MOL231: Characterization of Type I Interferons (IFNs) in Atlantic Salmon

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BACKGROUND & AIM

Type I interferons (IFNs) are key antiviral cytokines that bridge innate and adaptive immunity. In Atlantic salmon (*Salmo salar*), a uniquely large and diverse repertoire of type I IFN genes suggests specialized immune functions[1]. These IFNs are critical for controlling viral infections[2] and may influence the efficacy of mRNA vaccines by modulating immune activation[3]. However, the specific roles of each IFN subtype in salmon remain poorly understood.

This project aims to characterize Atlantic salmon type I IFNs by cloning and expressing selected subtypes as recombinant proteins. Their antiviral activity will be evaluated by stimulating leukocytes with Pathogen-associated molecular patterns (PAMPs) and analyzing the expression of interferon-stimulated genes (ISGs), providing insights relevant to fish immunology and vaccine development.

MATERIALS & METHODS

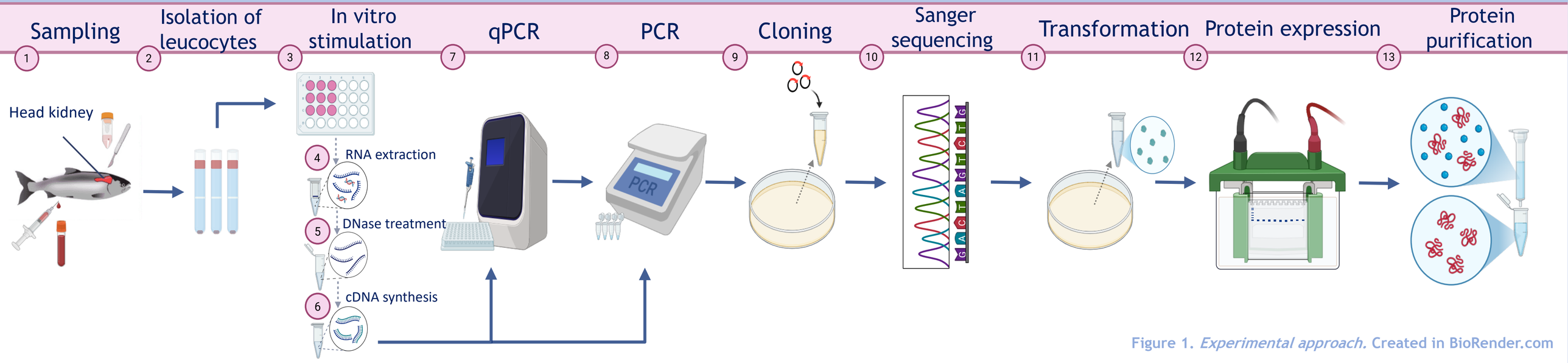

Figure 1. *Experimental approach.* Created in BioRender.com

RESULTS & DISCUSSION

1

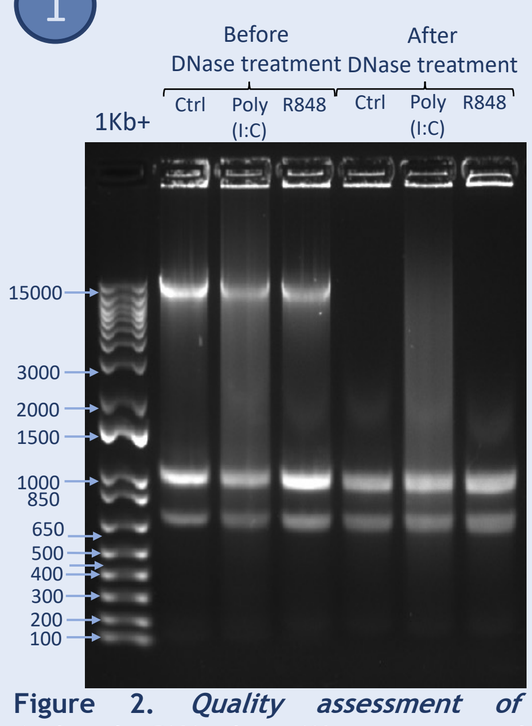

Figure 2. ***Quality assessment of isolated RNA by 1% agarose gel electrophoresis.*** Representative agarose gel image showing total RNA from blood cells stimulated with poly(I:C) or R848, before and after DNase I treatment. Loss of high molecular weight DNA confirms efficient degradation. Similar results were obtained from head kidney samples.

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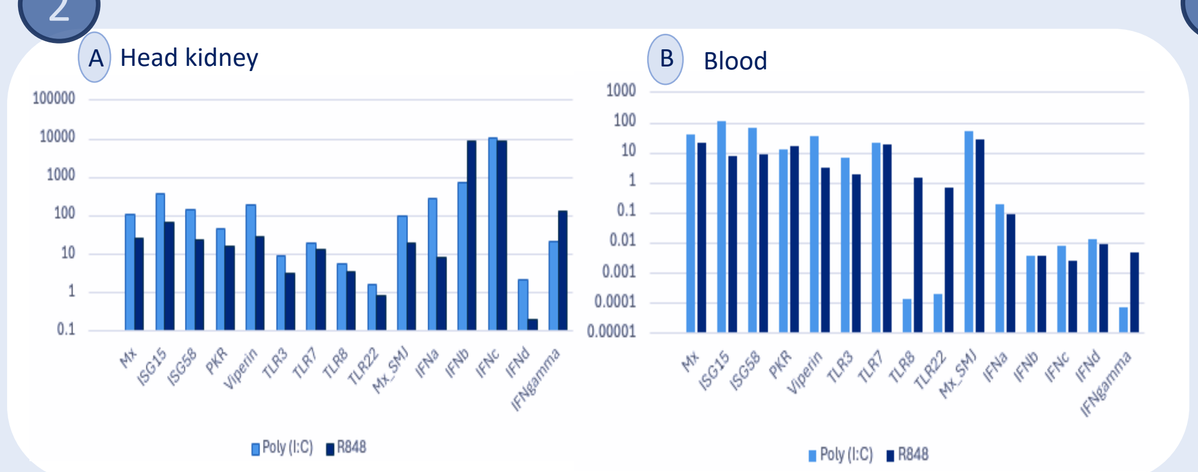

Figure 3. ***Normalized expression of antiviral and immune-related genes in leucocytes isolated from head kidney (A) and blood (B) ,following Poly (I:C) or R848 stimulation.*** Gene expression was quantified by qPCR using RPS20 as a reference gene and is presented on a logarithmic scale.

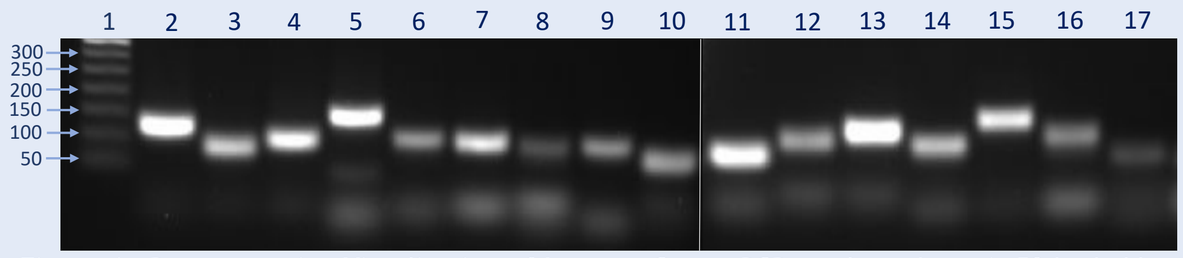

Figure 4. ***Representative Visualization of Immune Gene qPCR products.*** Lane 1: 50 bp ladder. Lanes 2-17 show the reference gene RPS20 (2), immune-related genes Mx (2), ISG15 (4), ISG58 (5), PKR (6), Viperin (7), TLR3 (8), TLR7(9), TLR8 (10), TLR22 (11), Mx_SMJ (12), and interferons IFNa (13), IFNb (14), IFNc (15), IFNd (16), IFNg (17). Amplicon sizes are consistent with expected lengths, confirming specific amplification. This gel is representative of head kidney cDNA stimulated with Poly (I:C); similar results were obtained for blood-derived Poly (I:C) stimulated cDNA.

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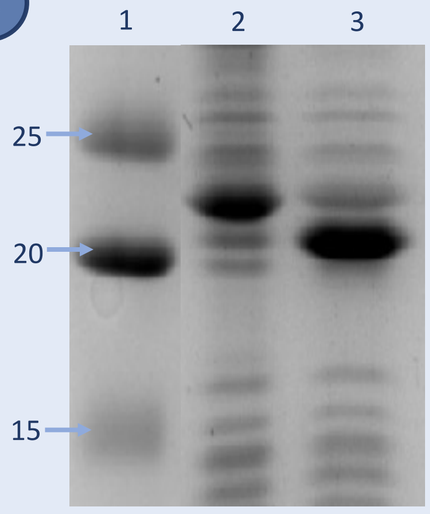

Figure 6. ***SDS-PAGE analysis of recombinant Atlantic salmon type I interferon b expression (IFNb).*** Lane 1: Standard - Precision Plus Protein™ Kaleidoscope molecular weight marker (Bio-Rad). Lane 2: cell extract of non-induced (-IPTG). Lane 3: Cell extract of IPTG-induced. Coomassie staining confirms IPTG-induced expression of His-tagged IFNb at approximately 20 kDa.

1. High quality RNA was extracted from Atlantic salmon head kidney and blood, as confirmed by 1% agarose gel electrophoresis (figure 2).
2. Poly (I:C) stimulation induced higher antiviral gene expression than R848 in both blood and head kidney cDNA, with the highest overall expression in head kidney (figure 3). Specific amplifications was confirmed by gel electrophoresis (figure 4). Selected head kidney Poly (I:C) stimulated cDNA for further research.
3. Multiple sequence alignment showed that the newly obtained IFNs sequences closely match known IFNs, with conserved domains and some isoform-specific variations (figure 5)
4. SDS-PAGE analysis confirmed successful IPTG-induced expression of recombinant Atlantic salmon His-tagged IFNb (~20 kDa)

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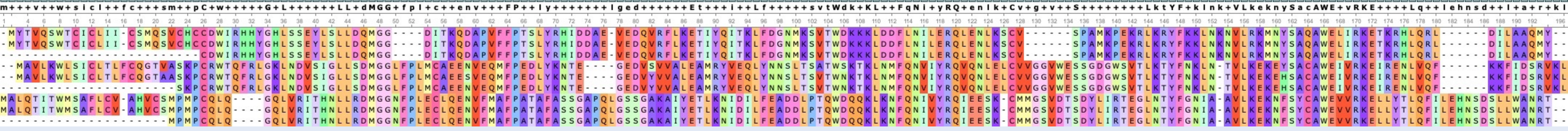

Figure 5. ***Multiple Sequence alignment of Atlantic salmon type I interferons (IFNa, IFNb and IFNc).*** MUSCLE (UGENE) alignment of IFNa1 (Accession no. ACE75690.1), IFNa1.1 (NP_001117182), newly obtained IFNa, IFNb1 (ACE756991.1), IFNb1.2 (NP_001266024), newly obtained IFNb, IFNc1 (ACE756921.1), IFNc1.1 (XP_045570937) and newly obtained IFNc reveals conserved regions characteristic of type I interferons, with colored shading indicating sequence similarity. Conserved domains suggest shared structural and functional features, while sequence variations may relate to differences in antiviral activity among isoforms.

CONCLUSION & FUTURE WORK

In this investigation, type I interferons (IFNs) from Atlantic salmon were effectively cloned, expressed, and characterized. SDS-PAGE analysis confirmed recombinant protein expression at the predicted molecular weights (~20 kDa) and multiple sequence alignment revealed conserved domains indicating that IFN isoforms possess common structural and functional features. Induction of key significant ISGs such as Mx, ISG15, and PKR etc. when primary leukocytes were stimulated with poly(I:C) and R848 further validated the cellular immune response and the feasibility of the experimental approach. Consequently, we aim to utilize purified proteins for dose-response and time-course assays, investigate downstream ISG activation, and express more IFN variants. This research lays the groundwork for employing interferons as potential adjuvants in fish vaccinology, particularly in the development of mRNA vaccines for fish.

REFERENCES

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2. Svingerud T, Solstad T, Sun B, Nyrud ML, Kileng O, Greiner-Tollersrud L, Robertsen B. Atlantic salmon type I IFN subtypes show differences in antiviral activity and cell-dependent expression: evidence for high IFNb/IFNc-producing cells in fish lymphoid tissues. *J Immunol*. 2012;189:5912-23.
3. Linares-Fernández S, Lacroix C, Exposito JY, Verrier B. Tailoring mRNA vaccine to balance innate/adaptive immune response. *Trends Mol Med*. 2020;26(3):311-23.

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