

**EXPRESSION OF THE OCEAN POUT (*Macrozoarces
americanus*) ANTIFREEZE PROTEIN GENE IN TRANSGENIC
ATLANTIC SALMON (*Salmo salar*).**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

A current problem for aquaculture of salmonid fish in Atlantic Canada is the susceptibility of these fish to cold water temperatures and ice. The development of freeze resistant salmon, therefore, would extend the range of salmon sea-pen aquaculture in Atlantic Canada. Previous attempts at producing freeze resistant transgenic salmon by introducing the type I antifreeze protein (AFP) gene from winter flounder have yielded much optimism (Fletcher *et al.*, 1988). The AFP activity in the resulting transgenic salmon, however, was too low to confer freeze resistance to the fish (Hew *et al.*, 1992). One possible reason for this may be due to the lack of an enzyme in salmon necessary to cleave the proAFP form of the type I AFP to produce the fully active mature protein. As an alternative, the gene encoding a type III AFP from ocean pout is being examined for the production of freeze resistance in salmon. The ocean pout AFP lacks the pro-sequence and is therefore fully active post-translationally and is constantly expressed in a variety of tissues (Fletcher *et al.*, 1992). The ocean pout AFP gene was previously introduced into Atlantic salmon and shown to demonstrate Mendelian inheritance.

Methodology

Adult ocean pout (*Macrozoarces americanus*) were caught by divers in Conception Bay, Newfoundland in the winter of 2002 and maintained in a large 300-liter aquarium. Adult Atlantic salmon transgenic for either the ocean pout AFP (opAFP) gene or Chinook salmon growth hormone (csGH) gene were

kindly provided by Drs. Garth Fletcher and Margaret Shears at the Ocean Sciences Centre, Logy Bay, NF. In March/April 2002, the fish (two of each, total of six) were killed by an overdose of anesthetic (3-aminobenzoic acid ethyl ester). Various tissues were removed immediately, frozen in liquid nitrogen and stored at -70°C until needed. RNA from each tissue sample was extracted using TRIzol[®] reagent. Reverse transcription reactions were performed on all RNA samples using the GibcoBRL[®] Superscript II[™] RNase H⁻ Reverse Transcriptase kit. Tissue specific expression was examined using polymerase chain reaction (PCR) and gene specific primers. Reactions were performed in 500 μl micro test tubes containing two units Taq polymerase, 1x PCR buffer, 1.5 mM MgCl_2 , 0.2mM each dNTP, 0.2 μM primer and 1 ng template cDNA. Two sets of primers were used in the analyses: one amplified the housekeeping beta-actin gene and the second amplified the ocean pout AFP gene. The denaturation stage of the PCR was held at 94°C for three minutes. This was followed by amplification at 94°C for 15 seconds, 57°C for 15 seconds and 72°C for 30 seconds and was repeated 34 times. Total samples were analyzed by agarose gel electrophoresis. Expression levels in the tissues were analyzed using Northern blot analyses as outlined in Mehindate *et al.* (2001).

Results

All samples were initially tested using primers specific to a beta actin gene and

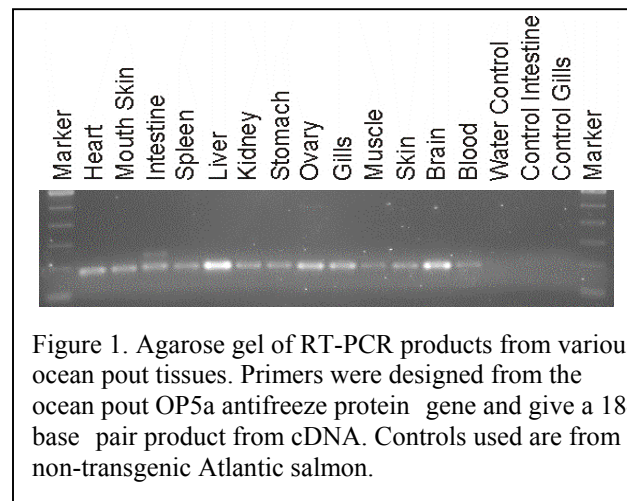


Figure 1. Agarose gel of RT-PCR products from various ocean pout tissues. Primers were designed from the ocean pout OP5a antifreeze protein gene and give a 184 base pair product from cDNA. Controls used are from non-transgenic Atlantic salmon.

results indicated that the RNA samples used had not degraded. Subsequent PCR analyses on cDNA from ocean pout and Atlantic salmon transgenic for the opAFP gene using opAFP specific primers have been completed and the results are outlined in figures 1 and 2. A 184 base pair

band on the gel indicates a positive result for presence of the opAFP transcript in

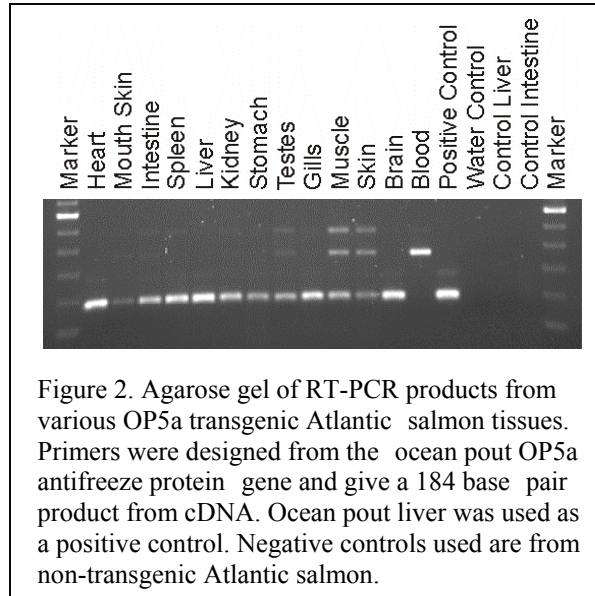


Figure 2. Agarose gel of RT-PCR products from various OP5a transgenic Atlantic salmon tissues. Primers were designed from the ocean pout OP5a antifreeze protein gene and give a 184 base pair product from cDNA. Ocean pout liver was used as a positive control. Negative controls used are from non-transgenic Atlantic salmon.

cDNA. Figure 1 shows expression of the opAFP gene in ocean pout in all tissues tested. Figure 2 shows expression of the opAFP gene in transgenic salmon in all tissues except for the blood. Northern blots are currently being conducted to determine the relative amount of expression in each tissue. Also, these expression patterns will be compared to those of Atlantic salmon transgenic for a

Chinook salmon growth hormone (csGH) gene. The reason for this comparison is that in the transgenic Atlantic salmon, both the opAFP and csGH transgenes utilize the same promoter region, which was obtained from the opAFP gene. By comparing the expression patterns from the two transgenes, it may shed some light on the relative importance of this promoter region to gene expression.

Conclusions

Ocean pout express the type III AFP in all of their tissues. This is in contrast to previous findings by Gong *et al.* (1992) where no expression was found in testes or muscle tissue. In Atlantic salmon transgenic for the type III AFP of the ocean pout, expression was found in all tissues except for the blood.

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