한국어병학회지 제36권 제1호 (2023)
J. Fish Pathol., 36(1) : 009~019

www.ksfp.org
pISSN 1226-0819, eISSN 2233-5412
http://dx.doi.org/10.7847/jfp.2023.36.1.009

Molecular characterization of interferon regulatory factors (IRFs) 8 of red sea bream (*Pagrus major*) and expression analysis after RSIV infection

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Interferon regulatory factors (IRFs) exhibit antiviral and antibacterial properties. In this study, we cloned the IRF8 genes of red sea bream (*Pagrus major*) and analyzed their expression following red sea bream iridovirus (RSIV) infection. Multi-sequence analysis revealed conserved features, such as the IRF-association domain, nuclear localization signal, and DNA-binding domain. Phylogenetic analysis demonstrated that PmIRF8 clustered with IRF8 from other fish species. Quantitative real-time PCR was employed to examine IRF8 expression in normal tissues, revealing high expression levels in the gill (26.7-fold), spleen (25.7-fold) and brain (22.6-fold). This finding aligns with previous research, which has established that IRF8 is associated with immune cell development and is highly expressed in lymphomyeloid tissues. Additionally, temporal expression of PmIRF8 following RSIV infection demonstrated an initial low expression in the gills, followed by an upregulation at 36 hours post-infection (hpi). A similar expression pattern was observed in the spleen. The liver showed variable expression, while the kidney exhibited an increase at 6 hpi, followed by another upregulation at 3 days post-infection. These results suggest that PmIRF8 plays a role in regulation and suppression during the immune response. These findings are corroborated by previous studies on other fish species. Overall, this investigation presents the characterization of IRF genes in red sea bream and provides foundational data for comparative analysis of IRF genes.

Key words: *Pagrus major*, Red sea bream iridovirus, IRF8, Interferon regulatory factors, IRF

Introduction

The innate and adaptive immune systems have evolved over time to enable vertebrates to combat the invasion of foreign pathogens. These systems encompass organic binding sites and the stimulation of various expression factors, including interferon regulatory factors (IRFs). First identified in humans, IRFs are a family of proteins responsible for regulating the transcriptional and inducible gene expression of interferons (IFNs) (Antonczyk et al., 2019). Since the initial discovery of IRF1 in 1988, the identification of IRFs has expanded to encompass IRF1-11. Studies

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have reported that IRF1-9 are found in mammals, IRF10 in birds and fish, and IRF11 exclusively in fish (Suzuki et al., 2011; Shu et al., 2015; Guan et al., 2020).

The IRF family features an N-terminal DNA-binding domain (DBD), a peptide linker (LK), and an IRF-association domain (IAD), which contains five tryptophan repeats essential for recognizing similar DNA motifs in the highly conserved DBD (Antonczyk et al., 2019). Phylogenetic analysis divides the IRF family into four subfamilies: IRF1-G (IRF1, IRF2); IRF3-G (IRF3, IRF7); IRF4-G (IRF4, IRF8, IRF9, IRF10); and IRF5-G (IRF5, IRF6) (Nehyba et al., 2002). The primary function of the IRF family is to regulate the antiviral response of innate immunity in vertebrates by participating in the transcription of IFN and interferon-stimulated genes (ISG) (Paun & Pitha, 2007). In vertebrates, IRFs detect viral infections by recognizing pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) (Medzhitov & Janeway Jr, 2000). Moreover, IRFs differentiate and regulate dendritic cells (Gabriele & Ozato, 2007), as well as modulate key cytokines and transcription factors involved in T helper cell differentiation (Zhang et al., 2012).

In fish, IRF8 has been identified in species such as rock bream (*Oplegnathus fasciatus*), grass carp (*Ctenopharyngodon idella*), turbot (Scophthalmus maximus), rainbow trout (*Oncorhynchus mykiss*), olive flounder (*Paralichthys olivaceus*), zebrafish (*Danio rerio*), malabar grouper (*Epinephelus malabaricus*), and golden pompano (*Trachinotus ovatus*) (Holland et al., 2010; Li et al., 2011; Bathige et al., 2012; Chen et al., 2012; Hu et al., 2013; Periyasamy & Lu, 2018; Zhu et al., 2019; Chang et al., 2021). Phylogenetic analysis indicates that IRF8 belongs to the IRF-4G subfamily. IRF8, also known as interferon consensus sequence-binding protein (ICSBP), regulates the transcription of IFN-γ (Levi et al., 2002). It exhibits strong binding affinity to the interferon-stimulated response element (ISRE) domain, particularly when heterodimerized with IRF1, IRF2, or IRF4 (Alter-Koltunoff et al., 2008). IRF8 interacts with the ISRE domain and modulates type I IFN expression in dendritic cells. Deletion of IRF1 and IRF8 results in reduced IFN expression, which is crucial for macrophage activity and function in mammals (Langlais et al., 2016).

First identified in Japan in 1990 in red sea bream, red sea bream iridovirus (RSIV) is a linear double-stranded DNA virus that belongs to the family Iridoviridae and the genus Megalocytivirus (Inouye et al., 1992). Annually, RSIV causes significant damage to various fish species, with susceptible populations consistently reported to harbor the virus (Kim et al., 2018; Lopez-Porras et al., 2018; Girisha et al., 2020; Puneeth et al., 2021).

Red sea bream is a fast-growing fish with excellent meat quality, and as a result, it is in high demand. Consequently, it has become a major aquaculture species in Korea, Japan, and China. To date, numerous studies have focused on red sea bream in relation to growth and stocking density (Seo et al., 2020). However, among existing investigations on immunity, very few reports have addressed IRF (Haque et al., 2021; Joo et al., 2022; Kim et al., 2023).

Given that IRFs have been demonstrated to influence infection with viral pathogens, understanding the characteristics and expression of the IRF gene could greatly contribute to research on innate immunity and viral infections. Therefore, this study aimed to examine the molecular characteristics of IRF8 (members of the IRF-4G subfamily) in red sea bream and characterize their expression during RSIV infection. This report presents the first data on IRF8 in relation to innate immunity and viral infection in sea bream.

Materials and methods

Fish and virus

Red sea bream were obtained from a net cage cul-

ture in Tongyeong, Gyeongsangnam-do, South Korea. The fish had an average weight of 52.1 ± 4.6 g and a total length of 12.5 ± 1.6 cm. Upon transportation to the laboratory, the fish were acclimatized with aeration in a flowing water tank at 22 ± 1°C. To confirm the absence of bacterial, parasitic, and viral diseases, five fish were randomly selected and meticulously examined. The external symptoms of the fish were scrutinized using both microscopic and visual inspection methods to ascertain the absence of parasitic infestations. Following this, the spleen and kidney were excised, and tissue samples were applied to brain and heart infusion agar medium to evaluate the presence or lack of bacterial infections. The potential RSIV infection was assessed through DNA extraction from the spleen, followed by PCR utilizing primers recommended by qPCR analysis (Kim et al., 2022b). As no diseases were detected in these tests, the fish were deemed appropriate for subsequent experimentation.

RSIV was isolated from rock bream at a fish farm near Tongyeong in August 2019. Subsequent sequencing analyses confirmed that the virus belonged to the predominant genotype II in Korea (accession number: AY532608) (Kim et al., 2022a). The virus was propagated utilizing L-15 medium (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% antibiotic-antimycotic solution (Gibco, USA). To facilitate viral replication, RSIV was inoculated onto confluent monolayers of the PMF (*Pagrus major* fin) cell line and maintained at 25°C (Kwon et al., 2020). Culture supernatants displaying complete cytopathic effects were collected, followed by centrifugation at 10,000 × g for 10 minutes at 4 °C. The acquired supernatant was employed to ascertain the viral titer, as outlined in a previous study (Kim et al., 2022b). The virus was stored at -80°C for subsequent applications.

Molecular characteristics and phylogenetic analysis

Utilizing RNA extracted from the spleen of red sea bream stimulated with RSIV, the coding sequence (CDS) of PmIRF8 was obtained through next-generation sequencing (NGS) analysis using the RNA-seq method. To test the integrity of the gene sequence obtained through genome sequencing, subcloning was carried out using *Escherichia coli* (*E. coli*) JM109 and the pGEM T-easy vector (Promega, USA). The sequence was validated using Sanger sequencing and then translated into amino acid sequences with the aid of GENETYX software version 8.0 (SDC Software Development, Japan). The National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast) search method was employed to identify relevant sequences, while ClustalX 2.1 and MEGA 6.0 were used for multiple sequence alignment and phylogenetic analysis, respectively. The Expert Protein Analysis System PROSITE Scan program (http://prosite.expasy.org) was utilized to detect specific domains and motifs.

Tissue collection and cDNA synthesis

Tissue samples from five healthy red sea breams were aseptically collected and preserved at -80 °C to investigate the mRNA distribution of PmIRF8. The samples included head kidney, trunk kidney, skin, stomach, gill, heart, liver, spleen, eye, brain, and intestine.

To examine the mRNA expression of red sea bream IRF8 following RSIV infection, Fish were experimentally infected intraperitoneally with 1×10^7 copies/fish of RSIV suspended in PBS. These RSIV-infected red sea breams were employed to assess the temporal expression changes of PmIRF8. Gill, spleen, liver, and trunk kidney samples were aseptically collected from five randomly selected red sea breams at 0 (control), 1, 3, 6, 12, 24, and 36 hours, and 3, 5, and 7 days post-RSIV infection.

All collected samples were processed using the easy-spin™ Total RNA Extraction Kit (iNtRON Biotechnology, South Korea) according to the manu-

facturer's instructions to extract total RNA. Subsequently, cDNA synthesis was performed using the PrimeScript™ 1st strand cDNA Synthesis Kit (Takara, Japan) following the manufacturer's manual. Synthesized cDNA was stored at -80°C until further experimentation.

Reverse transcription–quantitative real–time PCR (RT–qPCR) analysis

To determine the mRNA expression levels for each gene, RT-qPCR was performed using TB Green™ Premix Ex Taq™ (Takara). The sequences and details of the primer sets employed can be found in Table 1. The PCR reactions were carried out using a Thermal Cycler Dice® Real-Time System III (Takara). The $2^{-\Delta\Delta Ct}$ method was applied to quantify the relative mRNA expression, utilizing EF-1α (AY190693.1) as the reference gene (Livak & Schmittgen, 2001). These experiments were conducted in triplicate.

Statistical analysis

The expression of PmIRF8 in RSIV-infected tissues was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test, employing IBM SPSS software version 19 (IBM, USA). The standard deviation (SD) of the experimental results is denoted, and all samples were examined in triplicate.

Results

PmIRF8 Gene Structure and Amino Acid Sequence Characterization

Genome sequencing of PmIRF8 revealed molecular properties, such as mRNA structure and amino acid (aa) sequences (Fig. 1). The predicted molecular weight of PmIRF8 (OK340061.1) was 48 kDa, with a theoretical pI of 5.98. The PmIRF8 sequence spanned 1269 bp (423 aa) and included a DNA-binding domain (2-114 aa) and an IRF-association domain (189-371 aa) (Fig. 1A).

Multiple alignments and phylogenetic analysis of PmIRF8

Multiple alignment analyses of PmIRF8 revealed that the five tryptophan residues and the DBD and IAD domains were highly conserved across all species examined (Fig. 2). Upon analysis, PmIRF8 displayed the highest homology with European seabass (91.71%) and large yellow croaker (90.99%) (Fig. 2). Phylogenetic analysis confirmed that PmIRF8 formed distinct clusters and constituted an outgroup separate

Table 1. Primer sequences and condition were used in this study

Primer name	Primer sequence (5′-3′)	Usage	Condition
RsbIRF8-F	AACACAGGAGGTCGCAGACT	PCR amplification	95°C/5 minutes 95°C/30 seconds 57,59°C/30 seconds 72°C/30 seconds 72°C/5 minutes 4°C/∞ Number of cysles: 35
RsbIRF8-R	ACAGCCCAGGCCTTAAAAAT		
RsbIRF8-F-2	CCAACAAGCTCTGGTGACCT		
RsbIRF8-R-2	CCTGGATGTTACAGCCTTCGT		
qPCR-PmEF-1α-F	ACGTGTCCGTCAAGGAAATC	qPCR amplification	95°C/5 minutes 95°C/30 seconds 60°C/30 seconds 72°C/30 seconds 72°C/5 minutes 4°C/∞ Number of cysles: 35
qPCR-PmEF-1α-R	TGATGACCTGAGCGTTGAAG		
qPCR-PmIRF8-F	TCCGAATTCCTTGGAAACAC		
qPCR-PmIRF8-R	GTCCCCCTCCTTAAACTTGC		

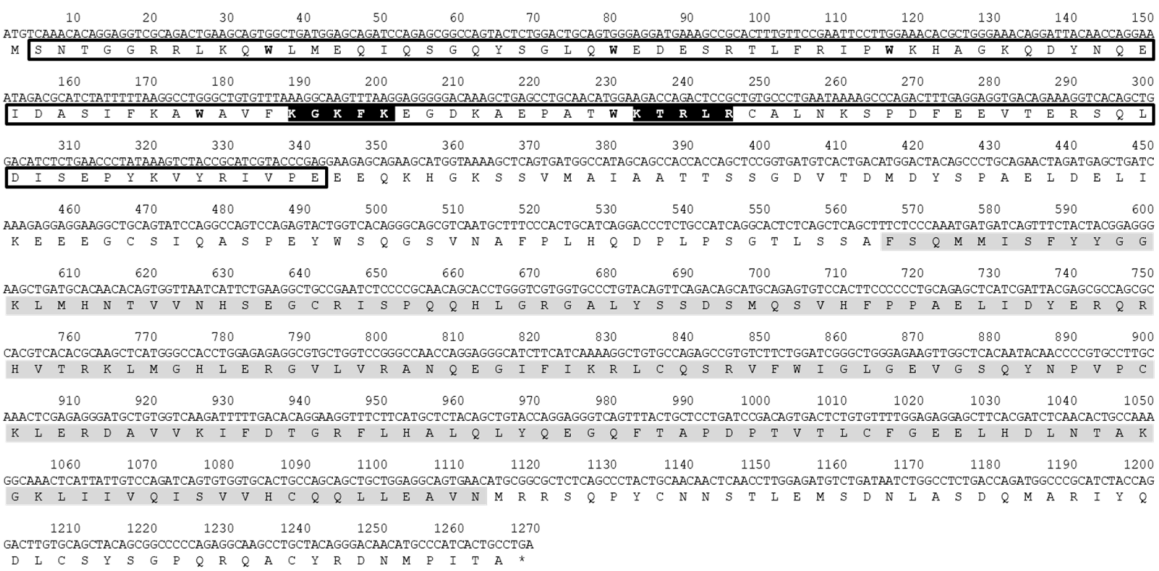

Fig. 1. IRF8 transcript sequences from red sea bream. The nucleotide sequence cDNA of PmIRF8 (GenBank accession: OK340061.1). The nucleotide sequence number is indicated above the sequence. An asterisk (*) indicates the stop codon. Black empty boxes indicate DNA binding domain (DBD), and red letters indicate five tryptophans. White text in black boxes represents nuclear localization signals (NLS), and gray boxes represent the IRF-association domain (IAD).

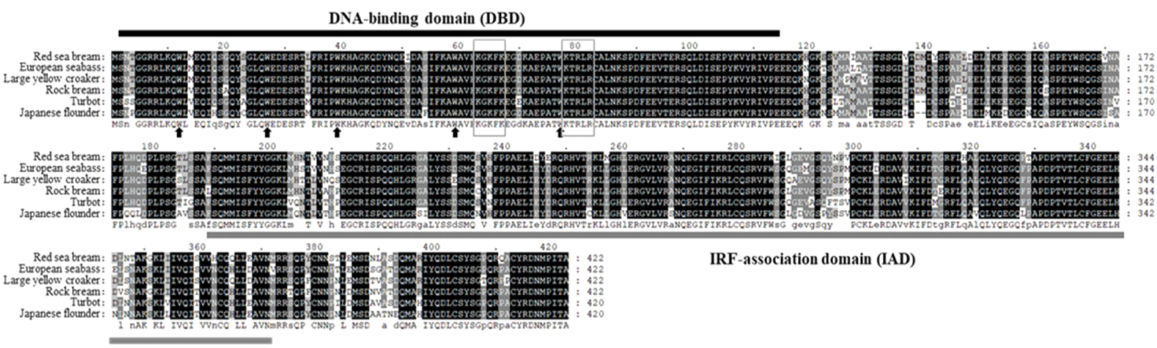

Fig. 2. Multiple sequence alignments are analyzed in red sea bream IRF8 with homologs of other vertebrates. Black lines indicate DNA binding domains and arrows indicate five tryptophans. Gray boxes represent nuclear localization signals, and dark gray lines represent IRF-association domains.

from mammals (Fig. 3).

Analysis of PmIRF8 mRNA expression in healthy red sea bream

The analysis of IRF8 expression in healthy red sea bream revealed that PmIRF8 exhibited elevated expression levels in the gills (26.7-fold), spleen (25.7-fold), and brain (22.6-fold) compared to EF1-a (Fig. 4). Expression in the head kidney was 9.1-fold, while it was 7.9-fold in the intestine. In the liver, the expression was 5.4-fold, 4.2-fold in the eyes, 4.2-fold in the heart, 3.8-fold in the skin, 2.4-fold in the trunk kidney, and 1.2-fold in the stomach.

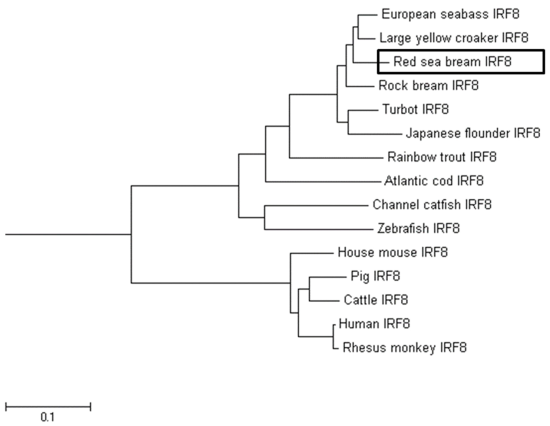

Fig. 3. IRF family proteins from several vertebrate taxa were analyzed phylogenetically. The phylogenetic tree was constructed using the Neighbor-Joining method. Table 2 lists the gene accession numbers of the sequences utilized in the analysis.

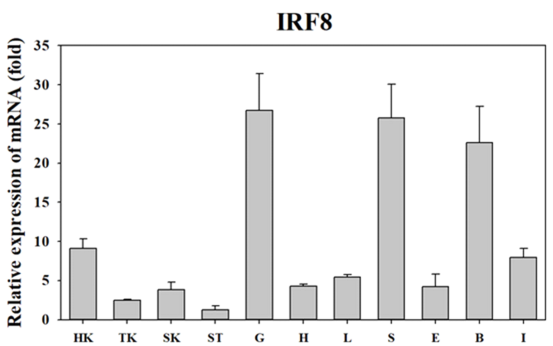

Fig. 4. Expression analysis of PmIRF8 in normal tissue. The relative expression level for each transcript was calculated as fold units after it was first normalized to the expression of a house-keeping gene (EF-1a). HK, TK, SK, ST, G, H, L, S, E, B, and I represent head kidney, trunk kidney, skin, stomach, gill, heart, liver, spleen, eye, brain, intestine. The results represent the mean ± SD of five fish.

PmIRF8 mRNA expression in red sea bream following RSIV infection

Hourly expression analysis of PmIRF8 following RSIV infection revealed that PmIRF8 exhibited significantly lower expression in the gills at 1 hour post-infection (hpi) and significantly higher expression at 36 hpi. An initial increase was observed in the spleen but increased from 36 hpi after downregulation; the expression level was significantly lower at 7 days post-infection (dpi). The expression of PmIRF8 in the liver was variable, with the lowest expression observed at 36 hpi and 7 dpi. In the kidney, the upregulation was significant at 6 hpi, and after downregulation, the highest significant expression was again observed at 3 dpi, followed by downregulation at 7 dpi (Fig. 5).

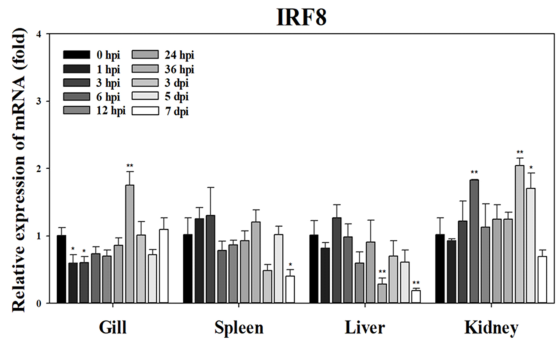

Fig. 5. Expression analysis of PmIRF8 in RSIV infection tissue. The relative expression level for each transcript was calculated as fold units after it was first normalized to the expression of control (0 hpi). The results represent the mean ± SD of five fish. Asterisks (*) above each bar indicate a significant difference from the control group (*, $P \leq 0.05$; **, $P \leq 0.01$).

Discussion

Interferon Regulatory Factors (IRFs), a well-studied class of transcription factors, are noted for their antiviral and antibacterial properties in animals, as they respond to viral infections and modulate apoptosis or the development of immune responses (Savitsky et al., 2010). IRFs bind to the Interferon-Stimulated Response Element (ISRE) as both homodimers and heterodimers via the DNA Binding Domain (DBD), interacting with the ISRE half-site on the opposite side of the DNA in a proximal direction to activate Toll-Like Receptor (TLR) and Interferon (IFN) signaling pathways (Andrilenas et al., 2018). Five tryptophan residues in the DBD bind to the promoters of

Table 2. IRF 8 sequences are used for phylogenetic tree construction

Gene	Species	Scientific name	Protein accession
IRF8	European seabass	*Dicentrarchus labrax*	AKC57349.1
	Large yellow croaker	*Larimichthys crocea*	ATE88517.1
	Rock bream	*Oplegnathus fasciatus*	AFU81292.1
	Turbot	*Scophthalmus maximus*	AFE88897.1
	Japanese flounder	*Paralichthys olivaceus*	AFE18695.2
	Atlantic cod	*Gadus morhua*	AJR33029.1
	Rainbow trout	*Oncorhynchus mykiss*	ALS92677.1
	Channel catfish	*Ictalurus punctatus*	AHH39223.1
	Zebrafish	*Danio rerio*	NP_001002622.1
	Pig	*Sus scrofa*	NP_001239356.1
	Human	*Homo sapiens*	EAW95435.1
	Cattle	*Bos taurus*	NP_001077238.1
	House mouse	*Mus musculus*	NP_001288740.1
	Rhesus monkey	*Macaca mulatta*	NP_001252887.1
	Red sea bream	*Pagrus major*	UIR15471.1

target genes, such as IFN regulatory elements and ISRE. Although extensive research has been conducted in mammals, studies on IRFs in fish are relatively limited, and the variability in gene expression among different fish species is greater than that observed in mammals. Consequently, investigating IRFs in each fish species is vital as it enables a more comprehensive understanding of unique immune mechanisms within different species, offering valuable insights into their antiviral and antibacterial defense strategies.

In this study, we identified the nucleotide sequence of IRF8 in red sea bream, which showed considerable similarity to sequences from other fish species like European seabass, large yellow croaker, rock bream, turbot, and Japanese flounder. Additionally, the DBD, a critical domain of IRF, was found to contain five tryptophan residues. The Nuclear Localization Signal (NLS) sequence and the IRF Association Domain (IAD) were confirmed to be highly conserved, suggesting that the identified sequence belongs to the IRF family.

IRF8 tissue expression is known to be elevated in lymphomyeloid or immune-related tissues (Wang & Morse, 2009). It is predominantly expressed in organs such as the spleen, kidney, and head kidney, as well as in muscles and the brain in certain species (Holland et al., 2010; Bathige et al., 2012). In this study, the highest expression of PmIRF8 was detected in the gills and spleen, primary immunological organs in fish. Previous research has demonstrated that IRF8 is crucial for the formation of B and T cells, is important for the rearrangement of the immunoglobulin light chain gene, and shows elevated expression in lymphomyeloid tissues, such as the gills (Lu, 2008; Hu et al., 2013). We observed increased expression in the spleen, a significant producer of T lymphocytes, and in the gills, a lymphomyeloid tissue. In other fish, IRF8 was found to have high expression in the kidneys, gills, and brain of the golden pompano, as well as in the spleen and skin of the malabar grouper (Periyasamy & Lu, 2018; Zhu et al., 2019). High expression of IRF8 was also detected in the spleen, head kidney, and gills of the mandarin fish, and high expression of IRF8 was observed in the spleen, kidney, and head kidney of turbot, corroborating the findings of this study (Chen et al., 2012; Laghari et al., 2018).

A previous study investigated the role of IRF8 in Japanese encephalitis virus infection and reported in-

creased susceptibility to viral pathogen infection in mice lacking IRF8 (Tripathi et al., 2021). These results suggested that IRF8 plays a pivotal role in modulating immune activity during viral infection (Holtschke et al., 1996). Previous investigations have demonstrated that mutations in IRF8 result in an ineffective antiviral response due to the production of defective type I IFN during the immune process, which is initiated in pDCs (Das et al., 2021). Previous research exploring the antiviral response of IRF8 in fish has shown an upregulation of IRF8 expression in the spleen after turbot were inoculated with poly (I:C) and turbot reddish body iridovirus (TRBIV) (Chen et al., 2012). IRF8 expression was confirmed in the brain, spleen, and kidney following poly (I:C) administration in the malabar grouper (Periyasamy & Lu, 2018). After poly (I:C) administration to grass carp CIK cells, IRF8 expression was significantly higher at the initial 6 hpi and 12 hpi (Chang et al., 2021). In the present study, we compared the upregulation of PmIRF8 following RSIV infection with previous studies. We found that the activity of IRF8 is required in red sea bream, as IRF8 is necessary for the existing antiviral response of fish. However, following the initial infection in the gills, the expression was dramatically reduced, and variable expression was observed in other organs. Literature suggests that IRF8 either represses or activates transcription, depending on the form of the complex (Sharf et al., 1995; Rosenbauer et al., 1999). IRF8 shows suppressed expression in CIK cells when it forms a heterodimer with IRF2 (Chang et al., 2021). While it is challenging to deduce the exact reason for downregulation from the findings of this study, previous research suggests that IRF8 forms a heterodimer with other IRFs in the antiviral immune response and is thought to induce inhibition and activation in the process of regulating the expression of type I IFN (Chang et al., 2021). IRF8 modulates the immune response by decreasing the expression of the Myd88 and NF-kB signaling pathways, which could explain the decreased expression (Yan et al., 2020).

Conclusion

We have verified the molecular characteristics and expression analysis of IRF8 in red sea bream. Although further investigation is required to ascertain their precise functions, both PmIRF8 appear to exhibit distinct roles in the antiviral immune response, consistent with observations of IRF8 in previous studies. Consequently, this research provides a foundational dataset for understanding IRF characteristics in red sea bream and expands upon existing reports in teleost fish. Moreover, PmIRF8 are crucial immune genes in red sea bream, and additional immune-related studies are warranted to thoroughly elucidate their gene functions.

Acknowledgments

This research was supported by the National Institute of Fisheries Science, Ministry of Oceans and Fisheries, Korea (R2023024)

Reference

Alter-Koltunoff, M., Goren, S., Nousbeck, J., Feng, C. G., Sher, A., Ozato, K., Azriel, A., & Levi, B.-Z. Innate immunity to intraphagosomal pathogens is mediated by interferon regulatory factor 8 (IRF-8) that stimulates the expression of macrophage-specific Nramp1 through antagonizing repression by c-Myc. *Journal of Biological Chemistry*, *283*(5), 2724-2733. https://doi.org/10.1074/jbc.M707704200. 2008.

Andrilenas, K. K., Ramlall, V., Kurland, J., Leung, B., Harbaugh, A. G., & Siggers, T. DNA-binding landscape of IRF3, IRF5 and IRF7 dimers: implications for dimer-specific gene regulation. *Nucleic acids research*, *46*(5), 2509-2520. https://doi.org/10.1093/nar/gky002 2018.

Antonczyk, A., Krist, B., Sajek, M., Michalska, A., Piaszyk-Borychowska, A., Plens-Galaska, M., Wesoly, J., & Bluyssen, H. A. R. Direct Inhibition of IRF-Dependent Transcriptional Regulatory Mechanisms

Associated With Disease. *Front Immunol*, *10*, 1176. https://doi.org/10.3389/fimmu.2019.01176 2019.

Bathige, S., Whang, I., Umasuthan, N., Lim, B.-S., Park, M.-A., Kim, E., Park, H.-C., & Lee, J. Interferon regulatory factors 4 and 8 in rock bream, Oplegnathus fasciatus: structural and expressional evidence for their antimicrobial role in teleosts. *Fish & Shellfish Immunology*, *33*(4), 857-871. 2012.

Chang, K., Han, K., Qiu, W., Hu, Z., Chen, X., Chen, X., Xie, X., Wang, S., Hu, C., & Mao, H. Grass carp (Ctenopharyngodon idella) interferon regulatory factor 8 down-regulates interferon1 expression via interaction with interferon regulatory factor 2 in vitro. *Molecular Immunology*, *137*, 202-211. https://www.sciencedirect.com/science/article/pii/S0161589021001371?via%3Dihub 2021.

Chen, X., Hu, G., Dong, X., Liu, Q., & Zhang, S. Molecular cloning and expression analysis of interferon regulatory factor 8 (IRF8) in turbot, Scophthalmus maximus. *Veterinary immunology and immunopathology*, *149*(1-2), 143-150. 2012.

Das, A., Chauhan, K. S., Kumar, H., & Tailor, P. Mutation in Irf8 Gene (Irf8R294C) Impairs Type I IFN-Mediated Antiviral Immune Response by Murine pDCs [Original Research]. *Frontiers in immunology*, *12*. https://doi.org/10.3389/fimmu.2021.758190 2021.

Gabriele, L., & Ozato, K. The role of the interferon regulatory factor (IRF) family in dendritic cell development and function. *Cytokine & growth factor reviews*, *18*(5-6), 503-510. 2007.

Girisha, S., Puneeth, T., Nithin, M., Kumar, B. N., Ajay, S., Vinay, T., Suresh, T., Venugopal, M., & Ramesh, K. Red sea bream iridovirus disease (RSIVD) outbreak in Asian seabass (Lates calcarifer) cultured in open estuarine cages along the west coast of India: First report. *Aquaculture*, *520*, 734712. https://doi.org/10.1016/j.aquaculture.2019.734712 2020.

Guan, Y., Chen, X., Luo, T., Ao, J., Ai, C., & Chen, X. Molecular characterization of the interferon regulatory factor (IRF) family and functional analysis of IRF11 in the large yellow croaker (Larimichthys crocea). *Fish & Shellfish Immunology*, *107*, 218-229. https://doi.org/10.1016/j.fsi.2020.10.001. 2020.

Haque, M. N., Nam, S.-E., Shin, Y. K., & Rhee, J.-S. The dinoflagellate Alexandrium affine acutely induces significant modulations on innate immunity, hepatic function, and antioxidant defense system in the gill and liver tissues of red seabream. *Aquatic Toxicology*, *240*, 105985. https://doi.org/10.1016/j.aquatox.2021.105985 2021.

Holland, J. W., Karim, A., Wang, T., Alnabulsi, A., Scott, J., Collet, B., Mughal, M. S., Secombes, C. J., & Bird, S. Molecular cloning and characterization of interferon regulatory factors 4 and 8 (IRF-4 and IRF-8) in rainbow trout, Oncorhynchus mykiss. *Fish & Shellfish Immunology*, *29*(1), 157-166. 2010.

Holtschke, T., Löhler, J., Kanno, Y., Fehr, T., Giese, N., Rosenbauer, F., Lou, J., Knobeloch, K.-P., Gabriele, L., & Waring, J. F. Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. *Cell*, *87*(2), 307-317. 1996.

Hu, G., Chen, X., Gong, Q., Liu, Q., Zhang, S., & Dong, X. Structural and expression studies of interferon regulatory factor 8 in Japanese flounder, Paralichthys olivaceus. *Fish & Shellfish Immunology*, *35*(3), 1016-1024. 2013.

Inouye, K., Yamano, K., Maeno, Y., Nakajima, K., Matsuoka, M., Wada, Y., & Sorimachi, M. Iridovirus infection of cultured red sea bream, Pagrus major. *Fish Pathology*, *27*(1), 19-27. 1992.

Joo, M.-S., Choi, K.-M., Kang, G., Woo, W.-S., Kim, K.-H., Sohn, M.-Y., Son, H.-J., Lee, J.-H., & Park, C.-I. Molecular characteristics, expression analysis and antiviral activity against RSIV of red sea bream IRF3 and IRF7. *Aquaculture Reports*, *27*, 101415. https://doi.org/10.1016/j.aqrep.2022.101415 2022.

Kim, K.-H., Choi, K.-M., Joo, M.-S., Kang, G., Woo, W.-S., Sohn, M.-Y., Son, H.-J., Kwon, M.-G., Kim, J.-O., & Kim, D.-H. Red Sea Bream Iridovirus (RSIV) Kinetics in Rock Bream (Oplegnathus fasciatus) at Various Fish-Rearing Seawater Temperatures. *Animals*, *12*(15), 1978. 2022a.

Kim, K.-H., Choi, K.-M., Kang, G., Woo, W.-S., Sohn, M.-Y., Son, H.-J., Yun, D., Kim, D.-H., & Park, C.-I. Development and Validation of a Quantitative Polymerase Chain Reaction Assay for the Detection of Red Sea Bream Iridovirus. *Fishes*, *7*(5), 236. 2022b.

Kim, K.-H., Joo, M.-S., Kang, G., Woo, W.-S., Sohn, M.-Y., Son, H.-J., & Park, C.-I. Characterization of Red Sea Bream (Pagrus major) Interferon Regulatory Factor 5 and 6 Genes and Their Expression in Response to RSIV Infection. *Fishes*, *8*(2), 114. https://doi.org/10.3390/fishes8020114 2023.

Kim, K., Hwang, S., Cho, M., Jung, S., Kim, Y., & Jeong, H. A natural infection by the red sea bream iridovirus-type Megalocytivirus in the golden mandarin fish Siniperca scherzeri. *Journal of fish diseases*, *41*(8), 1229-1233. https://doi.org/10.1111/jfd.12815

2018.

Kwon, W. J., Yoon, M. J., Jin, J. W., Kim, K. I., Kim, Y. C., Hong, S., Jeong, J. B., & Do Jeong, H. Development and characterization of megalocytivirus persistently-infected cell cultures for high yield of virus. *Tissue and Cell*, *66*, 101387. 2020.

Laghari, Z. A., Li, L., Chen, S. N., Huo, H. J., Huang, B., Zhou, Y., & Nie, P. Composition and transcription of all interferon regulatory factors (IRFs), IRF1–11 in a perciform fish, the mandarin fish, Siniperca chuatsi. *Developmental & Comparative Immunology*, *81*, 127-140. 2018.

Langlais, D., Barreiro, L. B., & Gros, P. The macrophage IRF8/IRF1 regulome is required for protection against infections and is associated with chronic inflammation. *Journal of Experimental Medicine*, *213*(4), 585-603. 2016.

Levi, B.-Z., Hashmueli, S., Gleit-Kielmanowicz, M., Azriel, A., & Meraro, D. ICSBP/IRF-8 transactivation: a tale of protein-protein interaction. *Journal of interferon & cytokine research*, *22*(1), 153-160. 2002.

Li, L., Jin, H., Xu, J., Shi, Y., & Wen, Z. Irf8 regulates macrophage versus neutrophil fate during zebrafish primitive myelopoiesis. *Blood, The Journal of the American Society of Hematology*, *117*(4), 1359-1369. 2011.

Livak, K. J., & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods*, *25*(4), 402-408. 2001.

Lopez-Porras, A., Morales, J. A., Alvarado, G., Koda, S. A., Camus, A., Subramaniam, K., Waltzek, T. B., & Soto, E. Red seabream iridovirus associated with cultured Florida pompano Trachinotus carolinus mortality in Central America. *Diseases of aquatic organisms*, *130*(2), 109-115. https://doi.org/10.3354/dao03267. 2018.

Lu, R. Interferon regulatory factor 4 and 8 in B-cell development. *Trends Immunol*, *29*(10), 487-492. https://doi.org/10.1016/j.it.2008.07.006 2008.

Medzhitov, R., & Janeway Jr, C. The Toll receptor family and microbial recognition. *Trends in microbiology*, *8*(10), 452-456. 2000.

Nehyba, J., Hrdlička, R., Burnside, J., & Bose Jr, H. R. A novel interferon regulatory factor (IRF), IRF-10, has a unique role in immune defense and is induced by the v-Rel oncoprotein. *Molecular and cellular biology*, *22*(11), 3942-3957. 2002.

Paun, A., & Pitha, P. The IRF family, revisited. *Biochimie*, *89*(6-7), 744-753. 2007.

Periyasamy, T., & Lu, M.-W. Molecular characterization and expression of malabar grouper (Epinephelus malabaricus) IRF8 gene response to immuno-stimulants and nervous necrosis virus. *pathways*, *8*, 16. 2018.

Puneeth, T., Baliga, P., Girisha, S., Shekar, M., Nithin, M., Suresh, T., & Kumar, B. N. Complete genome analysis of a red seabream iridovirus (RSIV) isolated from Asian seabass (Lates calcarifer) in India. *Virus research*, *291*, 198199. https://doi.org/10.1016/j.virusres.2020.198199 2021.

Rosenbauer, F., Waring, J. F., Foerster, J., Wietstruk, M., Philipp, D., & Horak, I. Interferon consensus sequence binding protein and interferon regulatory factor-4/Pip form a complex that represses the expression of the interferon-stimulated gene-15 in macrophages. *Blood, The Journal of the American Society of Hematology*, *94*(12), 4274-4281. 1999.

Savitsky, D., Tamura, T., Yanai, H., & Taniguchi, T. Regulation of immunity and oncogenesis by the IRF transcription factor family. *Cancer immunology, immunotherapy*, *59*(4), 489-510. https://doi.org/10.1007/s00262-009-0804-6. 2010.

Seo, J. S., Haque, M. N., Nam, S.-E., Kim, B.-M., & Rhee, J.-S. Inorganic nitrogen compounds reduce immunity and induce oxidative stress in red seabream. *Fish & Shellfish Immunology*, *104*, 237-244. https://doi.org/10.1016/j.fsi.2020.05.072 2020.

Sharf, R., Azriel, A., Lejbkowicz, F., Winograd, S. S., Ehrlich, R., & Levi, B.-Z. Functional Domain Analysis of Interferon Consensus Sequence Binding Protein (ICSBP) and Its Association with Interferon Regulatory Factors∗. *Journal of Biological Chemistry*, *270*(22), 13063-13069. 1995.

Shu, C., Sun, Y., & Xu, T. Molecular characterization of three IRF1 subfamily members reveals evolutionary significance of IRF11 in miiuy croaker. *Developmental & Comparative Immunology*, *53*(2), 385-391. https://doi.org/10.1016/j.dci.2015.07.009. 2015.

Suzuki, Y., Yasuike, M., Kondo, H., Aoki, T., & Hirono, I. Molecular cloning and expression analysis of interferon regulatory factor 10 (IRF10) in Japanese flounder, Paralichthys olivaceus. *Fish & Shellfish Immunology*, *30*(1), 67-76. https://doi.org/10.1016/j.fsi.2010.09.010. 2011.

Tripathi, A., Singh Rawat, B., Addya, S., Surjit, M., Tailor, P., Vrati, S., & Banerjee, A. Lack of Interferon (IFN) Regulatory Factor 8 Associated with Restricted IFN-γ Response Augmented Japanese Encephalitis Virus Replication in the Mouse Brain. *J Virol*, *95*

(21), e0040621. https://doi.org/10.1128/jvi.00406-21 2021.

Wang, H., & Morse, H. C., 3rd. IRF8 regulates myeloid and B lymphoid lineage diversification. *Immunologic research*, *43*(1-3), 109-117. https://doi.org/10.1007/s12026-008-8055-8 2009.

Yan, X., Zhao, X., Huo, R., & Xu, T. IRF3 and IRF8 regulate NF-κB signaling by targeting MyD88 in teleost fish. *Frontiers in immunology*, *11*, 606. 2020.

Zhang, R., Chen, K., Peng, L., & Xiong, H. Regulation of T helper cell differentiation by interferon regulatory factor family members. *Immunologic research*, *54*(1), 169-176. https://doi.org/10.1007/s12026-012-8328-0. 2012.

Zhu, K.-C., Guo, H.-Y., Zhang, N., Liu, B.-S., Guo, L., Jiang, S.-G., & Zhang, D.-C. Functional characterization of IRF8 regulation of type II IFN in golden pompano (Trachinotus ovatus). *Fish & Shellfish Immunology*, *94*, 1-9. 2019.

Manuscript Received : Apr 27, 2023
Revised : May 09, 2023
Accepted : May 11, 2023