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.





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.



IL-17A/F2 nap25a Znf395 Znf39: Danio rerio Salmo salar Cyclopterus lumpus Oryzias latipes

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.

	110	115	120	125	130	135	140	145	150	155	160	65	170	175 19	97 200	205	210 214	4
IL 17a/f2 [Ctenopharyngodon idella]	IHTR	SLS	AWTWI	- P K I S	SHD <mark>R</mark> I	PQVIY	EAQC	K S E Y <mark>C</mark>	T - L P T (<mark>3</mark> V		SLPI	YQDI	LVLK	ods-	FRTTFER	V I V G C	TCVWA-
IL 17a/f2 precursor [Danio Rerio]	IHNR	SLS	AWNWI	- P K F S	SPHRI	POVIF	EAOC	S S E Y <mark>C</mark>	I-LPT	<mark>3</mark> V		SVPI	YODI	ινι κ	OEM-	FRAMFEK	VI <mark>VGC</mark>	TCVRA-
IL-17A/F2 isoform A [Salmo salar]	IHTR	SLS	Ρ <mark>ΨΤ</mark> ΨΚ	- T T T \		. РОТМW	EAEC	S S M Y <mark>C</mark>	V - Y P T I	v s s	QYEQN	SVPI	YQQV	VVLY	TSA-	Y S A T F L S	V A V G C	TCAWA -
IL 17 isoform A [Oncorhyncus mykiss]	IHTR	SLS	р <mark>ит</mark> ик	- S T T \		POTMW	EAEC	S S M Y <mark>C</mark>	V - Y P T I	NRSOYM	RYRON	SVPI	YOOV	VVLY	TSA-	Y S A S F L S		TCAWA -
IL-17A/F2 isoform B [Salmo salar]	INTR	SLS	амтик	- P I T \		PSTIW	EAKC	SSMYC	V - Y P T I	vssovv	GYRON	SVPI	γοκν	LVLH	ISA-	Y S V S F L S		TCAWA -
IL-17A/F2 isoform B [Oncorhyncus mykiss]	INTR	SLS	ритик	- P I T \		P P T I W	ΕΑΚΟ	S S M Y <mark>C</mark>	V - Y P T I	NGSOAV	GYOON	SVPI	YOOV	м <mark>у</mark> цн	SSA-	Y N V T F L S		TCAWA -
IL-17A/F-2 isoform X1 [Orvzias latipes]	IHOR	SMS	PWRWR	- S T T \	/ R H <mark>R</mark> I	PSTLW	EAEC	dsif <mark>c</mark>	S - N P T S	с 5 G - ОРК		SVPI	YONI		нvк-	ΥΤΑΣΥΗΙ	V A V G C	TCVWA-
IL-17A-like [Takifugu rubripes]	IHSR	SLS	PWRWR	- S T T \	/ K N R I	PTTLW		TSRFS	S - G P R I	L G - Q P <mark>E</mark>	VHNLN	SVPI	YONI	LVLT	RQN-	Y T A S F Q L	V A V G C	TSVRA-
IL 17a/f2 [Cyclopterus Lumpus]	INLR	SLS	PWSWR	-ssts	SWT <mark>R</mark> I	PSTLW	EAEC	S S S F <mark>C</mark>	S-GLD	<u> </u>	GLD <mark>L</mark> I	SVPI	YQNV		RQA-	FTVSYRS	V A V G C	TCVWA-
IL 17A [Dicentrarchus labrax]	IHSR	SLS	AWSWR	- S T T \	/ K D <mark>R</mark> I	PSTLW	EAEC	SSSFC	S - S P N I	PG-QTD	GHNLN	SVPI	YONV	LVLN	RQE-	ΥΤΑΣΥΥΣ		TCVWA-
IL-17A [Larimichthys crocea]	IHR	SIS	PWSWR	-SST\	/ K D R I	ΡΑΤΙΨ	E A E C	S S S F <mark>C</mark>	S-SPN	PG-HTD	EHNIN	SVPV	YONV	ΙVΙΤ	ROP-	Y T A S Y R S	VAVGC	TCVWA-
IL 17A/F2 [Miichtys miiuy]	IHRR	SLS	PWSWR	-sst\	/ K D <mark>R</mark> I	PATLW	E A E C	S S S F <mark>C</mark>	S - S P N I	PG-QMD	EHNLN	S V P V	YQNV	LVLT	RQP-	Y T A S Y R S	V A V <mark>G</mark> C	TCVWA-
IL 17a/f1 [Ictalurus punctatus]	IQND	SIS	PWEYI	- s t t <mark>(</mark>	SNRI	PSQLH		LLT <mark>G</mark> C		G V E	тмеже	SRRI	FRQI	ΡΙ Ι Ο	RVRY	FRLEYKT	vsvgc	TCVRP-
IL 17AF1 [Ctenopharyngodon idella]		SIS	PWTYT	- F Т Н <mark>с</mark>	ENLY	PSSIA	<mark>Ε Α Κ </mark>	S L T <mark>G C</mark>	L I D (.	SEDYL	SQPI	Ϋ́́́́́́ТQ́II	MVLR	R I R S	FRIEYKT	I A <mark>V G C</mark>	TCIRP-
IL 17a/f1 precursor [Danio rerio]	INND	SIS	PWTYM	- F T H N	NESLY	[^] P T S I A	ΕΑΚΟ	S L T <mark>G C</mark>	L I D (G V <mark>E</mark>	VQDYE	SKPI	YTQII	MVLR	RIRS	F К <mark>L</mark> Е Ү К Т	I A <mark>V G C</mark>	TCVRP-
IL-17F-like predicted [Takifugu rubripes]	LENV	/ S I S	PWTYN	- I S R	ASLF	PP-LA	E A R C	l f r <mark>g c</mark>	L - D S E	G Q E	DQSLE	SKPI	MRQV	LLLR	кvsн	F R <mark>L</mark> E S R L	V A V G C	TCIRP-
IL 17AF1 [Cyclopterus lumpus]	MQNT	svs	PWTYN	-vsh	SALF	P P V L S	E A R C	L L R <mark>G C</mark>		G R E		S R P I I	иноу	LLLR	RVKH	<mark>Y</mark> R <mark>L</mark> E S R L	LA <mark>VGC</mark>	TCIRP-
IL 17A/F1 [Miichtys miiuy]	LENA	SIS	PWTYN	-vsh	ESLF	P P M L S	E A R C	LLQ <mark>GC</mark>	L - DSK	G Q E		S R P I I	мноv	LLLR	RVRH	F R <mark>L</mark> E S R L	. I T <mark>V G C</mark>	TCVRP-
IL 17A/F [Dicentrarchus labrax]	LENS	5 T S	PWTYN	- ISH	FSLF	P P M L S	F A R C		L - D S E (G R E		S R P I I	иноу	LLLR	r v r 🗄	Y R L E S R L	I A <mark>V G C</mark>	TCVRP-
IL-17A/F1 isoform B [Salmo salar]	IWNH	IS I S	Ρ <mark>ΨΤ</mark> ΥΝ	- T T Y <mark>I</mark>	D R R F	P P I <mark>I</mark> S	EVRC	SLK <mark>GC</mark>	L - N I K	<mark>3</mark> К <mark>Е</mark>		<mark>S Κ Ρ Ι</mark>	FYQI	LVLR	мvмн	Y R L E S K V	' I S <mark>V G C</mark>	TCVRP-
IL-17A/F1 isoform B [Oncorhynchus mykiss]	ΙΥΝΗ	IS I S	PWTYN	- T T Y <mark>I</mark>	D R <mark>R</mark> F	PAIIS	E V R C	SLK <mark>GC</mark>	L-NIK	<mark>3</mark> К <mark>Е</mark>		SKPI	FYQI		к v мн	Y R L E S K V	' I S <mark>V G C</mark>	TCVRP-
IL-17A/F1 isoform A [Salmo salar]	IGNH	IS I S	PWT TN	- T T Y <mark>I</mark>	ESRF	PQTIS	E V R C	S L E <mark>G C</mark>	L - NVA	<mark>3</mark> К <mark>Е</mark>		SKPI	Y H Q I		күмү	Y R <mark>L</mark> E S K I	I V <mark>V G C</mark>	TCVRP-
IL-17A/F1 isoform A [Oncorhynchus mykiss]	I G N H	IS I S	PWTTN	- T T Y <mark>I</mark>	E S R F	PNTIS	ενκς	S L E <mark>G C</mark>	L - NVA	<mark>3</mark> К <mark>Е</mark>		SKPI	ЧНQІ	LVLR	күмү	Y R L E A K I	I A <mark>V G C</mark>	TCVRP-
IL 17a/f3 [Ictalurus punctatus]	SPNQ	SIS	PWTYE	- V S Y <mark>I</mark>	ESRI	PSHIF	E A K C	e r t <mark>g c</mark>	M-NKD	<mark>3</mark> Н <mark>Е</mark>	DAGLE	SKPI	FYQI	LVLR	RVKS	FRLEKYT	TR <mark>VGC</mark>	TCVLP-
IL 17a/f3 precursor [Danio rerio]	SPDR	SLS	Ρ <mark>ΨΤ</mark> ΥΤ	- т s v <mark>г</mark>	ESRI	PSTIS	E A K C	e k r <mark>g c</mark>	<mark>L</mark> - ткр <mark>(</mark>	<mark>3</mark> E <mark>E</mark>		SQPI	YYQI	NILR	RVKA	l k <mark>l</mark> e t k k	vsvgc	TCVLP-
IL 17a/f3 [Ctenopharyngodon idella]	SPSR	SLS	PWTIE	- T S F <mark>I</mark>	NSRI	P V Q I S	ΕΑΚΟ	E K R R <mark>C</mark>	I - TKD	<mark>3</mark> К <mark>Е</mark>		SKPI	YYEI	NVLR	R V MA	l k <mark>l</mark> e t k i	VSLGC	TCVLP-
IL-17A/F3 [Oncorhynchys mykiss]	KAT <mark>R</mark>	SLS	PWTYS	- D T Y <mark>I</mark>	ETRV	PQHIS	QAQC	Q R S <mark>G C</mark>	L - T P G (G E <mark>E</mark>	DMGLE	<mark>S</mark> Κ Ρ Ι	LΥQΤ	LVLR	RVQF	Y K <mark>L</mark> D S E S	V N V G C	TCVRP-
IL 17a/f3 precursor [Oryzias latipes]	LANS	SLS	PWTYR	- ENY		PKSIS	EAEC	Q T S <mark>G C</mark>	I RD(G V <mark>E</mark>	D D A L E	AKPI	QYQI	LVLY	RVQD	F M <mark>L</mark> G T Q V	' I T <mark>V G C</mark>	TCVRP-
IL 17AF3_X1 [Cyclopterus lumpus]	IANM	1 <mark>5 L</mark> S	PWTYR	- ESR\	/ E S <mark>R</mark> L	. PRQIF	ΗΑVC	LTSGC	L-SLQ	G A <mark>E</mark>	NAGLE	AKPI	Y HQV	<mark>с v</mark> с н	KISS	L R <mark>L</mark> G T E V	' I S <mark>V G C</mark>	TCVRP-
IL 17a/f3 isoform X3 [Cyclopterus lumpus]	ΙΑΝΜ	1 <mark>5 L</mark> S	PWTYR	- ESR\	/ E S <mark>R</mark> L	PRQIF	н <mark>а</mark> vс	L T S <mark>G C</mark>	L-SLQ	G A <mark>E</mark>	NAGLE	AKPI	ЧНQV	<mark>с v с</mark> н	KILL	ILYSSAH		
IL-17F [Larimichthys crocea]	IANM	1515	PWTYR	- <u>0 S C I</u>		PQQIA	наос	ітт <mark>с</mark>	L - S L Q	<u> </u>	DASLV	AKPI	YYQV	<mark>с v</mark> с н	R V P 🖸	F R <mark>L</mark> G M F V	V T V <mark>G C</mark>	TCVRP-
IL 17A/F3 [Miichtys miiuy]	IANM	1615	PWTYR	- ESCN	1 E S <mark>R</mark> F	PROIA	HAOC	LTA <mark>GC</mark>	I - S I O	G - R F F	DASLV	AKPI	YOV	<mark>I V</mark> I Н	RIPD	F R <mark>L</mark> G M E V	v T <mark>V G C</mark>	TCVRP-
IL 17F [Dicentrarchus labrax]	IANM	1 <mark>5 L</mark> S	Ρ <mark>ΨΤΥ</mark> Κ	- ESCI	I A S <mark>R</mark> L		накс	L T S <mark>G C</mark>	L-SLQ	GEG <mark>E</mark>	DAGLV	AKPI	YYQV	<mark>с v</mark> с н	<mark>R</mark> V P Y	F Κ <mark>L</mark> G T E V	' I T <mark>V G C</mark>	TCVRP-
IL-17F-like [Xenopus laevis]		SIV	PWDYE	- L I K	NERI	PSKLH	NVTC	S N Н - <mark>С</mark>	V - G	Y	ERTLN	AVPI	QRQI	LVLR	кік-	<mark>Υ</mark> Τ <mark>Ι</mark> QΤVΙ	L T <mark>V G C</mark>	ТСVНР-
IL-17A precursor [Rattus norvegicus]	YLNR	STS	PWTLS	- R N E	P D R Y	[′] P S V I W	EAQC	r h q r <mark>c</mark>	V - N A E (<mark>3</mark> К L	D H H M N	SVLI	QQEI	LVLK	<mark>R</mark> E P T	FRVEKML	vgvgc	TCVSS-
IL-17A precursor [Homo Sapiens]	Y Y N R	STS	P W N L H		P E R Y	[′] P S V I W	EAKC	RHL <mark>G</mark> C	I - NAD	<mark>g</mark> N V	DYHMN	SVPI	QQEI		REPS	FR <mark>L</mark> EKIL	vsvgc	<mark>Т С V</mark> Т <mark>Р</mark> -
IL 17F [Homo sapiens]	IESR	STS	PWNYT	- v т w <mark>г</mark>	P N R Y	' P S E V V	QAQC	R N L <mark>G C</mark>	I - NAQ	<mark>3</mark> К <mark>Е</mark>	DISMN	SVPI	QQET	LVVR	<mark>к</mark> кнѕ	F Q <mark>L</mark> E K V L	V T V G C	ТСVТР-
IL-17F precursor [Rattus norvegicus]	FQNR	s s s	PWDYN	- I T R I		PSEIA	EAQC	R H S <mark>G C</mark>	I - NAO	G Q E	DGSMN	SVPI	QQEI	LVLR	REPS	FRLEKML	ΙΚ <mark>VG</mark> C	ТСVТР-
IL-17A [Xenopus laevis]	ISKR	SLA	PWDYS	- Y D M		PSMIA	EAKC	RYNNC		G K L	DLSIN	SVPI	ROEI		REMS	FKLEKKM	V T V G C	TCVLP-

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).



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.