Detection of Transgene in Tilapia (*Oreochromis Niloticus*) by Polymerase Chain Reaction (Pcr) Technique

P. C. Ray[2], M. S. Alam[1], and M. S. Islam [2*]

[1]Department of Fisheries Biology and Genetics
[2]Department of Biotechnology, Bangladesh Agricultural University, Mymensingh- 2202
*Corresponding author: shahidj1@yahoo.com

Abstract
Polymerase Chain Reaction (PCR) technique using specific primer can be used to detect transgenes. The present study was undertaken to detect salmon growth hormone (GH) gene in transgenic tilapia (*Oreochromis niloticus*) by PCR. DNA was extracted from F1 Tilapia generated by crossing transgenic parents. Two primers were designed to amplify a part of the region of GH gene sequence, which was used to make transgenic tilapia. To confirm the specificity of the selected primer, PCR was performed on diluted DNAs, extracted from tilapia fin tissues. GH transgene sequences (1500 bp) were successfully amplified from transgenic fish in this study. The specificity of the primers was found to be high in detecting the salmon GH transgenes. The PCR-based method therefore, could be used for fast and easy screening of transgenic fish for this gene.

Key words: Growth hormone (GH) gene, PCR amplification, Transgenic tilapia

Introduction

The tilapia, *Oreochromis niloticus* (Linnaeus, 1758). Is a tropical food fish used extensively in aquaculture in developing countries. It is a cheap source of animal protein especially in underdeveloped tropical and subtropical countries (FAO, 2010). However, it is also a useful laboratory animal of robust disease-free constitution, relatively short generation time, and extremely rapid growth (Alam *et al.,* 1996). Transgenic fish offer considerable potential for improvement of aquaculture production. The term "transgenic" is used to describe an organism which has exogenous genetic material. A "transgenic" animal can be produced by inserting the gene into the nucleus of newly fertilized ovum, selecting for incorporation, and then breeding positives. Transgenic fish have been produced in several species using growth hormone (GH) coding transgenes (Rahman *et al*., 1997). GH is a polypeptide hormone synthesized in the anterior portion of the pituitary glands of all vertebrates, from which source it is released into blood circulation and exerts stimulation influences over growth and development. GH sequences have been isolated from Chinook salmon (*Oncorhynchus tschawytscha*) and introduced into Nile tilapia to produce transgenic tilapia (Rahman and Maclean, 1992).

Transgenic fish of many species have been produced by egg microinjection and other methods. Such species include Atlantic salmon, *Salmo salar* (Shears *et al*., 1991); rainbow trout, *O. mykiss* (Guyomard *et al*., 1989); carp, *Cyprinus carpio* (Zhang *et al.,* 1990); medaka, *Oryzias latipes* (Ozato *et al*., 1986); zebra fish, *Danio rerio* (Stuart *et al*., 1990) and tilapia, *O. niloticus* (Brem *et al*., 1988; Rahman and Maclean, 1992; Alam *et al*., 1996). There are several methods to transgene detection, such as polymerase chain reaction (PCR) analysis, reverse transcriptase PCR (RT-PCR) analysis, multiplex PCR analysis, southern blot analysis, slot blot analysis (Culp *et al.,* 1991; Rahman and Maclean, 1992; Alam *et al.,* 1996; Stuart *et al*., 1990; Martinez *et al*., 2000). The PCR is a commonly applied nucleic acid amplification method that is specific to detect even tiny amounts of organism-specific DNA sequences. The amplified DNA can be detected as a band after electrophoresis on agarose gel. The amplification of selected transgene sequence by PCR promises to be a powerful tool for the identification of transgene (Shears *et al.,* 1991; Guyomard *et al*., 1989; Gadani *et al*., 2000) and this method have been developed to detect transgenic fish (Rahman and Maclean, 1992). The objective of the present study was to detect salmon growth hormone (GH) transgenes in tilapia (*O. niloticus*) using PCR technique.

Materials and Methods

Fish sample collection

Fingerlings of transgenic tilapia (*O. niloticus*) were collected from the University of Southampton, U.K. in January 2006 and grown in aquarium in the Department of Fisheries Biology and Genetics (Fig.1). The experiment was carried out at the Department of Fisheries Biology and Genetics and Professor Mohammad Hossain's Central Laboratory, Bangladesh Agricultural University, Mymensingh.

Fig. 1. Thirty week old transgenic- (above) and non-transgenic (below) full sibling tilapia (*O. niloticus*). The average mass of transgenic and non-transgenic fish was 615 and 105 g, respectively. Scale=15 cm.

Extraction of genomic DNA

Genomic DNA was isolated from fin tissues of fish. Fin tissues were collected from 12 fishes and stored separately in 95% ethanol at room temperature. DNA was isolated from these samples by the phenol-chloroform extraction and ethanol precipitation method (Islam and Alam, 2004). In brief, approximately 20 mg of fin tissues were cut into small pieces and taken into 1.5 ml microcentrifuge tube containing extraction buffer (100mM Tris-HCl, 10 mM EDTA, 250 mM NaCl, pH 8.0 and 1% SDS) and proteinase-K (10 mg/ml). After homogenization, samples were kept at 37°C overnight. Then phenol, chloroform and isoamylalcohol (25:24:1, v/v/v) purification and ethanol precipitation was performed. DNAs pellets were diluted in TE (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) and stored at -20°C. Isolated DNAs were confirmed by running on 0.8% agarose gel and quantified by using a spectrophotometer (Spectronic® Genesys™).

PCR amplification

PCR analysis was performed using primers P_1 (5´-ACCTGTGGAGACTGTTGAGAT-3´**)** and P_2 (5´-CTACTTAGACCACTCAATTGG -3´) to detect GH transgene (Rahman *et al*., 1997). Since these primers amplify salmon GH transgene fragment only, this reduces the chances of obtaining false positive results from any endogenous fish DNA sequences. PCR reactions were performed on each DNA sample in a 10µl reaction mix containing 1µl of 10x ampli *Taq* polymerase buffer, 2.5 µl of each of primers P_1 and P_2 (10µM each), 1µl of dNTPs (250µM each), 2 µl of template DNA (25ng/µl), 0.2µl of ampli *Taq* DNA polymerase (1 unit, Genei, Bangalore, India) and 0.8 µl of sterile water. DNA amplification was performed in an oil-free thermal cycler (Master cycler Gradient, Eppendorf, Germany). The reaction mix was preheated at 95°C for 3 min denaturation followed by 30 cycles of 1 min at 95°C, annealing at 59°C for 1 min and elongation or extension at 72°C for 2 min. After the last cycle, a final step of 5 min at 72°C was added to allow complete extension of all amplified fragments. After completion of cycling program, reactions were held at 4°C.

Agarose gel electrophoresis and documentation

PCR products were run on 1% agarose gel at 120 volt for about1 hr 30 min. Molecular weight markers markers (λ DNA *Hind*III digest, Thermo Fisher Scientific and pUC18/*Taq* I digest, Genei, Bangalore, India) were run alongside the gel to ensure the size of PCR products. The gel was observed under UV light on a transilluminator and photographed by a BioDoc-It™ system.

Results and Discussion

Reliable protocols are required for detection of transgene in genetically modified organisms (GMOs) or transgenic organisms. There are various ways to detect transgenes or GMOs. For instances, Southern blotting, DNA sequencing, Northern blotting, Western blotting and bioassay test. Among them, PCR based detection of transgenes is an easy, cost-effective, and reliable method. Therefore, after production of transgenic fish or GMO, initial detection of transgenes is made by PCR based approach. The method is considered very suitable for detection of a specific DNA sequence (Gadani *et al*., 2000). In transgenic tilapia, the exogenous genes, such as the GH gene is expressed under the control of different promoters, introns and terminators. The present detection system was based on a PCR system to amplify parts of the promoter or intron. Primer P_1 and P_2 is used to detect GH contained transgenic tilapia (Fig. 2). Here, the primer pair P_1 and P_2 was able to detect 1500 bp DNA sequence from transgenic tilapia.

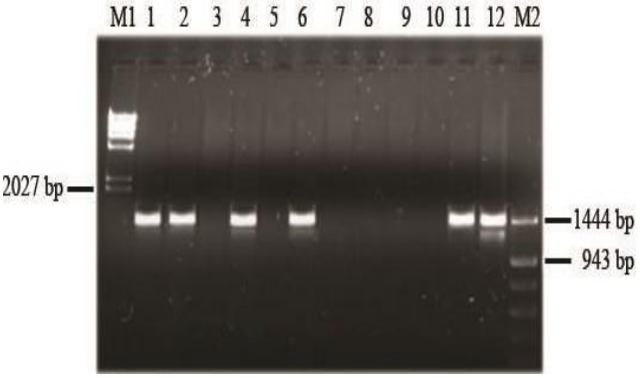

Fig. 2. Detection of GH transgene in tilapia (*O. niloticus*) by PCR using primer P_1 and P_2. P_1 (5'ACCTGTGGAGACTGTTGAGAT 3') derived from the 3' end sequences of OPAFPcsGH and P_2 (5'CTACTTAGACCACTCAATTGG 3') derived from 5' end sequences of carp *β*-actin/ *lacZ*. The expected fragment size using these primers was 1500 bp. Lane: 1-12 individual fish. M1 and M2: Molecular weight markers (λ-DNA Hind III digest and $P^{UC\ 18}$/Taq I digest, respectively). PCR analysis showed that 1, 2, 4, 6, 11 and 12 gave specific amplification band of 1500 bp which is identical to OPAFPcsGH (exogenous GH gene) (Fig. 2 & Table 1). GH specific amplification revealed that the sample DNA contained GH transgene and the fish were transgenic. However, no DNA band was found in lanes 3, 5, 7, 8 and 9. This suggests that these fish were not transgenic.

Table 1. Detection of GH transgene in tilapia (*O. niloticus*)

Fish species	Sample No.	PCR reaction
		P1/ P2 (Primer)
O. niloticus	1	+
	2	+
	3	-
	4	+
	5	-
	6	+
	7	-
	8	-
	9	-
	10	-
	11	+
	12	+

By analyzing the results it can be said that among the 12 samples 6 were positive with the primer P_1 and P_2

(1500 bp) and 6 were not positive (Table 1). Therefore, 50% of the fish were positive for the GH transgene. Similar phenomenon has been previously observed and reported in a number of transgenic fish species (Stuart *et al*., 1990; Culp *et al.*, 1991). Rahman *et al.* (1997) were able, by means of this technique, to differentiate transgenic and non-transgenic tilapia which was not possible morphologically. The absence of transgenes in some fishes might be due to the event of recombination and mutation during the course of integration and germline transmission (Alam *et al.*, 1996).

In conclusion, PCR has been provided to be a powerful tool for detection of transgens in transgenic fish. This could be used as a baseline study for future transgenes detection in transgenic plants, animals, fish or other organisms in Bangladesh.

Acknowledgements

We are grateful to Dr. Norman Maclean, Emeritus Professor of the University of Southampton, UK for providing fingerlings of transgenic tilapia.

References

Alam, M. S.; Popplewell, A. and MacLean, N. 1996. Germ line transmission and expression of a *lacZ* containing transgene in tilapia *(Oreochromis niloticus). Transgenic Research*, 5: 87-95.

Brem, G.; Brenig, B.; Horstgen-Schwark, G. and Winnacker, E. L. 1988. Gene transfer in tilapia (*Oreochromis niloticus). Aquaculture*, 68: 209-219.

Culp, P.; Nusslein-Volhard, C. and Hopkins, N. 1991. High frequency germ-line transmission of plasmid DNA sequences injected into fertilized zebrafish eggs. *Proceedings of the National Academy of Sciences of the United States of America*, 88: 7953-7.

FAO, 2010. Introduced species fact sheets. Fisheries and Aquaculture Department. Introduced species fact sheets. Fisheries and Aquaculture Department. Rome, Italy: FAO.http://www.fao.org/fishery/introsp/9144/en

Gadani, F., Bindler, G., Pijnenburg, H., Rossi, L. and Zuber, J. 2000. Current PCR methods for the detection, identification and quantification of genetically modified organisms (GMOs): a brief review. *Beiträge* zur Tabakforschung *International*, 19: 85–96.

Guyomard, R.; Chourrout, D.; Leroux, C.; Houdebine, L. M. and Pourrain, F. 1989. Integration and germline transmission of foreign genes microinjected into fertilized trout eggs. *Biochemie*, 71: 857-63.

Islam, M. S. and Alam, M. S. 2004. Randomly amplified polymorphic DNA analysis of four different populations of the Indian major carp, *Labeo rohita (Hamilton). Journal of Applied Ichthyology*, 20: 407-412.

Linnaeus, C. 1758. Systema naturae per regna tria naturae: secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis (in Latin) (10th ed.). Stockholm: Laurentius Salvius.

Martinez, R.; Juncal, J. and Zaldivar, C. 2000. Growth efficiency in transgenic tilapia (*Oreochromis* sp.) carrying a single copy of an homologous cDNA growth hormone. *Biochemical and Biophysical Research Communications*, 267: 466–472.

Ozato, K.; Kondoh, H.; Inohara, H.; lwamatsu, T.; Wakamatsu, Y. and Okada, T. S. 1986. Production of transgenic fish: Introduction and expression of chicken delta-crystalline gene in medaka embryos. *Cell Differentiation*, 19: 237-44.

Rahman, M. A.; Iyengar, A. and MacLean, N. 1997. Coinjection strategy improves integration efficiency of a growth hormone gene construct, resulting in lines of transgenic tilapia (*Oreochromis niloticus*) expressing an exogenous growth hormone gene. *Transgenic Research*, 6: 369–378.

Rahman, M. A. and Maclean, N. 1992. Production of transgenic tilapia *(Oreochromis niloticus)* by one-cell stage microinjection. *Aquaculture*, 105: 219-32.

Shears, M. A.; Fletcher, G. L.; Hew, C. L.; Gauthier, S. and Davies, E. L. 1991. Transfer, expression and stable inheritance of antifreeze protein genes in atlantic salmon (*Salmo salar*). *Molcular Marine Biology and Biotechnology*., 1: 58-63.

Stuart, G.W.; Vielkind, J. R.; McMurray, J. V. and Westfield, M. 1990. Stable lines of transgenic zebrafish exhibit reproducible patterns of transgene expression. Development, 109: 577- 84.

Zhang, E.; Hayat, M.; Joyce, C.; Gonzalez-Villasensor, L. I.; Lin, C. M.; Dunham, R. A.; Chen, T. T. and Powers, D. A. 1990. Gene transfer, expression and inheritance of pRSV-rainbow trout GH cDNA in the common carp, *Cyprinus carpio* (Linnaeus). *Molecular Reproduction and Development*, 25:3-13.