AQUABYTE SECTION

Aquabyte is the Newsletter of the Network of Tropical Aquaculture Scientists

Editorial

NTAS was formed in 1987 with the main objective of linking aquaculture scientists in tropical countries and providing them with information on recent developments in aquaculture, especially those who are working in isolation and lack access to journals and other publications. The principal vehicle for communication has been the Network's newsletter *Aquabyte* which was incorporated into the *Naga, the ICLARM Quarterly* in 1992. To date, the Network has a membership of 700 members in 105 countries. We are planning to put the Network on the Internet for faster dissemination of information and interaction among members. Before going on the Internet, the membership database will be updated. Re-registration forms have been sent to all members with the request to return them with details of their area of specialization. We also encourage those who are not members to join the Network and share their research outputs and/or benefit from the information sharing.

It is often debated whether aquaculture technologies should be developed through studies in research institutions (on-station) or through farmer participatory field trials. While both approaches have advantages and disadvantages, for collecting accurate information it is necessary to undertake on-station studies that take into consideration the ecological and socioeconomic conditions under which they are likely to be adopted. This issue contains a paper that indicates how field conditions could be simulated on-station.

M.V. Gupta

Transgene Expression in Seabass *(Lates calcarifer)* Following Muscular Injection of Plasmid DNA: a Strategy for Vaccine Development?

Zohrah Haji Sulaiman

Abstract

Muscular injection has become one of the direct methods for transferring foreign DNA into organisms. The technique has been recently introduced in the development of vaccines and gene therapy. Vaccine development, in particular, would be desirable in managing viral diseases in farmed fishes. In this study, the technique was performed on seabass and was found that the foreign gene could be transferred successfully through injection into the muscles.

Introduction

In recent years, gene transfer techniques have included direct injection of foreign DNA into muscle cells of organisms. Wolff et al. (1990) first developed the technique and showed that enzymes were expressed after injecting chloramphenicol acetyltransferase, luciferase, and β-galactosidase genes, separately and directly, into diaphragms of mice (Davis and Jasmin 1993) and muscles of fish like the common carp (Hansen et al. 1991), tilapia (Rahman and Maclean 1992) and rainbow trout (Anderson et al. 1996).

The technique of direct injection into muscles for the purpose of somatic correction (which is non-heritable) is simple and inexpensive, requiring only a hypodermic syringe

and genes of interest. Furthermore, expression assays could be performed using biopsy samples obtained from the organism within days of the injection. It is more reliable and much faster than other techniques of gene transfer, such as microinjecting DNA into nucleus of fertilized eggs for making transgenic fish (which is heritable) or using high intensity electric field pulses to transfer DNA from the surrounding media into host cells.

In addition to introducing transgene expression in host cells, the application of this technique may have potential as a therapeutic strategy for inherited primary myopathies such as Duchenne muscular dystrophy (Davis and Jasmin 1993) and for vaccination in fish with gene constructs whose products can elicit a direct immune response (Hansen et al. 1991). Fish scientists would be more interested in the second potential application of the technique for viral diseases such as lymphocystis, lateral line disease, and epizootic hematopoietic necrosis, the latter being a new type of viral disease discovered in southern Thailand three years ago (Danayadol et al. 1994). The disease infected seabass broodstocks (5-7 kg) and caused about 60% mortality within 2-3 days. The development of a vaccine may help to reduce the incidence of infectious viral diseases in fish farms.

Since the technique has been followed in other fish species, e.g., common carp, tilapia and rainbow trout, it would be interesting to find out whether it is also applicable to seabass *(Lates calcarifer)* which is one of the commercially important food fishes of Southeast Asia. This paper describes the application of the technique to seabass and its implications for developing vaccines as defense against viral diseases.

DNA Injection

In this study, plasmid DNA consisting of a cytomegalovirus promoter and *E. coli* β-galactosidase (Clontech Laboratories Inc., USA) was used as the reporter gene. The gene has been commonly used in studying *in situ* expression and proved to be a sensitive histochemical marker in cultured cells and transgenic mouse embryos (Mientjes et al. 1994). A total of 3 and 6 seabass aged 8 months old were injected with 110 µl PBS each using a 1 ml disposable Terumo syringe and a 26.5-gauge needle. The first 3 seabass were used as controls in the experiment. The fishes were anaesthesized in a 100 µg/ml concentration of tricaine methanesulfonate before injection. The point of injection was the muscle below the trailing edge of the dorsal fin, above the lateral line. The depth of injection was approximately 1 mm. The fishes were sacrificed 1, 2 and 7 days after injection by treating them in excess 2-phenoxyethanol.

Assaying for β-Gal Gene Expression

Using sterile scalpel blades, approximately 100 to 200 mm^3 of tissue was recovered from the control and test samples. The flourometric technique for assaying β-gal expression was developed by Hoefer Scientific Instruments (Minnesota, San Francisco). Each sample was homogenized in 50 µl 25 mM Tris-HCl (pH 7.85) and centrifuged at 13 000 g (ca. 9 750 rpm) for 5 minutes. After centrifugation, 40 µl of homogenate was added to 160 µl of the reaction cocktail (25 mM Tris-HCl, pH 7.85; 125 mM NaCl; 2 mM 2-Mercaptoethanol; 0.1 mg/ml 4-methylumbelliferyl-β-D-galactosidase dissolved in 0.5% ethanol). The mixture was incubated at 37°C and stopped after 30 minutes by adding 50 µl 25% TCA with immediate cooling on ice. The sample was then centrifuged at 13 000 g for 5 minutes and 0.1 ml supernatant was added to 1.9 ml of glycine-carbonate reagent (133 mM glycine, 83 mM Na_2CO_3, pH 10.7). The degree of flourescence was measured on DyNA Quant 200 flourometer (Hoefer, USA). The product of β-gal gene expression was determined based on a standard curve of 0-200 nm 4-methylumbelliferone (MU). For example, concentration of 50 nm MU was prepared by diluting 100 µl of the 1 µM MU solution (19.8 mg 4-MU was added to 100 ml sterile distilled water and diluted further into 1:1000 to make 1 µM MU stock) into 1.9 ml gly-

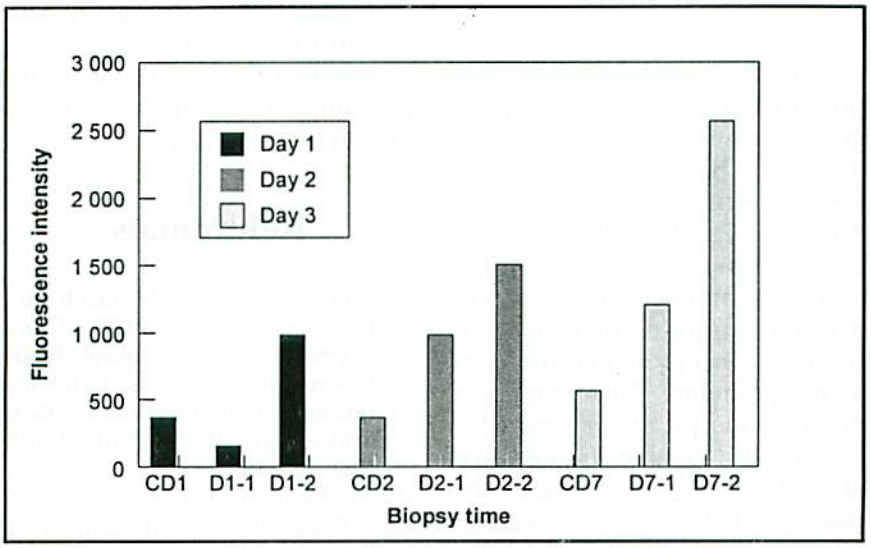

Fig. 1. Fluorescence intensity of muscle samples taken from injected seabass 1, 2 and 7 days after injection. CD1, CD2 and CD7 are controls at day 1, 2 and 7, respectively. D1-1 to D7-2 are test samples on the respective days.

cine-carbonate reagent stop buffer. The fluorescence intensity for this concentration is 500.

β-Gal Expression in Seabass

The study showed a high incidence of positives in the test samples, 5 out of 6 fishes (83.3%) were found to express β-galactosidase enzymes in their muscles. The fluorescence intensities in positive samples ranged from 977 to 2 568. Expression was still present in the injected seabass after 7 days (Fig. 1). None of the controls showed positive readings with intensity ranging from 365 to 569. The result clearly indicated that the technique is also adaptable to seabass. The result of the study was also comparable to the study done by Anderson et al. (1996), which found high expression on day 7 using the same cytomegalovirus promoter. The expression declined after 7 days. In the present study, 3 of the fishes showed β-gal expression in muscle tissues surrounding the injection path as well as in muscle tissues taken 2 mm anterior to the injection site.

Where Do We Go from Here?

The study indicates that it is possible to transfer and express genes in muscle cells of seabass. This could be used for better management of disease in farmed fishes. The genes can be tailored to one's needs. For example, structural genes that produce a vaccine against lymphocytosis may be fused to a fish promoter instead of a viral promoter. The efficiency of homologous promoter genes has been proved in other studies (Du et al. 1992). The present study also indicates that transgene expression may not be confined to the injection site, and that fish cells are capable of taking DNA (Anderson et al. 1996). In small fish (3-5 g), transgene expression had been recovered from other organs, e.g., kidney and spleen, which are important in fish immune response (Anderson et al. 1996). The other indication of this study is the possibility of using fish as "bioreactors" to produce pharmaceutical products, which has been explored recently by Wright's team in Halifax. The team injected human insulin gene into tilapia for production of human insulin (Mackenzie 1996). The only drawback in using a gene as vector for vaccine expression is the cost of the gene itself. The present cost of 25 μg pCMVβ-gal from Clontech is approximately US$400. This could change in the future as technology for making recombinant DNA becomes less expensive. Most of the other studies on fishes have indicated a decline in transgene expression after 7 days. This is desirable since the presence of a long-term transgene activity may not be acceptable for human consumption. Although we are still far from producing fish vaccines, it is nevertheless an interesting option in managing disease on fish farms.

Acknowledgment

I thank the Research and Consultancy Committee of Universiti Brunei Darussalam for providing a research grant (UBD/T/RG.96) for this study.

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