Mol231: Characterizing IL-17A/Fs from lumpfish

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Background

Introduction Interleukin-17 (IL-17) cytokines are involved in inflammation in mammals and fish. Fish possess three homologous genes (IL-17A/F1, 2, and 3), which produce proteins that regulate the immune system and protect against extracellular pathogens. Aquaculture is a fast increasing industry, and deterioration of water environment leads to increased amounts of invading pathogens. Thus, understanding the function and expression of genes involved in immune responses becomes increasingly important (Zhou et al., 2021).

Aim Study and characterize the IL-17A/F1, 2, and 3 cytokines in lumpfish (*Cyclopterus lumpus*) using both experimental methods and bioinformatical analyses.

Materials and Methods

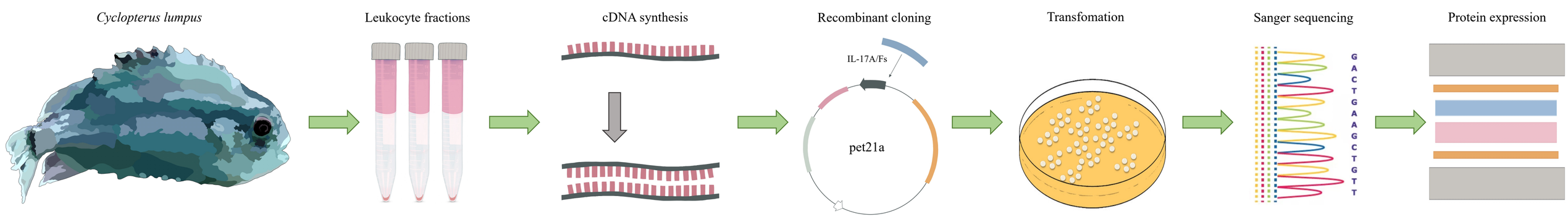

Figure 1. An outline of the main experimental methods employed in the study.

Results

Bioinformatic analysis

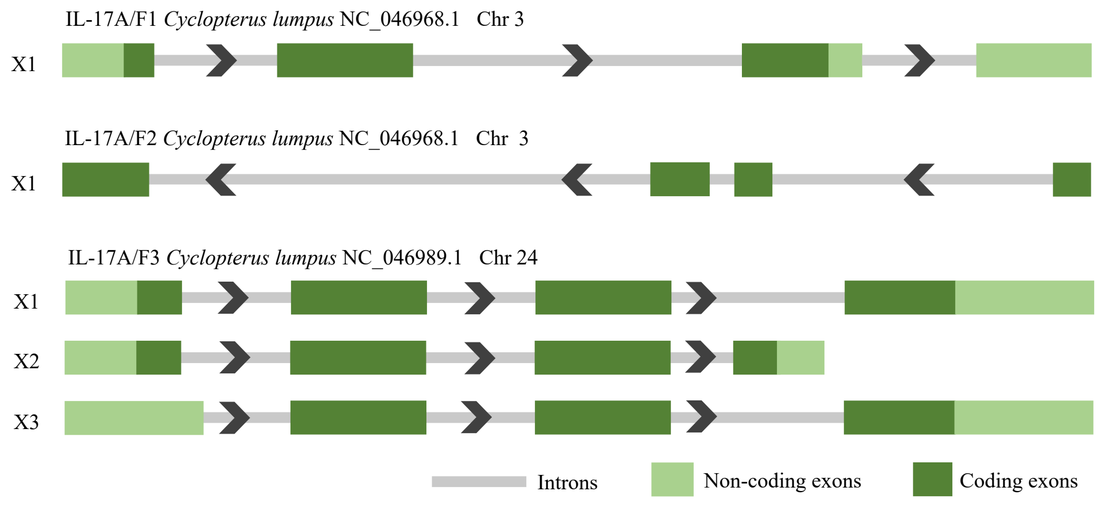

Figure 2. Representation of the IL17A/F1, IL17A/F2 and IL-17A/F3 genes including their introns, non-coding and coding exons. IL-17A/F3 exist in the three isoforms X1, X2 and X3.

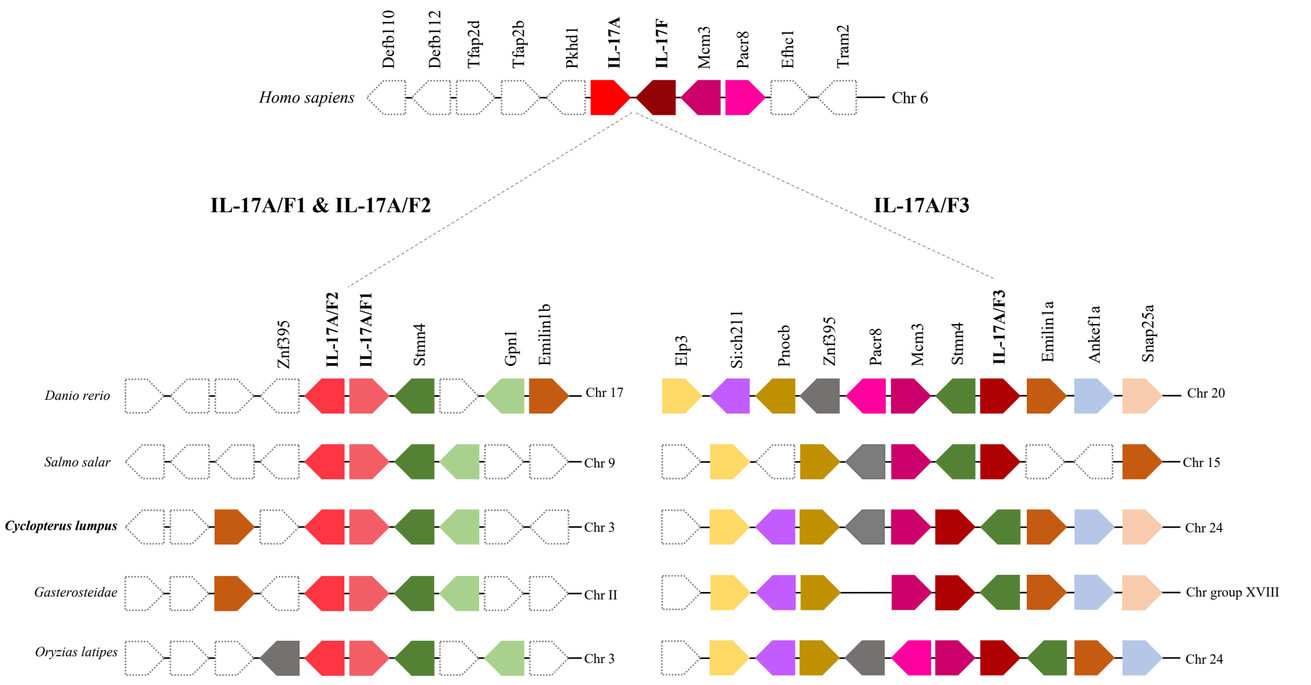

Figure 3. Synteny of IL-17A/F1, IL-17A/F2 and IL-17A/F3 and adjacent genes in human and various fish species. Different colours indicate conservation of the genes across species.

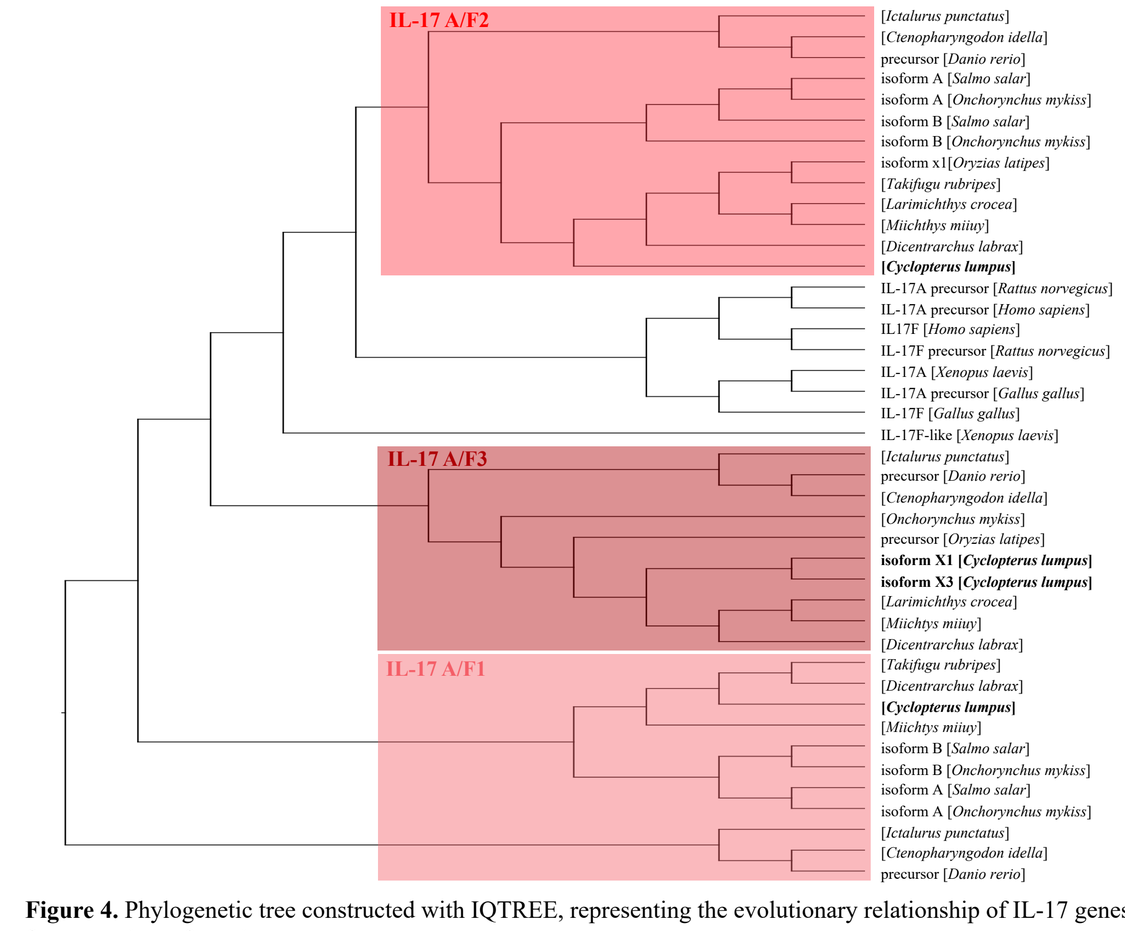

Figure 4. Phylogenetic tree constructed with IQTREE, representing the evolutionary relationship of IL-17 genes from a variety of species.

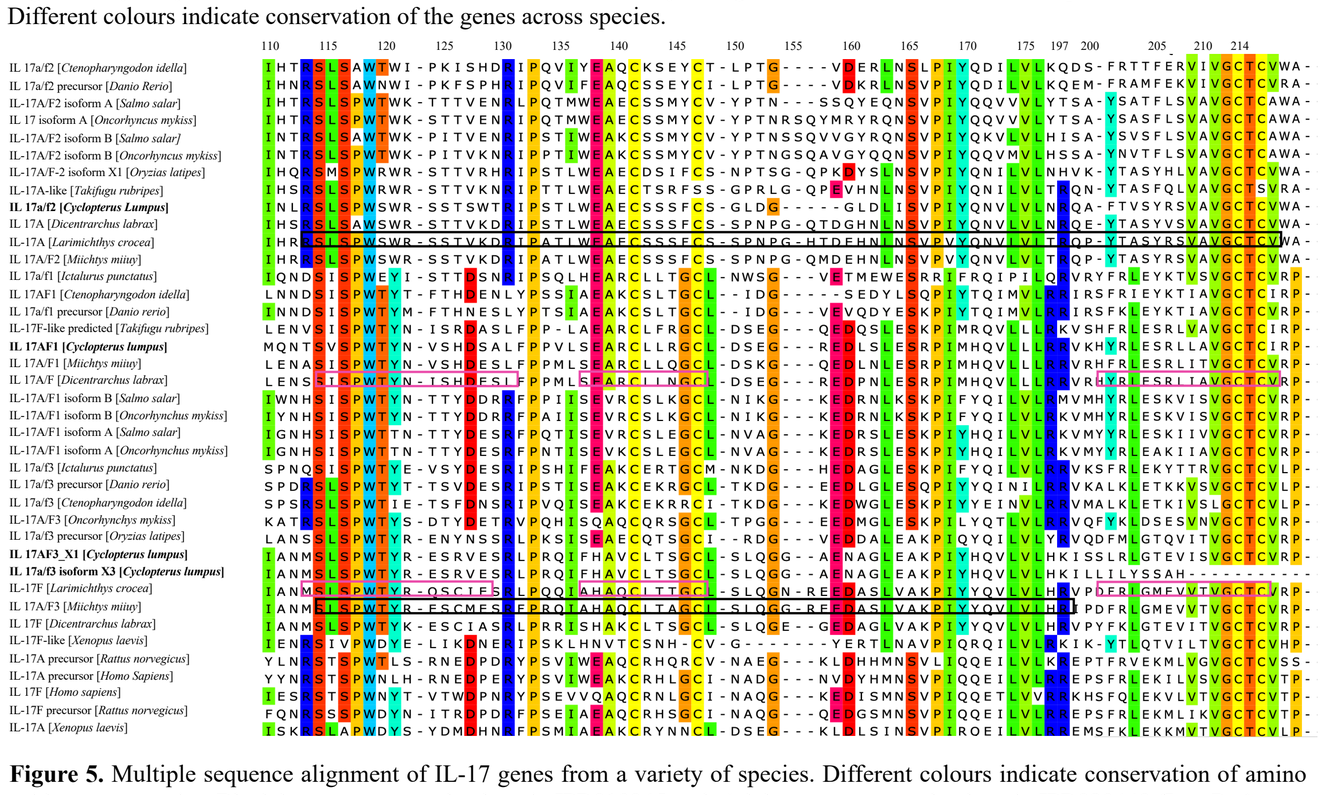

Figure 5. Multiple sequence alignment of IL-17 genes from a variety of species. Different colours indicate conservation of amino acids across species. Black boxes represent the domain IPR010345 and pink boxes represent the domain IPR020440 (InterPro).

Protein expression

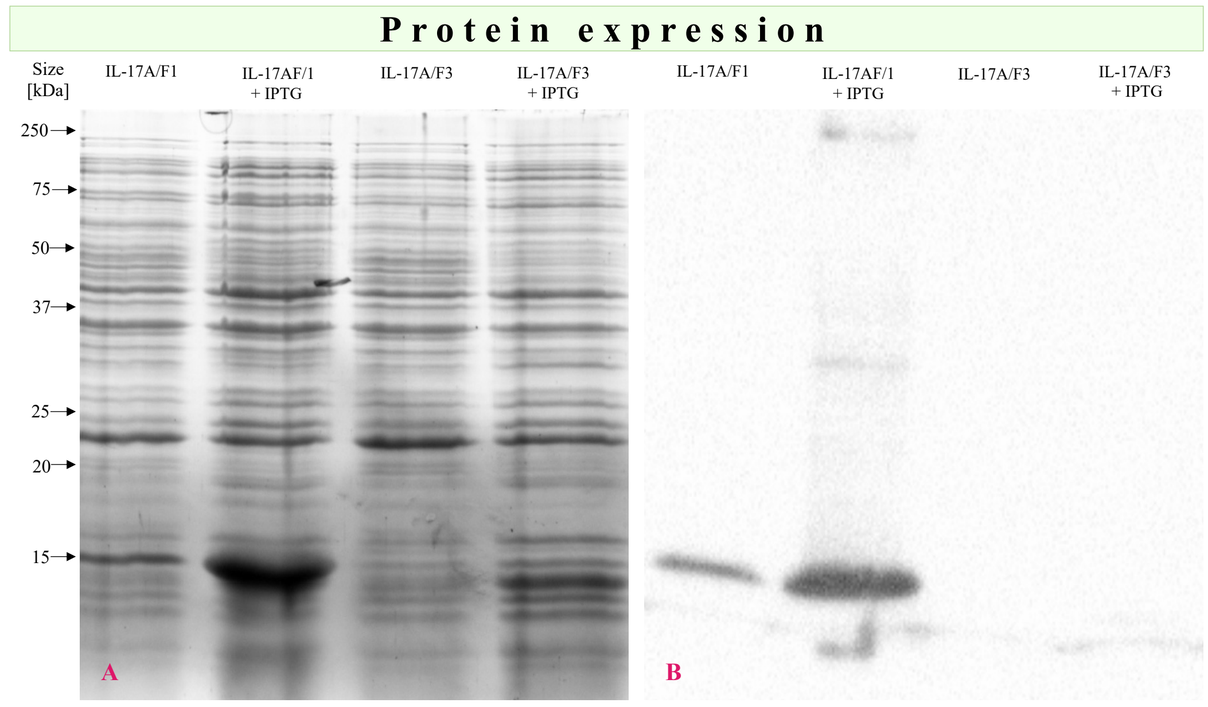

Figure 6. SDS-PAGE (A) and western blot (B) of recombinant IL-17A/F1 and IL-17A/F3_X1 proteins expressed in *E. coli codon+* in the presence and absence of IPTG. The SDS-PAGE revealed protein expression in IL-17A/F1 and IL-17A/F3_X1, while only IL-17A/F1 was expressed in the western blot. Remote expression of IL-17A/F1 in the absence of IPTG may be due to leaky wells.

Results The mRNA expression of IL-17A/Fs in lumpfish leukocytes were studied through experimental methodologies. Recombinant proteins of IL-17A/F1 and IL-17A/F3_X1 were constructed to explore the protein expression (Figure 1).

Bioinformatical analysis was performed for three IL-17A/F genes and their isoforms (Figure 2). Relatively high level of conservation for the genes adjacent to IL-17A/F1, 2 and 3 were observed in synteny analysis (Figure 3) and through phylogenic tree (Figure 4), shedding light on the evolutionary context of various IL-17 genes. Throughout, the signature sequences were found to be identical between IL-17A/F2 and IL-17A/F3_X3, as well as between IL-17A/F1 and IL-17A/F3_X1 (Figure 5).

The protein expression model through SDS-PAGE revealed an expression of both IL-17A/F1 and IL-17A/F3 in the presence of IPTG (15 kDa). Western blot analysis using His-tag revealed a higher expression of IL 17A/F1 in the presence of IPTG (Figure 6).

Conclusion Bioinformatical and experimental methods revealed insights into gene conservation of IL-17A/Fs and their recombinant protein expression, which highlights their importance in immune responses in lumpfish against invading pathogens in aquaculture.

Reference Zhou, X., *et al*. 2021. Expression and Function Analysis of Interleukin-17A/F1, 2, and 3 Genes in Yellow Catfish (*Pelteobagrus fulvidraco*): Distinct Bioactivity of Recombinant IL-17A/F1, 2, and 3. *Frontiers in Immunology,* 12.