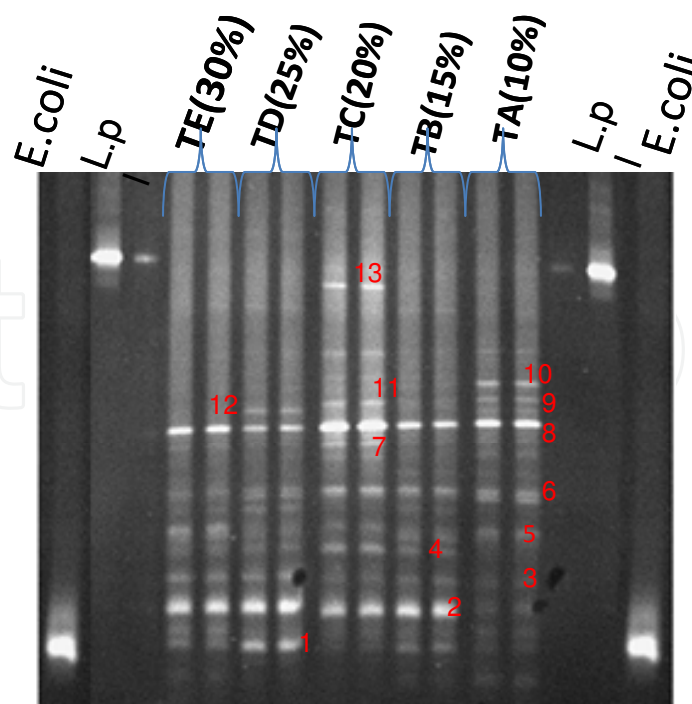


Fig. 6. Factorial variance analysis of 16S rDNA banding profiles of fish bacteria from 4 districts of An Giang province, Viet Nam in the dry season (D) and rainy season (R) 2006: CP: Chau Phu district; AP: An Phu district; CD: Chau Doc district; TC: Tan Chau district.



1: *P. putida*; 2: *B. licheiformis*; 3, 5: *Bacillus* spp.; 4: *B. subtilis*; 6: *Micrococcus luteus*; 7: *S. Piscifermentans*; 8: *S. saprophyticus*; 9, 11: *S. xylosus*; 12: *Staphylococcus* spp.; 10: *S. auricularis*; 13: *Streptococcus* spp.. (Abbreviations: B.; *Bacillus*; S, *Staphylococcus*. P, *Pseudomonas*).

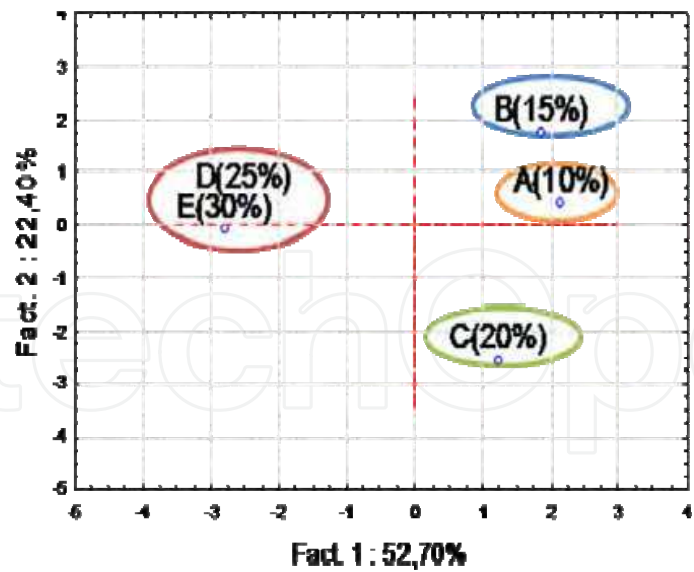
TA (10%): sample with 10% (w/w) of salt; TB (15%): sample with 15% (w/w) of salt; TC (20%): sample with 20% (w/w) of salt; TD (25%): sample with 25% (w/w) of salt; TE (30%): sample with 30% (w/w) of salt.

Fig. 7. PCR- DGGE of 16s rDNA profiles of bacteria from five samples at the third day of fermentation following the method 1 in Ivory Coast.

Factorial analysis of correspondences (FCA) was used to compare the levels of similarities between bacteria communities in function of salt content. Three different groups of samples were observed regardless the initial percentage of salt. Two variances described 75.1% of the variation (Fig. 8). More the percentage of salt was great, more differences between profiles were observed. Up to 25% of salt, DGGE profiles were almost identical and the same bacterial species were identified. This showed the influence of salt on the strains present on meat samples from method 1.

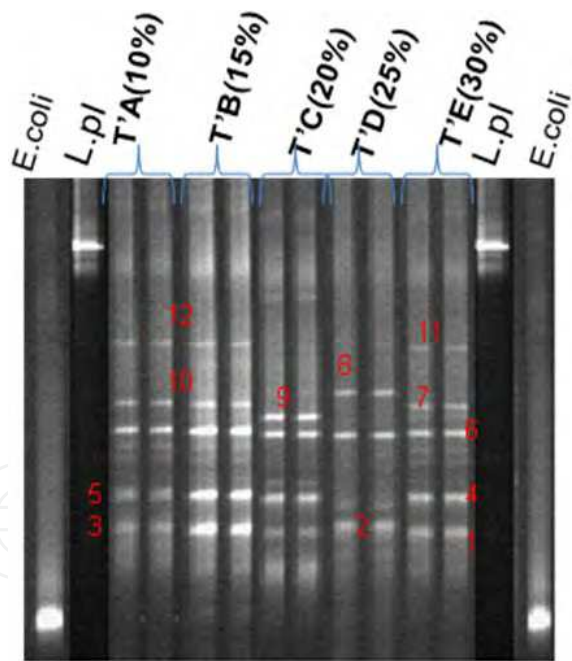
3.3.2 Microbiological changes following method 2

DGGE analyses conducted on fish after 24h of maturation gave two bands identified as *citrobacter freundii* and *Pseudomonas putida*, common to all samples. Five to 12 bands composed of 89.2% of gram positive bacteria with *Bacillus* spp. (49.8%), *Staphylococcus* spp. (17.3%), *Micrococcus* spp. (22.1%), 6.7% of aerobic mesophilic bacteria and 4.1% of unidentified bacteria (Fig.9) were also revealed the third day of fermentation by PCR-DGGE. The genera were *Bacillus subtilis*, *Bacillus licheniformis*, *Staphylococcus auricularis*, *Staphylococcus piscifermentans*, and *Micrococcus* spp. *Pseudomonas putida*, *Bacillus licheniformis*, *Bacillus subtilis* and *Micrococcus* spp. were predominant in all samples during fermentation and persisted up to the end of fermentation. Samples D (25% salt) and E



A (10%); B (15%); C (20%); D (25%); E (30%) five samples of fermented fish prepared with different percentages of salt.

Fig. 8. Factorial variance analysis of 16s rDNA banding profiles of bacteria from five samples at the third day of fermentation following method 1 in Ivory Coast.



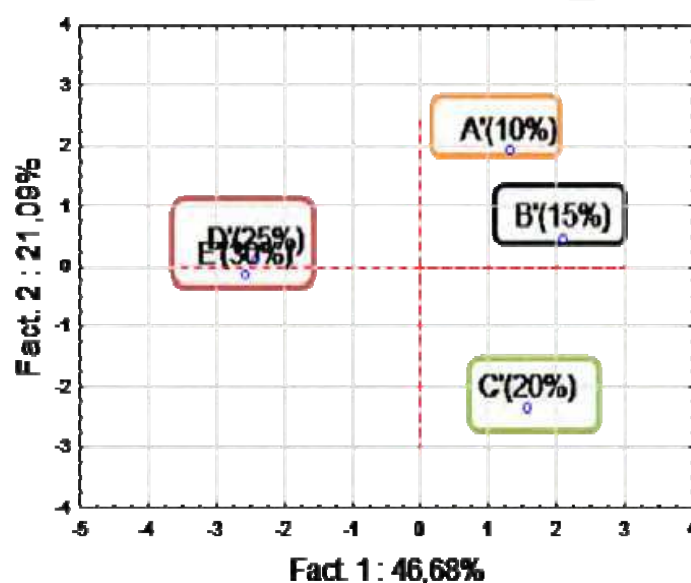
1: *Pseudomonas* spp.; 2: *P. fluorescens* ; 3: *P. putida*; 4,5: *B. licheformis*; 6: *B. subtilis*; 7,10: *S. piscifermentans*; 8: *Micrococcus* spp.; 9 : *Bacillus* spp.; 11,12: *S. auricularis*. (Abbreviations: B.; *Bacillus*; S, *Staphylococcus*; P, *Pseudomonas*).

T'A (10%): sample with 10% (w/w) of salt; T'B (15%): sample with 15% (w/w) of salt; T'C (20%): sample with 20% (w/w) of salt; T'D (25%): sample with 25% (w/w) of salt; T'E (30%): sample with 30% (w/w) of salt.

Fig. 9. PCR- DGGE 16s rDNA profiles of bacteria from five samples at the third day of fermentation following method 2 in Ivory Coast.

(30%) have common bands. A loss of only 10 % of bands was observed at the fifth day of fermentation.

Factorial analysis of correspondences (FCA) comparing the levels of similarities of bacterial communities at the third day of fermentation showed a variance described by 67.8% of the variation in the data set and a low difference between bacterial communities for all samples (Fig. 10). Overall flora varied very little from one sample to another but since 20% of salt, DGGE profiles were almost identical with the same bacterial species. All these results showed that the effect of salt was lower on the bacterial flora of method 2 than in method 1.



A' (10%); B' (15%); C' (20%); D' (25%); E' (30%) five samples of fermented fish prepared following different percentages of salt.

Fig. 10. Factorial variance analysis of 16s rDNA banding profiles of bacteria from five samples at the third day of fermentation following method 2 in June 2010 in Ivory Coast.

4. Discussion

Analysis of bacterial communities in fish samples has been often investigated using culture dependent methods and culture-independent methods by random amplified polymorphic DNA (RAPD) (Spanggaard et al., 2000). There are only a few published works that analyzed the bacterial communities in fish samples by PCR-DGGE methods (Spanggaard et al., 2000; Huber et al., 2004; Maiworé et al., 2009 a,b; Tatsadjieu et al., 2010).

In our study, we found that the band pattern of the bacterial communities isolated from fish obtained by PCR-DGGE was strongly linked to the microbial environment of the fish. The skin is in direct contact with the water, and the gills that filter the air from water that goes to the lung of fish is a good accumulator of the environmental bacteria. The intestine contains also a high amount of bacteria which is affected by the feeding habits of the fish.

The analysis of fish samples from different locations within the same period (rainy season) showed some significant differences in the migration patterns on the DGGE gel. However, the five replicates for each sampling location had statistically similar DGGE patterns throughout the study. The differences in the band profiles can be attributed to the differences of the feeding methods in between farms and the type of aquaculture system applied. The variations may also due to the water supply which can be affected by the pollution from urban life. Furthermore, the antibiotics needed to cure diseases and stress factors could also affect the microbial communities of the fish (Sarter et al., 2007). However, some common bands obtained by DGGE have been found in all the profiles within the same sampling periods and origin.

In fact, when seeing the different locations on the map of An Giang province on South Viet Nam, there is a separation of the Mekong River in two main branches which are then divided into many small canals. Chau Phu and Chau Doc are on the same branch in the west of the Mekong river and An Phu, Tan Chau and Phu Tan are on another branch in the east of the river. We could conclude that there were enough differences in the water quality and the environment of the fish to obtain a major effect on the bacterial ecology.

The study of the fish samples from the same locations in Viet Nam at two different seasons showed that there are some significant statistical variations in the DGGE bands profiles. This fact could result from the important variations due to the heavy rains in the rainy season which can greatly affect the salinity and the pH of the pond water. These factors will greatly affect the microbial communities of the fish which are dependent on the outside environment. These results suggested that the DGGE band profile of the bacterial community of the fish is unique and representative for a farm and a season. However, there was a relatively higher similarity between the samples of the same location across different sampling seasons than the samples from different locations. The DGGE profiles showed some dominant bands of the same locations which are present throughout the whole sampling time.

Concerning the fermented fish from Ivory Coast, our results showed that the salt content in the meat was influenced by the salt percentage in the brine and especially by the fermentation method used. It was also found, after sequencing DGGE bands, a strong dominance of different species of halophilic bacteria depending of the fermentation method. The variation of the bacterial flora of fermented fish was primarily influenced by the fermentation method and by the use of high salt content that conducted to at least 7% in meat. So the analysis of fermented fish bacteria communities by PCR-DGGE could be applied to differentiate the methods of fermentation.

In conclusion, PCR-DGGE technique could be applied to differentiate geographical location of fish production by using their bacterial community. This technique could be applied also to follow the influence of a process on the bacterial flora. Biological markers stayed stable for specific locations among the different seasons and that they showed sufficient statistical specificity per farm. This global technique is quicker (less than 24 h) than all of the classical microbial techniques and avoids the precise analysis of bacteria by biochemistry or molecular biology. Sequencing could be done directly from the DGGE gel extracts to identify bacteria. This method is a rapid analytical traceability tool for fish products and provides fish with a unique biological bar code.

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This book provides an understanding on a large variety of aquaculture related topics. The book is organized in four sections. The first section discusses fish nutrition second section is considers the application of genetic in aquaculture; section three takes a look at current techniques for controlling lipid oxidation and melanosis in Aquaculture products. The last section is focused on culture techniques and management, ,which is the larger part of the book. The book chapters are written by leading experts in their respective areas. Therefore, I am quite confident that this book will be equally useful for students and professionals in aquaculture and biotechnology.

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