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Determination of fish origin by using 16S rDNA fingerprinting of bacterial communities by PCR-DGGE: An application on Pangasius fish from Viet Nam

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Abstract

The determination of geographical origin is a demand of the traceability system of import–export food products. One hypothesis of tracing the source of a product is by analysing in a global way the bacterial communities of the food samples after their exportation. For this purpose, molecular techniques employing 16S rDNA profiles generated by PCR-DGGE were used to detect the variation in bacterial community structures of Pangasius fish from An Giang province, South Viet Nam harvested in different aquaculture farm and during two different seasons, the rainy season and the dry season. When the 16S rDNA profiles were analysed by multivariate analysis, distinct microbial communities were detected. The band profiles of the fish bacteria from different farms are different and are specific for each location and could be used as a bar code to certify the origin of the fish. When band profiles within the same location at different seasons were compared, we also observed the difference banding pattern for each season. Some common bands were noted which are stable throughout the seasons. These bands can be used as specific markers for this specific location. This method is a new traceability tool which provides fish products with a unique bar code and makes it possible to trace back the fish to their original location.

Keywords: Traceability; PCR-DGGE; Pangasius fish; Mekong River; Bacterial communities; Origin

1. Introduction

The issues surrounding food safety and security continue to be hot topics for concern throughout the supply chain. BSE, Salmonella and avian influenza remain embedded in the memories of European consumers. Regulations across Europe continue to be tightened in order to provide a greater degree of assurance in quality and safety. Meanwhile, the traceability and labelling of imported products in European countries remains a compulsory issue (UE regulation 178/2002). With similar scares occurring globally, the need for vigilance and strict monitoring is necessary.

One of the great concerns of the customers is the traceability of the products. Traceability is the capacity to find the history, use or origin of a food (activity, process and product, etc.) by registered methods (ISO 8402, 1994). For a long time the food industry has had simple traceability systems, but with the increasing implementation of current Good Manufacturing Practice and ISO 9000 quality management in food manufacture, traceability systems have become more important in the production chain. In view of the difficulties of installing these documentary systems in developing country as in South-East Asia, and to follow the product during processing, we propose to

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identify and validate some pertinent biological markers which come from the environment of the fish to assure traceability of aquaculture product during international trade. Currently, there are no existing scientific methods which permit to the origin of food to be followed or determined precisely.

The determination of geographical origin is one demand of the traceability of import–export products. One hypothesis of tracing the source of a product is by analysing in a global way the bacterial communities on the food 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, Leesing, Gemrot, & Loiseau, 2004).

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 (Al Harbi & Uddin, 2003; Grisez, Reyniers, Verdonck, Swings, & Ollevier, 1997; Leesing, 2005; Spanggaard et al., 2000). The water composition, temperature and weather conditions can influence the bacterial communities of fish. The research of de Sousa and Silva-Sousa (2001) on the bacterial communities of the Congonhas River in Brazil showed that there was a direct relation between the bacterial community of the river and the fish commensal bacteria. Wong, Chen, Liu, and Liu (1999) showed that Vibrio *parahaemolyticus* isolated from the shrimps imported from Asia were specific of the geographical capture zone.

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 (Leesing, 2005; Muyzer, De Waal, & Uitterlinden, 1993). PCR-DGGE has already been used to investigate several patterns of distribution of marine bacterial assemblages (Moeseneder, Arrieta, Muyzer, Winter, & Herndl, 1999; Murray, Hollibaugh, & Orrego, 1996; Øvreas, Forney, Dae, & Torsvik, 1997; Riemann et al., 1999) but this technique had not been previously applied to the study the bacteria on fresh water fish for the traceability.

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, Crowley, & Menge, 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 will be compared and analysed statistically to determine the fish origin.

2. Materials and methods

2.1. Fish sampling

The Pangasius fish samples *Pangasius hypophthalmus* were collected in a unique pond in five aquaculture farms of five different districts from the South Viet Nam namely Chau Phu, An Phu, Phu Tan, Chau Doc, Tan Chau of An Giang province (Fig. 1). 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 (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 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.

2.2. Total DNA extraction

DNA extraction was based on the methods of Ampe, Omar, Moizan, Wacher, and Guyot (1999) and Leesing (2005) but modified and optimised. Around 2 g 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 10,000*g* 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 50 μL 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 μL 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 μL of ultra pure water and stored at −20 °C until analysis.

2.3. PCR-Denaturing Gradient Gel Electrophoresis (DGGE) analysis

The V3 variable region of bacterial 16S rDNA from fish was amplified using primers gc338f (5′CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGACTCCTACGGGAGGCAGCAG, Sigma, France) and 518r (5′-ATTACCGCGGCTGCTGG, Sigma, France) (Ampe