BIO299: Unmasking the Immunology of Lumpfish

Characterization of IL-2 and IL-2Like in Lumpfish (*Cyclopterus lumpus*)

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Introduction:

The main aim of this project is to characterize the two cytokines Interleukin-2 (IL-2) and IL-2like (IL-2L) by using bioinformatical analysis and functional studies. (Fig. 1). Previous studies in lumpfish has mainly focused on innate immunity and B cell responses. T cell responses are hitherto poorly described.

IL-2 plays an important role in regulating immune responses and maintaining self-tolerance, and thus has both immunostimulatory and immune regulatory roles. It controls survival and proliferation of regulatory T-cells and other T cell subsets such as Th1, Th2 and Th17. In teleosts, both IL-2 and IL-2L have been described (ref).

Lumpfish plays a crucial role as a cleaner fish in aquaculture, serving as a key adversary against the salmon louse. A recent study conducted by the Norwegian Food Safety Authority has brought attention to the challenges faced by lumpfish, revealing a significant mortality rate of over 40% among those deployed in Atlantic salmon net-pens in Norway.

Methods:

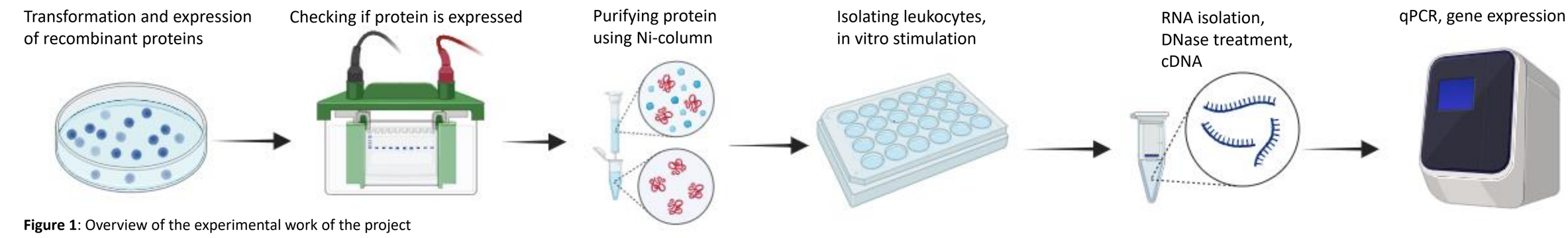


Figure 1: Overview of the experimental work of the project

Results & Discussion:

Both bioinformatic analyses and experimental methods were employed to characterize IL-2 and IL2-L in lumpfish (Cyclopterus lumpus) and to delineate their differences. Multiple sequence alignment (MSA) revealed distinctions in their amino acid sequences (Fig. 2). The protein structures indicated an additional alpha helix in IL-2 (Fig. 3). The phylogenetic tree traced the evolutionary lines of various species, demonstrating that the IL-2 and IL-2 candidates from lumpfish clustered with those from related species (Fig. 3). Protein gels were utilized to confirm the expression and purification of our proteins (Fig. 4). Additionally, to investigate biological function, blood leukocytes were stimulated with recombinant IL-2 and IL-2L (Fig. 5).

MSA:

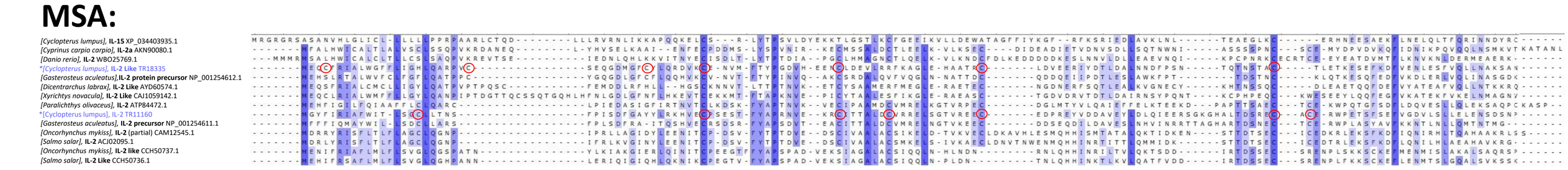


Figure 2: Multi Sequence Alignment containing amino acid chains of lumpfish(*Cyclopterus lumpus*) in purple and other species in black. The more similar the various amino acid chains, the stronger the color. IL-15 from lumpfish(*Cyclopterus lumpus*) is included as an outliner. Red circle shows Cysteine amino acids in IL-2 and IL-2L in lumpfish(*Cyclopterus lumpus*). MSA made in Unipro Ugene program.

Protein structure:

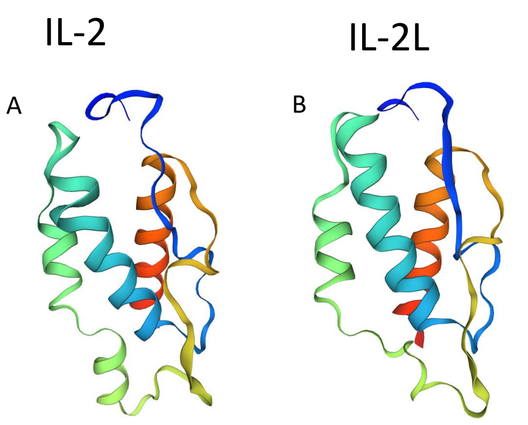


Figure 3: Protein structure of IL-2(A) and IL-2L(B) shows how IL-2 has four alfa helices and IL-2L has three. Template used to make A is 7d9m.1.A , and to make B was 7d9m.1.B.

Phylogenetic tree:

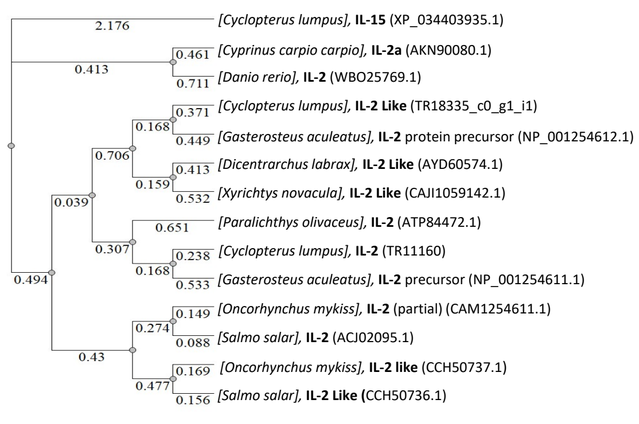


Figure 4: Phylogenetic tree, including IL-2 and IL-2L from Lumpfish (*Cyclopterus lumpus)* and from other species. IL-15 is included as an outliner. The higher number the more precise it is.

qPCR:

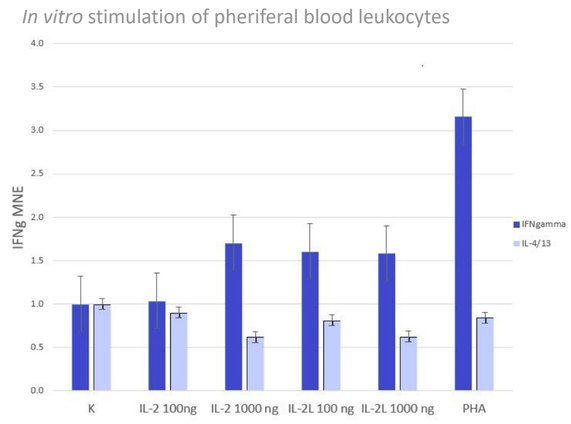
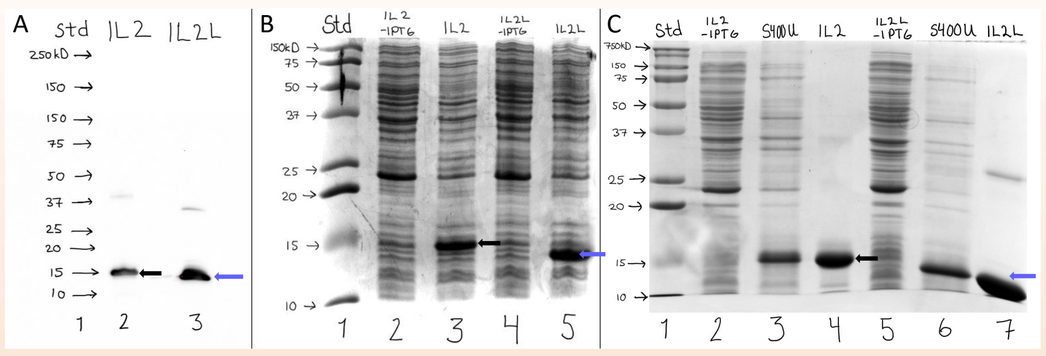


Figure 5:qPCR results. (K) is control gene (RP20). Second column with IL-2 100ng show a small increase in INFγ, third column IL-2 1000ng has a significant increase of INFγ. Same as for fourth and fifth column with IL-2L, which shows a significant increase of INFγ regardless of the concentration. IL-4/13 is decreased in all the columns except in k. PHA Shows a great increase of INFγ and a decrease of il-4/13.

Protein gels:



Figur 6: Western Blott (A), Well 1: Molecular weight standard. Well 2: Suspension of bacteria containing IL2(15,9kD) plasmid and added IPTG. Well 3: Suspension of bacteria containing IL-2L(15,3kD) plasmid and added IPTG. SDS-gel (B) stained with Comassie Blue, well 3: IL-2 plasmid and well 5: IL-2L plasmid. Comassie Blue stained SDS-gel (C) well 4: IL-2 cleansed with Urea. Well seven: IL-2L cleansed with Urea. The arrows indicate our recombinant proteins.

Conclusion:

IL-2 and IL-2L share several key characteristics, notably their ability to upregulate INFγ while reducing IL-4/13 levels. However, distinct differences emerge, such as variances in cysteine placement, potentially influencing protein structure dissimilarities. Notably, IL-2 features an additional alpha helix compared to IL-2L, likely due to these structural discrepancies. Moreover, IL-2 is slightly larger in size compared to IL-2L.

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Reference:

Wang, T., Hu, Y., Wangkahart, E., Liu, F., Wang, A., Zahran, E., ... & Secombes, C. J. (2011). Interleukin (IL)-2 Is a Key Regulator of T Helper 1 and T Helper 2 Cytokine Expression in Fish: Functional Characterization of Two Divergent IL2 Paralogs in Salmonids. Scottish Fish Immunology Research Centre, School of Biological Sciences, University of Aberdeen.