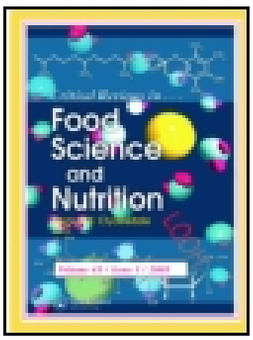

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REVIEW ARTICLE

How to Determine the Geographical Origin of Seafood?

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ABSTRACT

Traceability of seafood is a much needed service for the seafood industry. Current ways of tracing seafood are minimal while tracing of shellfish is nearly nonexistent. Tracing fish and shellfish are necessary for indicating where the fish and shellfish were fished from, farmed and packed from. This study reviews history of traceability of aquaculture and analytical approaches to verify the origin of seafood. It then describes the new molecular technique of the traceability by using PCR-DGGE to discriminate the geographical origin of fish (cases studies of Pangasius fish from Viet Nam and Sea bass fish from France) by analysis the DNA fragments of microorganisms (bacteria) on fish. This method is based on the assumption that the microbial communities of food are specific to a geographic area.

Key words: Seafood, traceability, analytical techniques, microbial communities, PCR-DGGE, geographical origin

HISTORICAL BACKGROUND: TRACEABILITY IN AQUACULTURE

Traceability systems have been present in Aquaculture for a long time. Many companies are part of quality shellfish, finfish and seafood schemes which require a tracing system. Additionally most of the health and hygiene regulations (EU directives 91/492 and 91/493) which apply to fish, shellfish and seafood products have measures in them to track and trace seafood product along various parts of the supply chain. It is only in recent years that traceability in its own right has become important. The first piece of EU traceability legislation (Regulation 178/2002) will come into force in January 2005. It is expected that further EU legislation will come into force which will replace a lot of the existing health and hygiene rules. The new legislation requires companies working in the food sector to have a minimum traceability capacity of one step forward and one step backwards. Complete traceability along the supply chain is theoretically possible (AquaTT, 2004).

GROWING DEMAND FOR SEAFOOD TRACEABILITY

While the meat industry has had quality assurance and traceability systems for some time - most dating back to the outbreak of BSE in the UK in the mid '90s - the fish and shellfish industries have shown a tendency to lag behind. With most fish in the past coming from the wild, traceability has been fraught with difficulties. Traceability is allocated to regions and is used more as a marketing tool rather than a designation of quality and safety. Scottish salmon, North Sea or North Atlantic Cod have been brands instead of assurance symbols. However, now with burgeoning aquaculture taking a more prominent role in the fish markets of the world, knowing where the fish, shellfish or crustaceans originate is becoming more important. As news of

problems with fish diseases in some fish farms becomes more publicly known, consumers want assurance that the products they are eating are safe. As more arguments appear in the media about the effect that fish farms and escapes from farms are having on wild fish stocks, the public will soon start to demand more and more knowledge about the welfare conditions of the farms and the manner in which the fish are reared. Globally, there is no single quality assurance system for fish and shellfish products, it is likely that it will be left to individual countries and industries to develop their own guarantees and set their own transparent standards with which the consumer can make a considered judgment (Moretti et al., 2003).

TRACEABILITY AND PRODUCT TRACING

Traceability System: Reliable Story of Product…. But When?

Food safety is an intrinsic part of food quality as are tracking and tracing systems. A reliable traceability system is the means by which a food company can track and trace any foodstuff which does not meet consumer expectations or the regulations in the country of production or consumption. The main objective of a traceability system is to tell the story of a product i.e. identifies a unique batch of product and the raw materials used in its production and follow that batch through the production and distribution process, to the retailer. Today tracking and traceability software tools are of major interest to the retail business (as a business to business communication tool). Tracking and traceability systems can be incorporated into information systems where consumers can get information on any product. Traceability systems enable efficient product recall and allow fewer products to be recalled. This brings important cost savings where the aim is to provide consumers with nutritious and healthy products which are

produced in a cost efficient way. Additional benefits of an efficient traceability system provide feedback on product quality to the supply chain and improve consumer confidence (Fig.1) (AquaTT, 2004).

Traceability in Seafood

Traceability and product tracing in seafood is not a new concept and for conservation and food safety reasons as well as providing consumers with more information on their product choices, it will expand in its use by the world's seafood industry and the trade in seafood and fish products (ACOA, 2004).

The fishing industry is characterized by the nature of its products that are essentially capture the quantities fished random, seasonality of certain species, the migratory nature for others, locating in different areas according to age or maturity sex for others, the variable delay between catching and landing due to the remoteness of the fishery (inshore and offshore) and the short shelf life products (Montet, 2004).

Capture fisheries and aquaculture supplied the world with about 145 million tons of fish in 2009. Of this, 117 million tons was used as human food, providing an estimated apparent per capita supply of about 17 kg (live weight equivalent), which is an all-time high. Aquaculture accounted for 46% of total food fish supply. In 2007, fish accounted for 15.7% of the global population's intake of animal protein and 6.1% of all protein consumed. Globally, fish provides more than 1.5 billion people with almost 20% of their average per capita intake of animal protein, and 3.0 billion people with at least 15% of such protein (FAO, 2010).

In recent years, international trade in fish products has faced the following major issues: the evolution of measurement quality control and safety in major importing countries, the introduction of new requirements of labeling and traceability adoption of the guidelines of FAO for the eco-labelling of fish and fishery products from marine capture and also by various trade between importing and exporting countries motivated by accusations of dumping of products from aquaculture and production subsidies (FAO/SOFIA, 2006).

International Market Regulations

Regulations in USA

Two major pieces of legislation in the USA are driving traceability systems in seafood production. United States, several regulations call for traceability systems in the field of production of fishery products. The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 and relevant regulations have several requirements to improve traceability. The Farm Security and Investment Act of the United States of 2002 (Farm Bill) requires retailers to notify their customers of the country of origin of fishery products since September 30, 2004. It is also noteworthy to consumers if the product is a “wild product” or “farm product”. This provision is not considered a food safety measure but as consumer information to help them choose the food they buy (ACOA, 2004).

Regulations in European Union

Several European Directives have introduced safety standards into the chain for fisheries and aquaculture products with the concept “from farm to fork”, usually based on the Codex

Alimentarius provisions. A labelling regulation for fishery and aquaculture products came into effect in the European Union in 2001 (Regulation No. 2065/2001), requiring identification of the official commercial and scientific name, the origin of the fish and its production method (farmed or wild). This regulation aims to provide consumers with a minimum of information on characteristics of such products and is enforced in Italy by Ministry of Agriculture Decree No. 27.03.02 on the labelling of fish products. In addition, in Regulation No. 178/2002, which lays down procedures in the matter of food safety and establishment of the European Food Safety Authority, the Commission defines traceability as "the ability to trace and follow a food, feed, food producing animal or substance intended to be or expected to be incorporated into a food or feed, through all stages of production, processing and distribution".

Identification of the Geographical Origin of Seafood…..Why?

There are many different issues concerning the traceability of fish which it may be desirable to check by performing chemical or physical analysis.

1. Species of origin (fish species),
2. Geographical origin (fish from different regions),
3. Method of production (wild or farmed, organic or intensive).

There is much debate and much concern over farmed fish versus wild fish. During the past few years the production of farmed fish has increased compared to wild fish capture. Furthermore, the quality of farmed fish is mainly influenced by the quality of the feed. Consequently there is a growing need to develop appropriate analytical methods allowing discrimination between wild and farmed fish, and determination of their geographical origin (Harris, 2008).

The European legislation establishes that the FAO area (Table 1) in which wild fish was caught should be part of the information available to consumers (EC regulation No 2065/2001). The FAO areas are wide regions and very often marketing is made based on a narrower and even local area where the fish comes from, because consumers usually appreciate more and are willing to pay higher prices for their local products. Another reason to establish methods to confirm the geographic origin lays in the quality, or toxicity of the fish. Fish may be marketed according to how pristine and unpolluted the farming region is perceived by the customers. Reports indicating high levels of environmental pollutants (lead, cadmium, dioxins or PCBs) in certain areas and lack of toxic compounds in others (Hites et al., 2004) may make the customers even more aware and demand more accurate information about the geographic origin of fish. It is evident that as long as there are no methods to demonstrate the geographic origin, false labels will be found in the markets offering fish from less attractive areas labelled as their more expensive counterpart (Harris, 2008).

Analytical Traceability Approaches to Verify the Origin of Seafood

Rapid identification techniques of the geographical origin are the current requirement for the purpose of fraudulent labeling prevention and food regulation control with the development of quality assurance systems. Differentiation of commercially important fish and shellfish species of various origins are of importance for the benefit of the product consumers (Yamashita et al., 2008).

The methods make possible the determination of the geographical origin based on the analysis of the product environment using discriminating criteria with reference to data banks of markers.

The choice of a technique strongly depends on the substrate studied. The characterization of origin could ensue from coupled analytical techniques. The results are then analyzed by mathematical/statistical methods to process the data. These methods can be categorized into two types: 1- Physicochemical techniques, which use some chemical compounds (i.e. fatty acids) and assume that these constituents characterize that particular origin of fish. From this point of view it seems to make sense that any modification of the diet or environment will modify the value of these ratios and will highlight a difference in its chemical composition. This approach is frequently associated with a large dataset and statistical analysis, in particular chemometric methods. Theoretically, after a multivariate analysis applied to many instrumental measurements, many similarities or differences can be found in the dataset and some particular samples can be distinguished from others for one or more variables. An alternative approach is to search for a specific marker in the fish, which could be a chemical constituent (i.e. antioxidants) that definitively proves the origin of the fish or fish product (Harris, 2008). 2- Biological techniques, which use the analysis of isoenzymes and more recently on the DNA (Hoelzel, 1992).

Physicochemical Techniques

Fatty Acid Profile

The effects of different types of lipids in the diet on growth and tissue fatty acid composition have been investigated for a number of cultured and wild species (Bergstrom, 1989; Argyropoulou et al., 1992; Haard, 1992; Shearer, 1994; Rueda et al., 1997; Serot et al., 1998, Grigorakis et al., 2002). In all cases the farmed fish were found to have much higher lipid content than their wild counterparts and the fatty acid profiles of farmed fish reflected the fatty

acid composition of the diet that the fish received. As a general rule cultured fish were characterized by higher levels of monounsaturated (especially 18:1n-9), n-9 and 18:2n-6 fatty acids, whilst wild fish were characterized by higher levels of n-3 fatty acids and higher n-3:n-6 ratios. The presence of increased amounts of 18:1n-9 and 18:2n-6 in cultured fish could be explained by the abundant presence of these fatty acids in plant oil used to partially substitute fish oil in fish feed formulations. These fatty acids are not normal constituents of the marine food chain. Soybean oil, sunflower oil and rapeseed oil are commonly used in aquafeeds and are particularly rich in these fatty acids (Turchini et al., 2000; Rosenlund et al., 2001). Comparison of the fatty acid profiles using multivariate data analysis (PCA) and linear regression showed that the fatty acid profiles in muscle tissue of fish fed different oils were clearly different from their wild counterparts and very similar to those of the diet (Rosenlund et al., 2001; Turchini et al., 2003).

The reports by Mooney et al. (2002) and Nichols et al. (2002) give the fatty acid composition of several Australian seafood, including that of Atlantic salmon, a species native of the Atlantic introduced in Tasmania some years ago. Comparison of the fatty acid profiles of oils from Atlantic salmon from different locations in the Atlantic and the Pacific permitted the identification of the origin, in our opinion reflecting that of the feed available at different locations (Aursand et al., 2004).

Antioxidant Profile

It is well known that antioxidants have been extensively used to prevent lipid oxidation in raw materials production and fish feed formulations. Of the chemical compounds that have been

investigated as antioxidants, three have been found to be effective for feeds and feed ingredients and can be used both efficiently and economically. These are ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline), BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene). EU legislation has established rules for authorizing, marketing and labelling feed antioxidant additives (Council Directive 70/524/EEC and subsequent measures). These antioxidants are lipophylic phenolic compounds, which inhibit the formation of fatty free radicals, which are fundamental to lipid oxidation. Only limited reports are available on the levels of these synthetic antioxidants in cultured fish tissue or fish-based products. Attempts were made by Hwang et al. (1995) to estimate the level of synthetic antioxidants and lipid quality in fish feeds and in cultured fish in Taiwan. 68 samples of six fish species, 37 feed samples for seven fish species and 33 samples of fish meal from six countries were collected from markets and manufacturers and were analyzed. BHT was the major antioxidant found in cultured fish, fishery feed and fish meal, ranging from 0–12.3, 0–59.8 and 0–150.0 mg/kg in fish liver (or shrimp hepatopancreas), feed and fish meal, respectively. According to the authors there was no residue of antioxidant found in fish muscle. The determination of authorized synthetic antioxidants in fish tissue has yet to be explored as a specific marker method to distinguish cultured fish from wild fish (Harris, 2008).

Biological Techniques

Genetic Analysis

Genetic analyses are useful to discriminate geographic origin of fish as long as different populations may be discriminated by these analyses and also segregate in space. As already

mentioned, traditional genetic studies are based on the analysis of isoenzymes and more recently on the DNA (Hoelzel, 1992). In the case of cod it is important to discriminate fish from protected endangered regions (Northwest Atlantic, the Baltic) and that from Barents and the Norwegian coastal fisheries. But it is the fish from Lofoten the one with the highest reputation and price. Fevolden and Pogson (Fevolden and Pogson, 1997; Pogson and Fevolden, 2003) showed profound allele frequency differences at the pantophysin (*PanI*) between coastal and north-east Arctic populations of cod (*Skrei*) in northern Norway. However, whereas the divergence between coastal and north-east Arctic populations is indisputable there may be less variation between Norwegian and Icelandic cod at this locus. Other markers like microsatellites are presently being used to discriminate those stocks (SEAFOOD plus, 2005).

Using RAPD fingerprinting, we have been able to find genetic markers to discriminate minke whales captured in the Atlantic from those captured in the Pacific (Martinez and Pastene 1999), but not between different areas in the Northeast and central Atlantic (Martinez et al., 1999), although probably the use of more primers would eventually have produced population-specific primers. However, in migratory species such as the minke whale, it is possible that different populations can be found in a given area at different periods of the year (Pastene et al., 2003), so the genetic stock would not indicate the geographic origin unless also the date of capture was known.

In any case, the usually high gene-flow between populations in highly abundant and widely distributed marine fish species (Waples, 1998; Ward et al., 1994) makes it difficult to find genetic markers to unequivocally identify the breeding stock as well as the geographic origin.

Traceability through the genetic characterization of DNA develops quickly enough to breeding farms and for wild populations. This technique has a very high degree of reliability to confirm or deny the origin, descent, or strain of animals or products and can also be used as evidence in court (Håstein et al., 2001).

Protein/Enzyme Profiles in Some Organs

As is the case for DNA analysis, proteins can be used as markers for the geographic origin when the protein expression is genetically determined and different allozymes are expressed in different stocks and different locations which is the classical approach used in population genetic studies (Drengstig et al., 2000; Galand and Fevolden, 2000; May, 1992, Mork and Giæver, 1999). In addition, we found that the structural myofibrilar proteins called myosin light chains have the potential to serve as genetic markers in the herring (Martinez et al., 1990) and the Arctic charr (Martinez and Christiansen, 1994).

Protein expression can also be used as indicator of geographic origin in those cases where certain compounds in the environment impose a stress on the organism that forces the expression of certain proteins. Novel applications of proteomics such as the characterization of changes induced in a live organism by environmental pollutants add new possibilities to using this set of techniques to ensuring correctly labelled and safe seafood. Thus, several works (Rodriguez-Ortega, 2003; Rodriguez-Ortega et al., 2003; Shepard and Bradley 2000; Shepard et al., 2000; Olsson et al., 2004) have identified protein markers in molluscs that can be used as indicators of the type and level of pollutant in the environment in which these organisms are reared. These

findings may help to identify the origin, if it is a polluted region, and to ensure that only specimens coming from clean areas reach the consumers.

The Microbial Community Betrays the Origins

In case of doubt or fraud, it is necessary to find a precise and fast analytical technique in order to determine their geographical origin. The skin of fresh foods is not sterile and can carry microorganisms or their fragments. The presence of various microorganisms depends on the external environment of the food (soil ecology, fungi, insects, diseases), but also those made by human activity (Sodeko et al., 1987). As example, skin of nuts could be attacked by insects and then open a hole for fungi contamination. Fish skin could be contaminated with bacteria as coliforms by the handling process. Chicken farms could be installing on fish ponds contaminating fish with salmonella. The idea was to create a "biological bar code" (Montet et al., 2004, **El Sheikha, 2010**) based on the analysis of DNA fragments of microorganisms on food products. This method is based on the assumption that the microbial communities of food are specific to a geographic area. Molecular techniques known as "genetic fingerprints" make it easier to compare samples and to quickly assess the microbial diversity of an ecosystem. The detection of 16S rDNA sequences is an imprint of the presence of microorganisms. It says nothing about the viability of the cells or their metabolic activity. The DNA of dead cells can persist in the environment temporarily and many cells may be in a dormant state. rRNA content in cells of a given population is correlated with the growth rate of these cells and reacts quickly to changes in environmental conditions (Ward et al., 1990). However, the sensitivity of rRNA

molecules to degradation by RNases makes the isolation and manipulation of RNA molecules more sensitive than the DNA molecules (Von Wintzingerode et al., 1997).

Why PCR-DGGE?

The PCR-DGGE is a molecular tool well established in environmental microbiology, which allows the study of complexity and behavior of microbial communities. The technique is reliable, reproducible, rapid, inexpensive and can analyze a large number of samples in one step. DGGE is applied to the study of microbial diversity and more can be coupled with techniques of cloning and subsequent sequencing (Muyzer et al., 1993).

The PCR-DGGE has the advantage that separation does not depend on the size of the fragment, but the melting behavior of the PCR product. DGGE is more discriminating than RFLP (Moeseneder et al., 1999). In addition, the banding pattern obtained from the PCR products is indicative of different species (Vaughan et al., 1999) or species assemblages (Muyzer et al., 1993) and allows to visualize the genetic diversity of populations microbial indices to quantify biodiversity (Simpson et al., 1999; McCracken et al., 2001) and the potential to find new non-cultural microorganisms (Simpson et al., 2000). One of the characteristics of strong DGGE is the ability to identify community members by sequencing and re-amplification bands excised directly from gels or by hybridization analysis with specific probes (McCracken et al., 2001; Temmerman et al., 2003), which is not possible with the RFLP (Muyzer, 1999; McCracken et al., 2001).

Like all molecular techniques, PCR-DGGE is also subject to numerous biases that can taint necessarily analyzing the microbial diversity of a sample. These biases can occur at different

stages of analysis: sampling, preservation of the sample, cell lysis during DNA extraction, amplification of the rDNA by PCR, electrophoretic migration of the DGGE gel and lack of sensitivity of the method for strains present in small quantities. Despite these limitations, DGGE is strongly preferred and is considered one of the best techniques for monitoring the microbial community of a food in a comprehensive, rapid and reproducible (Le Nguyen et al., 2008; El Sheikha et al., 2009; El Sheikha, 2010; El Sheikha, 2011; El Sheikha and Montet, 2011; El Sheikha et al., 2011a, b; El Sheikha et al., 2012).

Table 2 shows the advantages and the limitations of PCR-DGGE technique compared with other molecular-based techniques.

Principle of PCR-DGGE

To detect changes in the microbial community present on fish, a molecular biology approach that will permit to analyze in a unique step all microorganisms present on a fish, bacteria, yeasts or molds, is necessary. We proposed to combine PCR (Polymerase Chain Reaction) to DGGE Denaturing Gradient Gel Electrophoresis (Fig. 2) (Ampe et al., 1999; El Sheikha et al., 2009; El Sheikha, 2010; **Leesing et al., 2011**). The principle of DGGE is based on the partial separation of a fragment of double-stranded DNA under the action of heat or denaturant (Fischer and Lerman, 1983). A molecule composed in part of the classic double helix and part of two single-strands is greatly slowed down compared to a double-stranded molecule or completely denaturated.

DGGE separates DNA fragments of identical size but with different sequences. The separation is based on the electrophoretic mobility of double-stranded DNA in a polyacrylamide gel

containing linear gradient increasing denaturing agents (urea and formamide) (Fig. 3). Electrophoresis is performed at a constant temperature between 55 and 65°C, mostly at 60°C. However, if the gradient increases linearly, the denaturation of the DNA does not occur gradually throughout the molecule, but from estate to estate, which open more or less quickly depending on their composition AT bases (adenine-thymine) and GC (guanine-cytosine). The spatial conformation of the DNA fragment is changing along with the importance of denatured areas which limits its electrophoretic mobility (Muyzer et al., 1996). Optimal resolution is obtained when the fragments are not completely denatured. To avoid distortion of the total double-stranded structure with very high melting temperature (very rich in GC) is associated in the amplification. PCR is then performed with forward primer with 5' sequence of 40 GC: the GC clamp (Sheffield et al., 1989).

The development of the technique depends mainly on the choice of primers. The primers used for amplification determines the sharpness and specificity of the method. For bacteria, the target most often used for PCR amplification is highly conserved regions of the genome that also includes hyper variable regions. The primers are designed to hybridize but in the conserved regions spanning the variable regions to obtain PCR amplicons with differences in composition of base pairs that are specific bacterial species and can then be separated by DGGE (Ercolini, 2004). The primers used to amplify 100 to 500 bp in an area of low melting point. The "GC clamp" should be amplified near the end with the highest melting point. It is therefore necessary to know before amplification areas that have a melting point higher or lower. The behavior of the melting point of double-stranded DNA has been described by a computer model developed by Lerman et al. (1984). The computer software is available, as the MacMelt^{TM} (Biorad, Hercules,

USA) and can calculate the melting profile of DNA and showing the areas nominally high and low stability of a known sequence.

The PCR-DGGE is generally applied in microbial ecology because it is able to provide a genetic fingerprint of the bacterial community in an environmental sample or in a food product after direct extraction of DNA and comprehensive. First, the sample is subjected to the removal of all microorganisms and the microbial DNA is extracted in one step to obtain a mixture containing the total DNA of the bacterial species present in the sample. Then, total DNA is used as a target for PCR amplification of regions of interest. The amplified products obtained are composed of a mixture of amplicons of bacterial species in the initial sample. All amplicons are the same size but their sequences are different, therefore they can be separated by DGGE.

The final result is a specific genetic fingerprint of microbial species present in the sample. The electrophoretic profile presents a series of specific bands of the microbial populations of the analyzed sample that we consider that each band represents a microorganism (Muyzer et al., 1993, Ercolini, 2004).

In the case of fish, the predominant bacterial flora would permit the determination of the capture area, production process or hygienic conditions during post-harvest operations (Leesing, 2005; Le Nguyen et al., 2008). Aquatic microorganisms are known to be closely associated with the physiological status of fish (Al Harbi and Uddin, 2003; Grisez et al., 1997; Leesing, 2005; Spanggaard et al., 2000). Water composition, temperature and weather conditions can influence the bacterial communities (De Sousa and Silva-Sousa, 2001; Wong et al., 1999).

Pangasius hypophthalmus were collected in a unique pond in two aquaculture farms of two different districts from the South Viet Nam namely Chau Phu and An Phu of An Giang province.

This province supplies about 2/3 (about 80,000 MT in 2005) of Pangasius fish for export (Ministry of Aquaculture, Viet Nam, 2005). The samples were collected in two seasons in Viet Nam: the rainy season and the dry season. Bacterial DNA extraction and PCR-DGGE were based on the methods of Ampe et al. (1999) and Leesing (2005) but modified and optimized by Le Nguyen et al. (2008). The PCR-DGGE patterns of 5 replicates for each location revealed the presence of eight to 12 bands of bacteria in the fish (Fig. 4). Some of the bands are common to all the different regions. The bacterial communities for 5 replicates of the same pond of one farm in each district were totally similar among the same season. High similarities were also observed on bacteria patterns for the samples from the same districts. The statistical analysis of the DGGE gel patterns for the 5 replicates of fish samples from two different districts of An Giang province harvested in the rainy and dry seasons at six months difference showed 84.4% for the first two variances in between the bacterial communities (Fig. 5). Two different groups were clearly noted for the two different locations and the two seasons of harvest. The analysis of fish samples from different locations within the same period (rainy season) showed some significant differences in the migration patterns on DGGE. 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. However, some common bands obtained by DGGE have been found in all the profiles within the same sampling periods and origin (Le Nguyen et al., 2008).

Another study reported by Le Nguyen (2008) on *Dicentrarchus labrax* were collected from four fish farms in France: Ifremer Palavas (Montpellier, Mediterranean Sea), Les bars du soleil (Sète,

Mediterranean Sea), Aquanord (Gravelines, North Sea) and Viviers du Gois (Beauvoir sur Mer, Atlantic Sea).

The samples were taken from the same pond and aseptically transferred to storage bags, then maintained on 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 until analysis. On DGGE gel can be observed from four to six bands intense and distinct for each deposit. DGGE profiles of the same fish aquarium are identical and differ from the profiles obtained with fish of different origin by the number and position of bands (Fig. 6). Despite the limited number of bands observed, statistical analysis (Fig. 7) DGGE profiles showed that the profiles obtained with the fish of the same origin are grouped in a single cluster with 100% similarity. The profiles of sea bass from (Palavas, Montpellier, Mediterranean Sea) and (Les bars du soleil, Sète, Mediterranean Sea) gather in a large cluster with 88% similarity. Profiles of sea bass from (Aquanord, Gravelines, North Sea) and (Viviers du Gois, Beauvoir sur Mer, Atlantic Ocean) also come together in a large cluster with 82% similarity. Within these two groups, profiles of fish from different origins are well differentiated. Finally, the profiles of sea bass in the Mediterranean Sea and those of other origins can be clearly distinguished with only 70% similarity. The fish analyzed come from fish farms, with the exception of those that come from Palavas aquarium, which are directly related to the wilderness with three very different environments such as salinity, temperature, water quality but also by farming practices in particular food can vary from one company to another. It is therefore not surprising to find that differentiation of the profiles, namely a certain similarity between the profiles Mediterranean on the one hand and those of the ocean and the North Sea on the other hand, since studies on the

microbial flora aquatic fish caught in different geographical locations have shown that they were closely associated with the physiological status of fish, their hygienic quality and rearing conditions (Okpokwasili and Alpapiki, 1990; Grisez et al., 1997; Spanggaard et al., 2000; Leesing, 2005). The bacterial microflora on the surface of aquatic fish and in their intestines (Sugita et al., 1985). Studies have shown that the intestinal flora reflect the flora of the aquatic environment, particularly that of the diet (Campbell and Buswell, 1983, Nieto et al., 1984).

CONCLUSIONS

In conclusion, the PCR-DGGE analysis of fish bacterial community suggests that this technique could be applied to differentiate geographical location. A few publications relate the link between the analysis of microbial communities analyzed by PCR-DGGE and the geographical origin of fish (Leesing, 2005; Le Nguyen et al. 2008). Most jobs were done on the identification of pathogens by PCR or real-time PCR. The profile of the DNA bands from community of bacteria isolated from fish obtained by PCR-DGGE is strongly related to the microbial environment of fish. We showed that the biological markers for the specific locations stayed stable among the different seasons and that they show 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). This method can be proposed as a rapid analytical traceability tool for seafood and seafood products and could be considered as a provider of a unique biological bar code.

FUTURE PROSPECTS

The quality of seafood and processed products might depend on the quality of the raw materials and geographic origin of their species, which determines their commercial value in the marketplace. Information and databases on the chemical components concerning the determination of food quality, functionality, and safety are very important to assist consumers in selecting foods. Therefore, authentic food labeling regarding species contents and their principle origins should be combined with the labeling information so as to maintain the quality and chemical components of processed food with keeping consumer rights.

Universal scientific methods for the determination of the geographical origin of a foodstuff do not really exist. There are only indirect methods which often have to be coupled to increase their accuracy. The methods which permit the analysis of the microenvironment of food are very promising and have to be better studied by research teams in the world. The main problem will be the construction of data banks which are very necessary for all of these techniques. Some others techniques will be developed in the near future taking into account, for example, the micro-constitution of food. One could consider the micro-components of the lipids like tocopherols, phospholipids or sterols or other molecules brought by the environment like the pesticides, traces of insects, heavy metals, radioactives isotopes, etc.

ABBREVIATIONS

ACOA	Atlantic Canada Opportunities Agency
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene)
DGGE	Denaturing Gradient Gel Electrophoresis

FAO	Food and Agriculture Organization of the United Nations
PCR	Polymerase Chain Reaction
RAPD	Random Amplification of Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SOFIA	State of World Fisheries and Aquaculture

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Table 1 Catch area and identification of the area (Commission Regulation (EC) No 2065/2001)

Catch area	Identification area [1]
North-West Atlantic	FAO area 21
North-East Atlantic [2]	FAO area 27
Baltic Sea	FAO area 27.IIId
Central-Western Atlantic	FAO area 31
Central-Eastern Atlantic	FAO area 34
South-West Atlantic	FAO area 41
South-East Atlantic	FAO area 47
Mediterranean Sea	FAO area 37.1, 37.2 and 37.3
Black Sea	FAO area 37.4
Indian Ocean	FAO area 51 and 57
Pacific Ocean	FAO area 61, 67, 71, 77, 81 and 87
Antartic	FAO area 48,58 and 88

[1] FAO yearbook. Fishery Statistics. Catches. Vol. 86/1. 2000.
[2] Excluding the Baltic Sea.

Table 2 Advantages and limitations of PCR-DGGE compared with other molecular-based techniques to study the microbial diversity (Liu et al., 1997; Muyzer, 1999; Tiedje et al., 1999; Ranjard et al., 2000; Dorigo et al., 2005; Renouf, 2006)

Method	Advantages	Limitations
PCR-DGGE	- Very sensitive methods (differences of one base can be detected on fragments of several hundred base pairs) for the study of complex mixtures of species - Large number of samples can be analyzed simultaneously - Reliable, reproducible and rapid - Estimating the qualitative and semi quantitative diversity	- Optimization of migration on polyacrylamide gel required for any new gene used - Difficult to compare a large number of these gels - One band can represent more than one species (co-migration) - The amplified sequences should not exceed 500 bp
RAPD	- Rapid and sensitive technique to reveal differences between the prokaryotic complexes - Target all the genomic DNA	- Particularly sensitive to artifacts associated with PCR - Do not provide information on the composition of the community
ARDRA	- Detect structural changes in microbial community	- PCR biases - Banding patterns often too complex

RFLP/T-RFLP	- The combination of both discriminating criteria expands the pool of species identifiable (limitation of the problem of co-migration) - The possibility of associating relative abundance peaks - Reproducible method that can be used for qualitative and quantitative analysis of a particular gene of the community - Ability to detect rare members of the microbial community	- A species is a profile of several bands. During the analysis of complex mixtures of species profiles are very difficult to interpret. - It does not identify unknown species because we can extract the gel products of digestion - Training of pseudo restriction fragments that induce an overestimation of diversity (only valid for the T-RFLP) - Useful to see a community with low average wealth but not for complex communities, this is possible with the T-RFLP, which reduces the number of fragments
SSCP	- Obtain valuable information about changes in families of genes under different environmental conditions - Generally good detection of sequence variations - Ability to associate a peak when a	- The detection of sequence variations tends to decrease with increasing length of the strip - DNA molecules can migrate together resulting in an underestimation of the diversity and

	known sequence of capillary electrophoretic separation	the limit of detection sensitivity of the communities

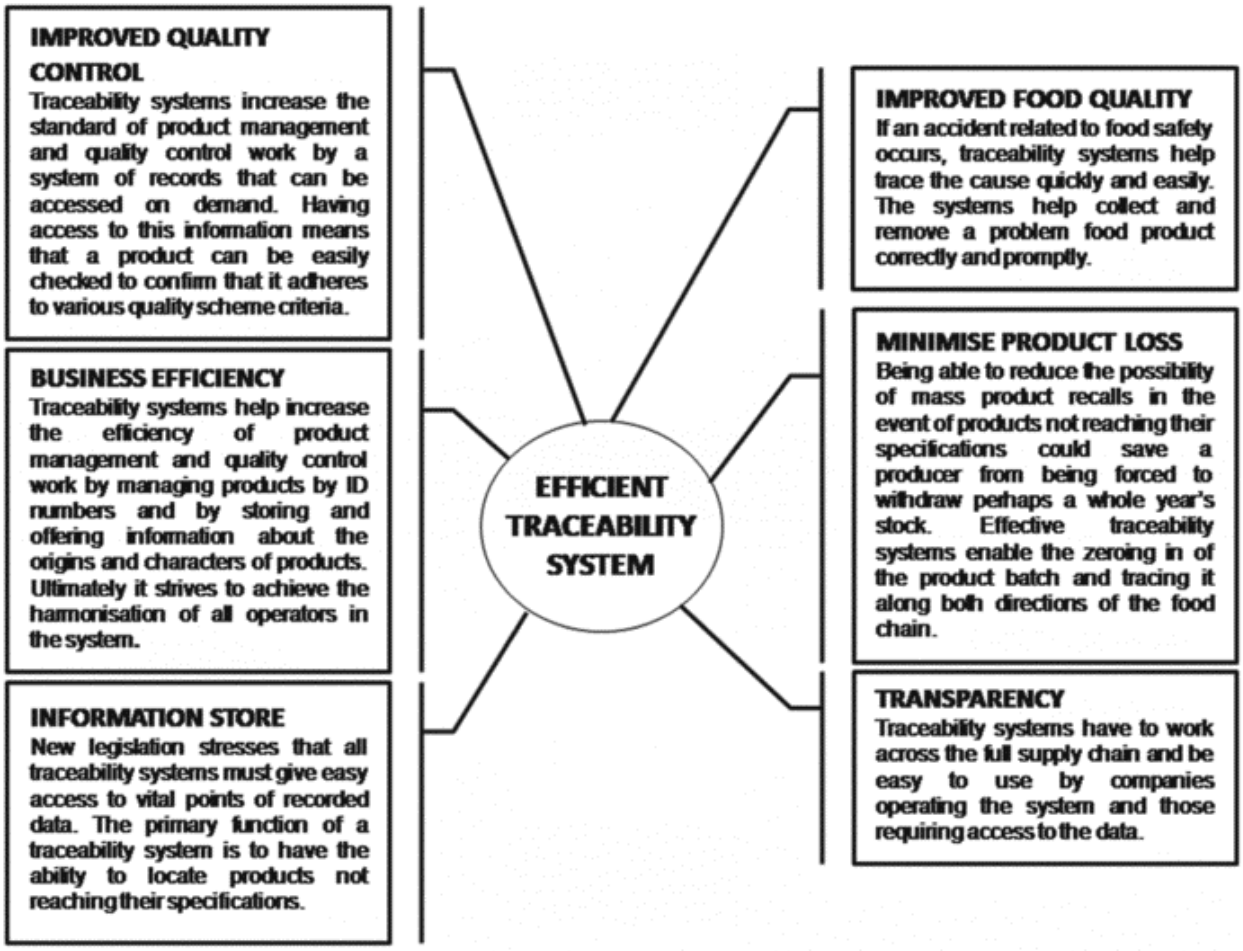

Figure 1 Schematic represent efficient traceability system attributes.

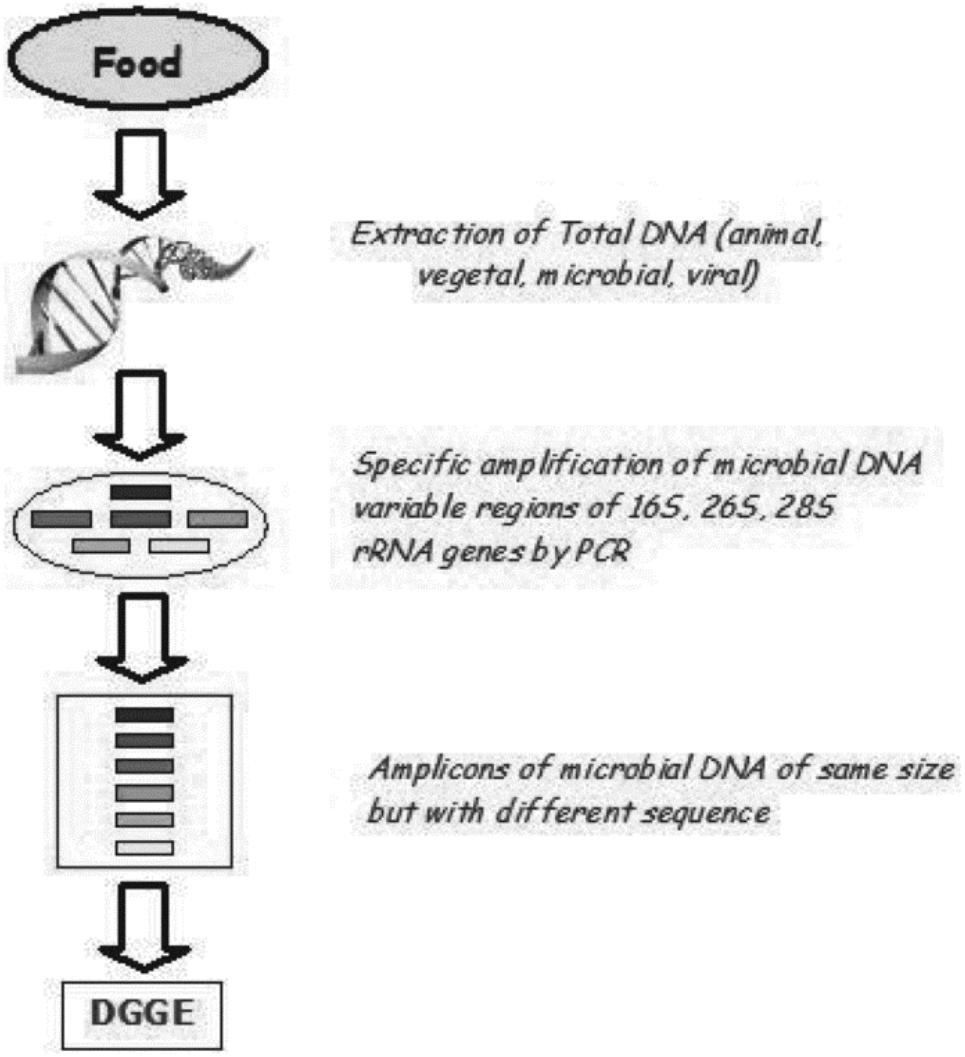

Figure 2 General schema of the analytical process.

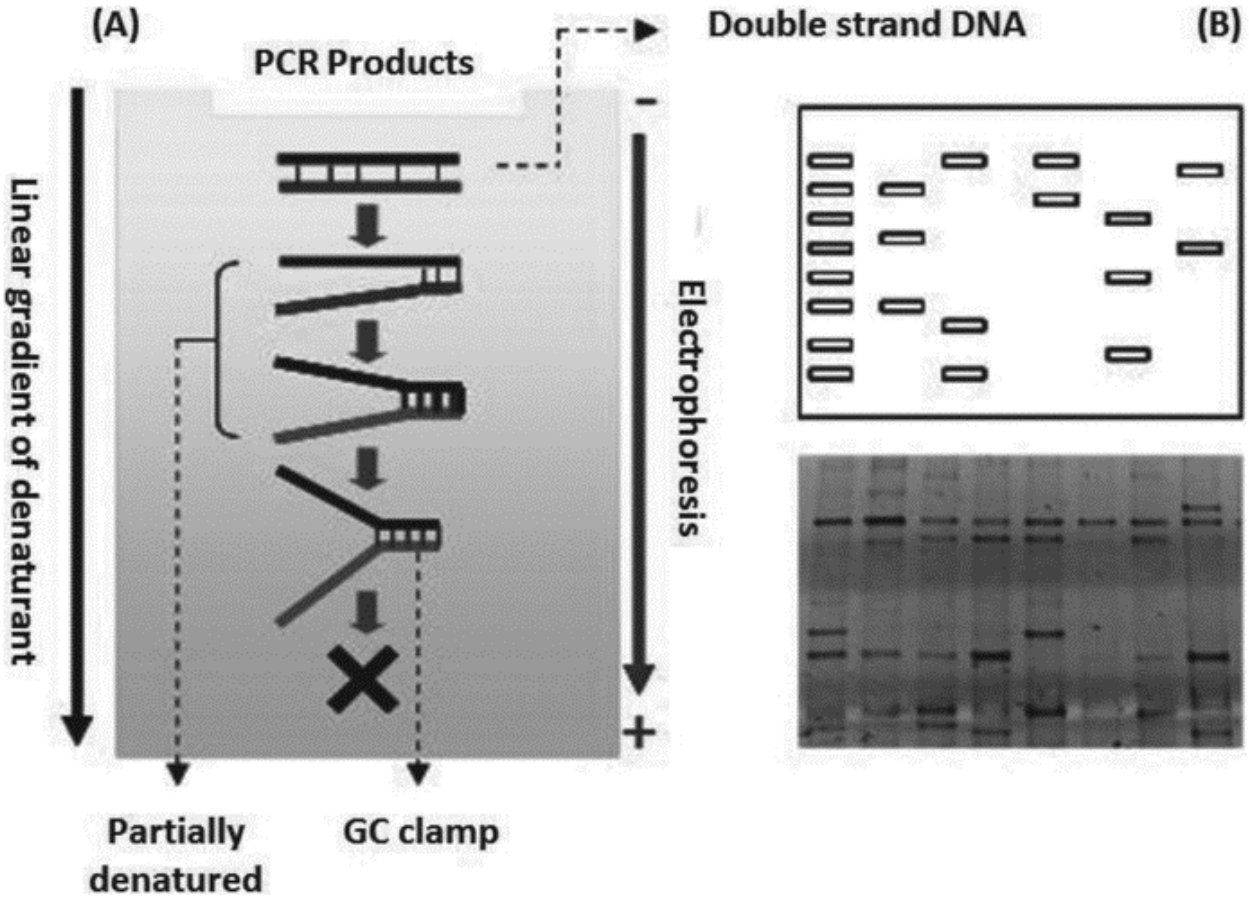

Figure 3 Schematic representation of PCR-DGGE. (A): behavior of a PCR product in DGGE; (B): DGGE profiles obtained after separation in DGGE gel and a photograph of a gel after staining with ethidium bromide.

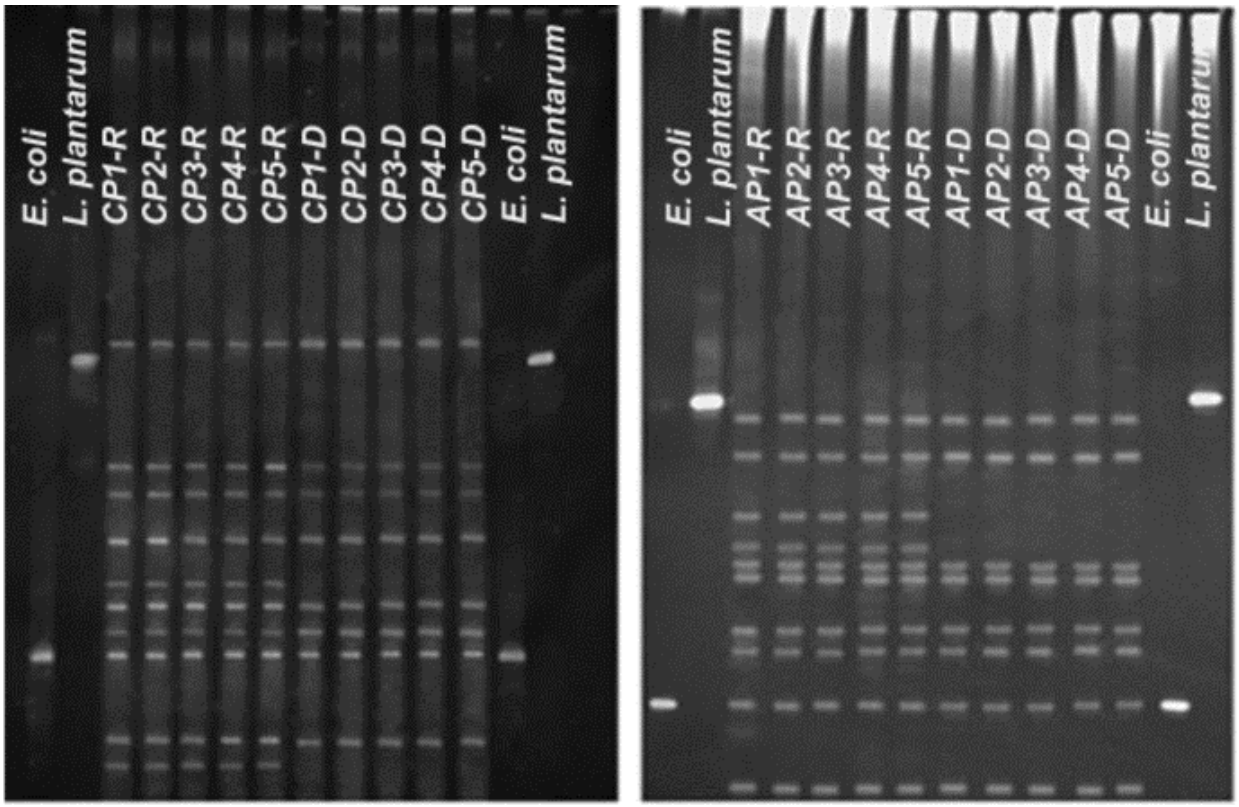

Figure 4 PCR-DGGE 16S rDNA banding profiles of Pangasius fish bacteria from two different districts of An Giang province (five fish from the same pond in the same farm in each district) of Viet Nam in rainy season (R) and dry season (D). (a) CP: Chau Phu district; (b) AP: An Phu district; 1–5: replicate of fish.

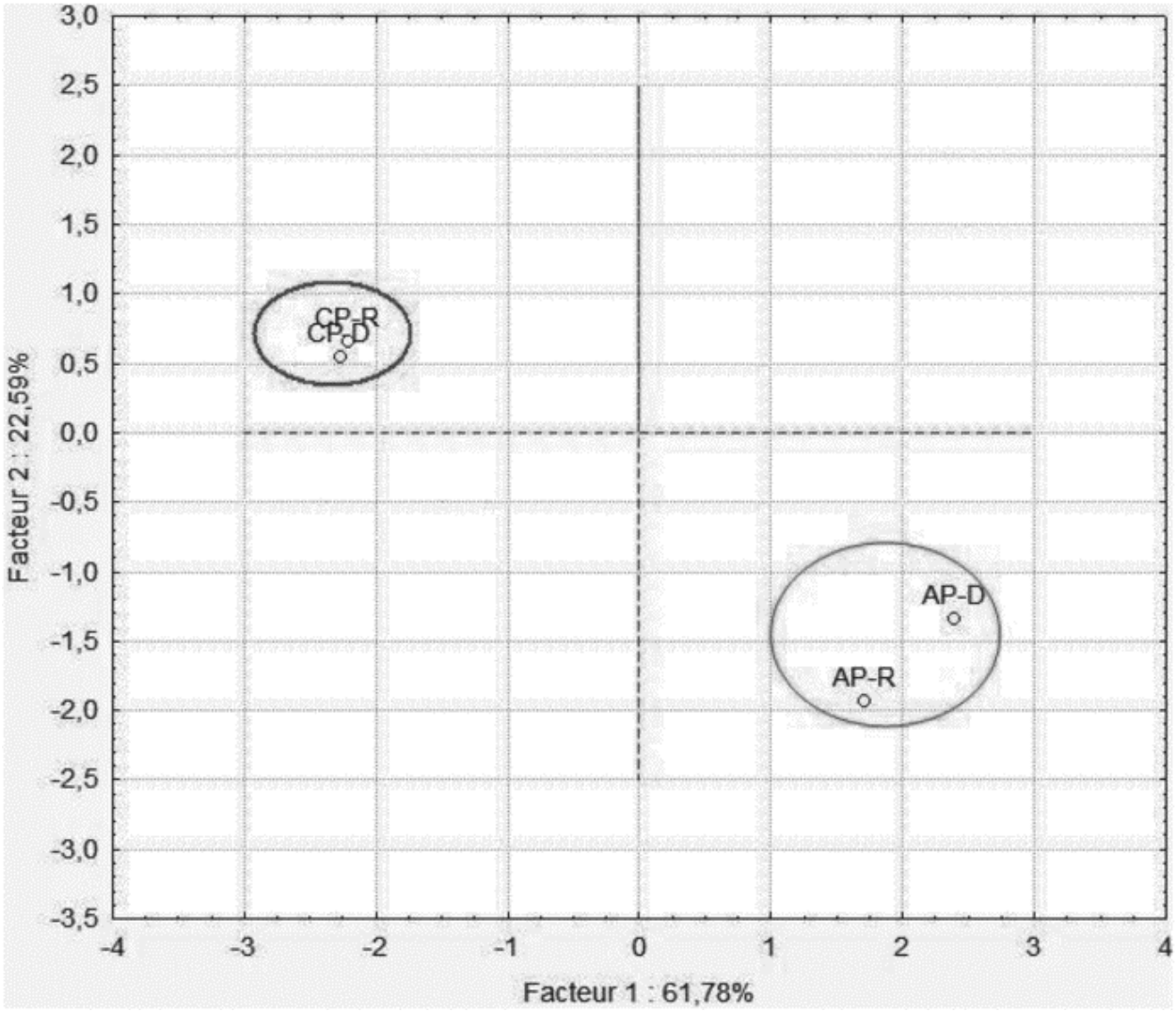

Figure 5 Factorial variance analysis of 16S rDNA banding profiles for Pangasius fish bacterial communities from two different districts of An Giang province of Viet Nam in rainy season (R) and dry season (D). CP: Chau Phu district; AP: An Phu district.

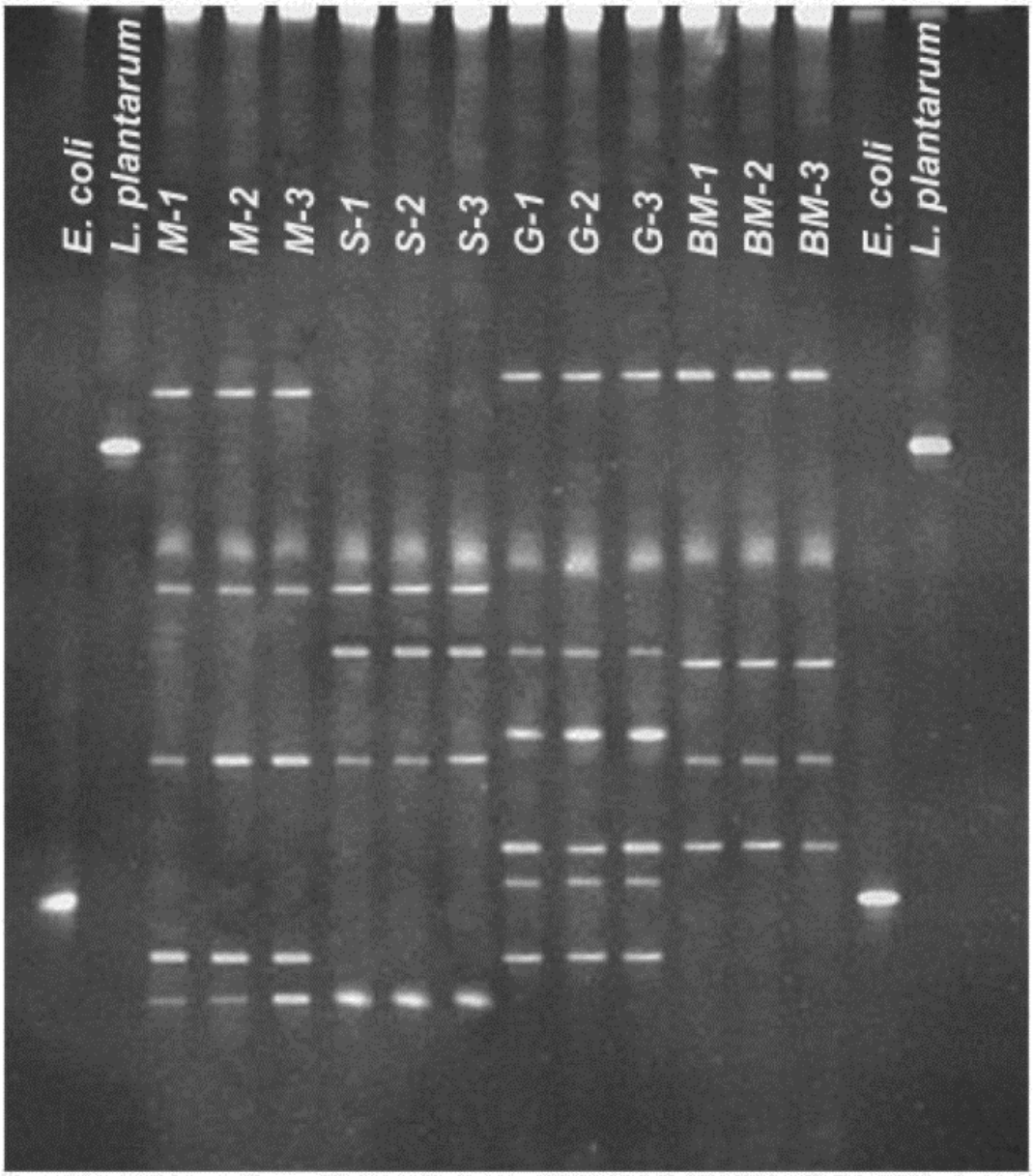

Figure 6 PCR-DGGE 16S rDNA banding profiles for Sea bass fish bacteria from four different districts of France. M: Sea bass fish from Ifremer Palavas (Montpellier) district; S: Sea bass from "Les bars du soleil" (Sète) district; G: Sea bass from Aquanord (Gravelines) district; BM: Sea bass from Viviers du Gois (Beauvoir sur Mer) district; 1–3: replicate of fish.

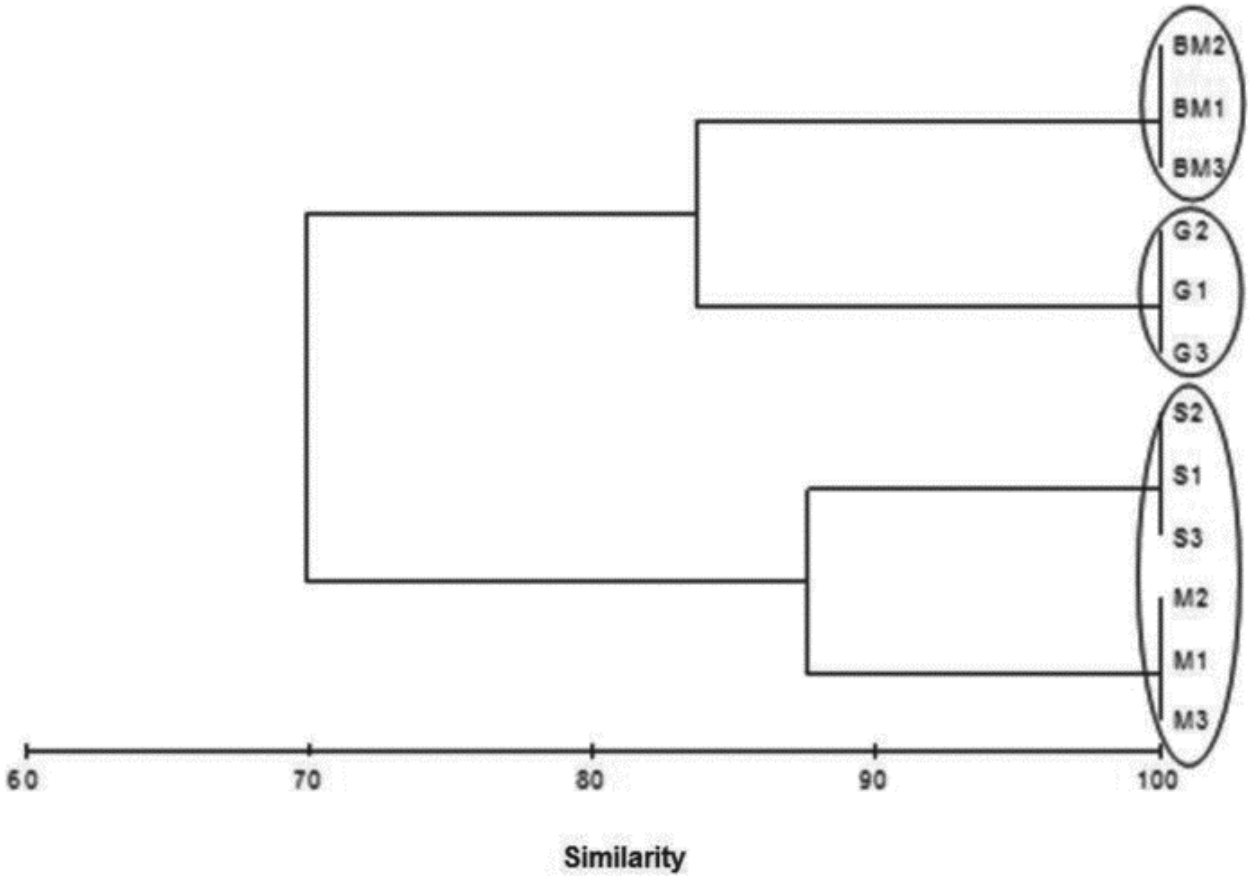

Figure 7 Cluster analysis of 16S rDNA banding profiles for Sea bass fish bacteria from four different districts of France. M: Sea bass fish from Ifremer Palavas (Montpellier) district; S: Sea bass from "Les bars du soleil" (Sète) district; G: Sea bass from Aquanord (Gravelines) district; BM: Sea bass from Viviers du Gois (Beauvoir sur Mer) district; 1–3: replicate of fish.