Growth hormone secretagogue peptide A233 upregulates Mx expression in teleost fish in vitro and in vivo

Rebeca Martinez1 · María Alejandra Fernández-Trujillo2 · Liz Hernández1 · Adrian Page2 · Julia Béjar2 · Mario Pablo Estrada1

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Abstract
Aquaculture constitutes an alternative source for food production and contributes to a reduction in the indiscriminate catching of aquatic organisms in their natural environment [2, 11]. However, high mortality during the larval state remains a challenge in this sector, mainly because of factors such as diet and diseases caused by pathogens [21, 41]. Therefore, growth and health management is a key strategy for sustainable aquaculture [31].

Synthetic growth hormone secretagogues (GHSs) are a family of ligands, initially termed “GH-releasing peptides” (GHRPs) [35]. These synthetic compounds were developed to release GH in vitro [3]. Ghrelin is the endogenous ligand of a previously orphan GHS receptor (GHS-R) and is a potent stimulator of pituitary GH release. Since its discovery, it has become increasingly clear that ghrelin plays an integral role not only in the neuroendocrine control of GH release but also in the regulation of feeding, energy metabolism, cardiovascular performance, and immune responses in a variety of vertebrates, including fish. The A233 decapeptide is a GHS with a demonstrated impact on growth, immune system function, and antioxidant defense in tilapia fish, but no antiviral activity has been described for this peptide. Here, using an in vitro model (TRG-2 cells) and two in vivo models (sea bream [Sparus aurata]) and zebrafish [Danio rerio]), we demonstrate for the first time the potential antiviral effect of A233 in teleost fish.

Aquaculture constitutes an alternative source for food production and contributes to a reduction in the indiscriminate catching of aquatic organisms in their natural environment [2, 11]. However, high mortality during the larval state remains a challenge in this sector, mainly because of factors such as diet and diseases caused by pathogens [21, 41]. Therefore, growth and health management is a key strategy for sustainable aquaculture [31].

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Synthetic GHS can modulate the functions described for ghrelin as well [20, 23]. On the other hand, GHS-R is expressed predominantly in the brain and pituitary gland, but it is also expressed in other peripheral tissues, such as gut, gills, spleen and head kidney [22]. This receptor and the growth hormone receptor (GHR) are expressed in cells of the immune system of mammals and fish, which suggests a relationship between ligands for this receptors and the immune system [20].

The fish immune system is composed of physical and chemical barriers as well as humoral and cellular factors that prevent the proliferation of pathogens. The interferon (IFN) system is essential as part of the first line of defense against animal viruses [44]. IFN I induces the transcription of hundreds of genes, some of which encode direct antiviral effectors, such as the Mx proteins [15]. Mx proteins are dynamin-like GTPases that are involved in basic cellular processes involving membrane remodeling [9]. They can
interfere with viral replication at different life stages of the virus by forming oligomers in which the GTPase activity is essential for viral inhibition [9, 26]. It has been shown that these proteins have antiviral specificity in several fish species [7, 28].

The A233 decapeptide is a GHS. Studies have demonstrated an impact of treatment with this peptide on growth, immune system function, and antioxidant defense in tilapia fish [34]. A233 peptide treatment also increases INF-γ levels in mouse splenocytes and decreased the viral load in mice challenged with dengue virus [32]. However, to our knowledge there have been no previous reports describing any GHSs with antiviral activity in fish.

The aim of the present study was to evaluate the ability of A233 to stimulate antiviral activity in vitro in the cell line RTG-2, as well as in vivo in sea bream (Sparus aurata) and zebrafish (Danio rerio). Our results demonstrate for the first time the potential antiviral effect of A233 on teleost fish.

The effect on the antiviral activity of A233 was first investigated using the cell line RTG-2 (a fibroblast cell line derived from gonad tissue of rainbow trout (Oncorhynchus mykiss)). Cells were incubated with A233 (1 nM) and/or poly(I:C) (10 µg/mL) for 0, 24, 48, or 72 hours. Expression of the Mx3 gene was measured by qPCR and normalized to that of the EF-1α gene. We chose the Mx3 gene because it has been reported previously that Mx3 is induced more strongly in RTG2 cells than the Mx1 and Mx2 genes [42]. A significant upregulation of the Mx3 gene was observed 24 hours after treatment with A233 mixed with poly(I:C) (Fig. 1A). Separately, poly(I:C) activated transcription of the Mx3 gene compared to the control, but when it was combined with A233 peptide, the level of stimulation was higher than with either treatment alone.

Based on these first results, an additional test was performed in which cells were incubated for 24 hours with different amounts of A233 (1, 10 and 100 nM) and/or poly(I:C). The antagonist peptide [D-Lys3]-GHRP6 (100nM) (Sigma) for GHS-R was included as a negative control. Once again, Mx3 was upregulated in cells incubated with both A233 (1 nM) and poly(I:C) in comparison with the response detected with the control + poly(I:C). Stimulation of cells with the antagonist peptide alone did not affect Mx3 expression (Fig. 1B).

The antiviral effect of this peptide in fish was then tested in vivo using gilthead sea bream (S. aurata). Fish were handled according to the European Union guidelines for the handling of laboratory animals (Directive 2010/63/UE). Optimized conditions of temperature (18 °C), light (8-h light and 16-h darkness), oxygen (6 ± 0.5 ppm), salinity (37%), and feeding were used to minimize stress. Fingerlings (n = 10) of 20 g were injected once intraperitoneally with 1 µg of A233 per gram of body weight in a total volume of 100 µL. The control group received the same volume of PBS. Samples from head kidney were collected at 24, 48, and 72 hours after the injection for total RNA extraction. Expression of the Mx2 [19] and IgM genes was measured by qPCR. Our results showed that Mx2 was upregulated 24 hours after injection with A233, and IgM was upregulated 72 hours after injection (Fig. 2).

The ability of A233 to modulate gene expression was then also tested in the intestine of zebrafish (D. rerio). Zebrafish of the wild-type AB lineage were maintained under standard conditions in the aquarium of the University of Málaga as described previously [13], and experiments were performed at 28.5°C. Juvenile zebrafish (n = 6) weighing 0.3 g were injected intraperitoneally daily for 3 days with 1 µg of A233 per gram of body weight in a total volume of 10 µL. The control group received the same volume of PBS. Samples from the intestine were collected 24 hours after the last injection for total RNA extraction, and expression of the MxAB, MxC, IFNγ, and IL-1β genes was measured by qPCR. After 24 hours, MxAB was upregulated, but MxC, IFN-γ, and IL-1-β were not significantly upregulated (Fig. 3).

In the present work, combined treatment of RTG-2 cells with the GHS A233 and poly(I:C) resulted in higher levels of expression of the Mx3 gene than in control and poly(I:C)-treated cells at 24 hours after treatment. RTG-2 cells are a validated experimental system for the functional characterization of virus–host interactions [18, 42]. The results suggest that an immune stimulatory effect against dsRNA viruses can be achieved in the presence of this secretagogue. However, treatment with A233 alone did not upregulate the transcription of this antiviral gene. Similar results were reported by Picchietti et al. [38], who showed that treatment of SAF-1 cells with aloe extract alone did not stimulate the transcription of Mx but had an effect in the presence of poly(I:C) and aloe. Kim et al. [25] and Falco et al. [14] also reported that after the administration of β-glucans in the diet of grass carp, another stimulus, such as virus or poly(I:C), was necessary to enhance the transcription of Mx. Thus, further studies should be conducted to explore the molecular mechanisms involved in the potentiating effect of A233 on poly(I:C)-activated cells. In the presence of an antagonist of the GHS-R, Mx3 expression was downregulated both with and without poly(I:C). This result agrees with that of Martinez et al. [32], who showed that the addition of this molecule in a mouse splenocyte cell culture decreased the secretion of IFN-γ compared to GHRP-6, another GHS. Previously, Martinez et al. [33] reported the action of another GHS as a candidate molecular adjuvant in mice and fish in the presence of different antigens. Considering this, future studies should be conducted in order to evaluate the possible actions of this secretagogue as a molecular adjuvant to enhance the effects of antigens in vaccines. The search for novel adjuvants that are safe and elicit the desired immune
Upregulation of Mx expression by A233

response in fish remains an active research area in this field (Dalmo et al., 2016; Wangkahart et al., 2019).

In the presence of the antagonist of the GHS-R, there was no upregulation of the Mx3 gene, which suggests that the effect of this GHS on the antiviral response is specific and mediated by the GHS-R. The expression of Mx2 in fingerlings of sea bream at 24 hours was upregulated by treatment with A233. Previously, it was demonstrated that this gene might play a major role in both the innate and adaptive immune responses in this species, being expressed in the head kidney, a lymph myeloid tissue [17]. In addition, it showed activity against DNA and RNA viruses [16]. Other authors have reported the effects of other immunostimulants (i.e., β-glucans or LPS) on the survival of RNA-virus-infected fish, where Mx expression also resulted in upregulation [4, 46]. Treatment with A233 also upregulated the expression of IgM after 72 h. This molecule is an indicator of specific immune responses and provides systemic
protection in fish [36]. Other authors have reported the effect of other molecules involved in the neuro-endocrine-immune regulation on antibody levels. In tilapia, Lugo et al. [29] reported that treatment with pituitary adenylate cyclase-activating polypeptide (PACAP) stimulated the secretion of IgM in serum. IgM levels have been shown to correlate with better disease outcome in infections. Caruffo et al. [5] demonstrated that an oral vaccine against infectious salmon anemia based on recombinant antigens modulated Mx expression levels in head kidney and stimulated IgM levels in serum, and this correlated with higher survival rates in a challenge trial. Similarly, in grass carp vaccinated against grass carp reovirus, Mx and IgM were upregulated in head kidney, while the survival rate was about 80% after challenge [48].

Further studies should be conducted to evaluate the effect of A233 treatment in challenge trials. The results obtained in the present work with IgM stimulation are in agreement with previous studies conducted in tilapia, where the growth hormone secretagogue peptide-6 stimulated IgM levels in serum when administrated with different antigens [33].

The intestine is a tissue that is continuously exposed to a complex community of microbiota and environmental pathogens. Thus, gut-associated lymphoid tissue plays essential roles in maintaining mucosal homeostasis in the digestive tract [39, 40]. In the present work, treatment with A233 upregulated MxAB in the intestine of zebrafish. The importance of this gene in the intestinal immune response against viral infections has been shown in previous studies. For example, Wang et al. [45] reported that, in rainbow trout, distinct Mx isoforms were upregulated after challenge with poly(I:C). Dong et al. [12] showed the importance of this gene in the digestive tract for the antiviral state developed after a bath infection with infectious hematopoietic necrosis virus (IHNV). Other studies have shown upregulation of Mx after treatment with immunostimulants in teleost. For example, Falco et al. [14] reported an increase in Mx expression levels in gut after feeding with β-glucans. Therefore, the results obtained in this work are of interest because different viruses can infect fish through the intestine, causing necrosis of epithelial cells, hemorrhage, or enteritis [8, 27, 30].

Previous in vitro studies with A233 have shown stimulation of the production of MAVS proteins in a murine macrophage cell line [32]. Meanwhile, in vitro, it stimulated the secretion of IFN-γ in mouse splenocyte cell culture, and in vivo, it decreased the viral load in DENV-challenged mice. These in vitro and in vivo effects were specifically mediated.
through the GHS-R [32, 33]. Other authors have reported the importance of MAVS proteins as adaptors in the IFN-mediated antiviral pathway, which leads to the transcription of different ISGs, such as Mx [6, 47, 49]. In fish, it has been reported that IFN-γ can stimulate the transcription of Mx proteins [19, 37, 43]. The results obtained in the present study show that, in the presence of A233, Mx expression was stimulated in RTG2-cells as well as in the head kidney of sea bream and in the intestine of zebrafish, which suggests the induction of an antiviral state. Further studies should be conducted to evaluate the effect of A233 on the infection status of fish after viral challenge, as well as its potential use as an adjuvant in vaccines. The number of different receptors that can be activated by this molecule is larger in in vivo experiments. In non-mammalian vertebrates, there are multiple isoforms of the ghrelin receptor due to genome duplication and polyploidization events, particularly in the Teleostei.

The fastest emerging global food industry currently is aquaculture. However, one of the main challenges in this sector is the high mortality of farmed fish in commercial aquaculture systems. This leads to economic losses due to outbreaks of infectious diseases, such as those caused by viruses [10]. Thus, the study of new molecules that are able to stimulate antiviral activity is important for the development of preventive measures against viral infections in this sector [1].

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Declarations

Conflict of interest The authors have not disclosed any competing interests.

References

1. Alvarez-Torres D, Garcia-Rosado E, Fernandez-Trujillo MA, Bejar J, Alvarez MC, Borrego JJ, Alonso MC (2013) Antiviral specificity of the Solea senegalensis Mx protein constitutively expressed in CHSE-214 cells. Mar Biotechnol 15:125–132. https://doi.org/10.1007/s10126-012-9478-8
2. Beveridge MCM, Thilsted SH, Phillips MJ, Metian M, Troell M, Hall SJ (2013) Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture. J Fish Biol 83(4):1067–1084. https://doi.org/10.1111/jfb.12187
3. Bowers CY, Chang J, Momany F, y F. K. (1977) Effect of the enkephalins and enkephalin analogs on release of pituitary hormones in vitro. Mol Endocrinol 287–292
4. Bravo J, Real F, Padilla D, Olveira JG, Grasso V, Román L, Acosta F (2013) Effect of lipopolysaccharides from Vibrio alginolyticus on the Mx gene expression and virus recovery from gilthead sea bream (Sparus aurata L.) experimentally infected with Nodavirus. Fish Shellfish Immunol 34(1):383–386. https://doi.org/10.1016/j.fsi.2012.10.012
5. Carufo M, Maturana C, Kambalapally S, Larenas J, Tobar JA (2016) Fish & Shellfish Immunology Protective oral vaccination against infectious salmon anaemia virus in Salmo salar. Fish Shellfish Immunol 54:54–59. https://doi.org/10.1016/j.fsi.2016.03.009
6. Chen WQ, Hu YW, Zou PF, Ren SS, Nie P, Chang MX (2015) MAVS splicing variants contribute to the induction of interferon and interferon-stimulated genes mediated by RIG-I-like receptors. Dev Comp Immunol 49(1):19–30. https://doi.org/10.1016/j.dci.2014.10.017
7. Chen YM, Su YL, Shie PS, Huang SL, Yang HL, Chen TY (2008) Grouper Mx confers resistance to nodavirus and interacts with coat protein. Dev Comp Immunol 32:825–836
8. Crane M, Hyatt A (2011) Viruses of fish: an overview of significant pathogens. Viruses 3(11):2025–2046. https://doi.org/10.3390/v3112025
9. Das BK, Roy P, Rout AK, Sahoo DR, Panda SP, Pattanaik S, Dehury B, Behera BK (2019) Molecular cloning, GTP recognition mechanism and tissue-specific expression profiling of myxovirus resistance (Mx) protein in Labeo rohita (Hamilton) after Poly I:C induction. Sci Rep. December 2018, 1–19. https://doi.org/10.1038/s41598-019-40323-0
10. Dadar M, Dhamra K, Vakharia VN, Hoseinifar SH, Karthik K, Tiwari R, Khandia R, Munjal A, Salgado-Miranda C, Joshi SK (2016) Advances in aquaculture vaccines against fish pathogens: global status and current trends. Rev Fish Sci Aquicult 25(3):184–217. https://doi.org/10.1080/23308239.2016.1261277
11. Diaz NF, Neira R (2005) Biotecnología aplicada a la acuicultura. Cien Inc Agr 32(1):45–49
12. Dong S, Ding LG, Cao JF, Liu X, Xu HY, Meng KF, Yu YY, Wang Q, Xu Z (2019) Viral-infected change of the digestive tract microbiota associated with mucosal immunity in teleost fish. Front Immunol 10(December):1–13. https://doi.org/10.3389/fimmu.2019.02878
13. Durán I, Mari-Befia M, Santamaria JA, Becerra J, Santos-Ruiz L (2011) Actinotrichia collagens and their role in fin formation. Dev Biol 354(1):160–172. https://doi.org/10.1016/j.ydbio.2011.03.014
14. Falco A, Miest JJ, Pionnier N, Pietretti D, Forlenza M, Wiegertjes GF, Hoole D (2014) β-Glucan-supplemented diets increase poly(I:C)-induced gene expression of Mx, possibly via Tlr3-mediated recognition mechanism in common carp (Cyprinus carpio). Fish Shellfish Immunol 36(2):494–502. https://doi.org/10.1016/j.fsi.2013.12.005
15. Fernández-Trujillo MA, García-Rosado E, Alonso MC, Álvarez MC, Béjar J (2015) Synergistic effects in the antiviral activity of the three Mx proteins from gilthead sea bream (Sparus aurata). Vet Immunol Immunopathol 168(1–2):83–90. https://doi.org/10.1016/j.vetimm.2015.08.007
16. Fernández-Trujillo MA, García-Rosado E, Alonso MC, Castro D, Álvarez MC, Béjar J (2013) Mx1, Mx2 and Mx3 proteins from the gilthead sea bream (Sparus aurata) show in vitro antiviral activity against RNA and DNA viruses. Mol Immunol 56(4):630–636. https://doi.org/10.1016/j.molimm.2013.06.018
17. Fernández-Trujillo MA, Novel P, Manchado M, Sepulcre MP, Mulero V, Borrego JJ, Álvarez MC, Béjar J (2011) Three Mx genes with differential response to VNNV infection have been identified in Gilthead sea bream (Sparus aurata). Mol Immunol 48(9–10):1216–1223. https://doi.org/10.1016/j.molimm.2011.03.008
18. González-Mariscal JA, Fernández-Trujillo MA, Alonso MC, García-Rosado E, Álvarez MC, Béjar J (2016) Gilthead sea bream (Sparus aurata) Mx gene promoters respond differentially to... Springer
IPNV and VHSV infections in RTG-2 cells. Vet Immunol Immunopathol 171:73–80. https://doi.org/10.1016/j.vetimm.2016.02.006

19. González-Mariscal JA, Gallardo-Gálvez JB, Méndez T, Alvarez MC, Béjar J (2014) Cloning and characterization of the Mx1, Mx2 and Mx3 promoters from gillhead sea bream (Sparus aurata). Fish Shellfish Immunol 38(2):311–317

20. Hattori N (2009) Expression, regulation and biological actions of growth hormone (GH) and ghrelin in the immune system. In: Growth Hormone and IGF Research (Vol. 19, Issue 3, pp. 187–197). Elsevier Ltd. https://doi.org/10.1016/j.ghir.2008.12.001

21. Helvik J, Hamre K, Hordvik I, van der Meeren T, Ressem H, Scharlt M, Tveiten H and Øie G (2009) The fish larvae: a transitional life form, the foundation for aquaculture and fisheries. Res Council Norway, 5, 7, 9, 13

22. Kiyah H, Kangawa K, Miyazato M, F(2013) Ghrelin receptors in non-mammalian vertebrates. Front Endocrinol 4(JUL):1–16. https://doi.org/10.3389/fendo.2013.00081

23. Kiyah H, Miyazato M, Kangawa K (2011) Recent advances in the phylogenetic study of ghrelin. Peptides 32(11):2155–2174. https://doi.org/10.1016/j.peptides.2011.04.027

24. Kaiya H, Miyazato M, Kangawa K, Peter RE, Unniappan S (2008) Ghrelin: a multifunctional hormone in non-mammalian vertebrates. Compr Biochem Physiol 149(2):109–128. https://doi.org/10.1016/j.cbpa.2007.12.004

25. Kim Y-S, Ke F, Zhang QY (2009) Effect of β-glucan on activity of antioxidant enzymes and Mx gene expression in virus infected grass carp. Fish Shellfish Immunol 27(2):336–340. https://doi.org/10.1016/j.fsi.2009.06.006

26. Kochs G, Reichelt M, Danino D, Hinshaw JE, Haller O (2005) As assay and functional analysis of dynamin-like Mx proteins. Methods Enzymol 404:632–643

27. Kopper G, Mirecki S, Kljujev IS, Raicevic VB, Lalevic BT, Kochs G, Reichelt M, Danino D, Hinshaw JE, Haller O (2005) Hygiene in primary production. Food Safety Manage. https://doi.org/10.1016/S2090-2180(04)00023-8

28. Lin CH, Christopher John JA, Lin CH, Chang CY (2006) Inhibition of nervous necrosis virus propagation by fish Mx proteins. Biochem Biophys Res Comm 351(2):534–539. https://doi.org/10.1016/j.bbrc.2006.10.063

29. Luco JM, Oliva A, Morales A, Reyes O, Garay HE, Herrera F, Cabrales A, Pérez E, Estrada MP (2010) The biological role of pituitary adenylyl cyclase-activating polypeptide (PACAP) in growth and feeding behavior in juvenile fish. J Pept Sci 16(11):633–643. https://doi.org/10.1002/jpsc.1275

30. Martin SAM, Dehler CE, Król E (2016) Transcriptomic responses in the fish intestine. Dev Comp Immunol 64:103–117. https://doi.org/10.1016/j.devimm.2016.03.014

31. Martínez R, Carpio Y, Morales A, Lugo JM, Herrera F, Zaldívar Nuez A, Rodriguez R, Reyes O, Oliva A, Estrada MP (2012) A novel GH secretagogue, A233, exhibits enhanced growth activity and innate immune system stimulation in teleosts fish. J Endocrinol 214(3):409–419. https://doi.org/10.1530/JEO-11-0373

32. Martínez R, Uieta K, Herrera F, Forellat A, Morales R, De la Nuez A, Rodríguez R, Reyes O, Oliva A, Estrada MP (2012) A novel GH secretagogue, A233, exhibits enhanced growth activity and innate immune system stimulation in teleosts fish. J Endocrinol 214(3):409–419. https://doi.org/10.1530/JEO-11-0373

33. Martínez R, Hernández L, Gil L, Carpio Y, Morales A, Herrera F, Rodríguez-Mallón A, Leal Y, Blanco A, Estrada MP (2017) Growth hormone releasing peptide-6 enhanced antibody titers against subunit antigens in mice (BALB/c), tilapia (Oreochromis niloticicus) and African catfish (Clarias gariepinus). Vaccine 35(42):5722–5728. https://doi.org/10.1016/j.vaccine.2017.07.060

34. Martínez R, Ubieta K, Herrera F, Forellat A, Morales R, De la Nuez A, Rodríguez R, Reyes O, Oliva A, Estrada MP (2012) A novel GH secretagogue, A233, exhibits enhanced growth activity and innate immune system stimulation in teleosts fish. J Endocrinol 214(3):409–419. https://doi.org/10.1530/JEO-11-0373

35. Momany F, Bowers CY, Reynolds GA, Chang D, Hong A, Newlander K (1981) Design, synthesis, and biological activity of peptides which release growth hormone in vitro. Endocrinology 108(1):31–39. https://doi.org/10.1210/endo-108-1-31

36. Munang’andu HM, Eversen Ø (2019) Correlates of protective immunity for fish vaccines. Fish Shellfish Immunol 85:132–140. https://doi.org/10.1016/j.fsi.2018.03.060

37. Peng W, Lu D-Q, Li G-F, Zhang X, Yao M, Zhang Y, Lin H-R (2016) Two distinct interferon-g genes in Tetraodon nigroviridis : functional analysis during Vibrio parahaemolyticus infection. Mol Immunol 70:34–46. https://doi.org/10.1016/j.molimm.2015.12.004

38. Piccietti S, Bernini C, Belardinelli MC, Ovidi E, Taddei AR, Guerra L, Abelli L, Fausto AM (2013) Immune modulatory effects of Aloe arborescens extract on the piscine SAF-1 cell line. Fish Shellfish Immunol 34(5):1–10. https://doi.org/10.1016/j.fsi.2013.02.019

39. Rombout JHWM, Abelli L, Piccietti S, Scaglioni G, Kiron V (2011) Teleost intestinal immunology. Fish Shellfish Immunol 31(5):616–626. https://doi.org/10.1016/j.fsi.2010.09.001

40. Salinas I (2015) The mucosal immune system of teleost fish. Biolog y 4(3):525–539. https://doi.org/10.3390/biology4030525

41. Stentiford GD, Sritunyalucksana K, Flegel TW, Williams BAP, Withychumman K, Itsathiphaitsam O, Bass D (2017) New paradigms to help solve the global aquaculture disease crisis. Pathogens 13(2):1–6. https://doi.org/10.1371/journal.ppat.1006160

42. Tafalla C, Chico V, Perez L, Coll JM, Estepa A (2007) In vitro and in vivo differential expression of rainbow trout (Oncorhynchus mykiss) Mx isoforms in response to viral haemorrhagic septicemia virus (VHSV) G gene, poly I: C and VHSV. Fish Shellfish Immunol 23(1):210–221

43. Velázquez J, Acosta J, Herrera N, Morales A, González O, Herrera F, Estrada MP, Carpio Y (2017) Novel IFNγ homologue identified in Nile tilapia (Oreochromis niloticus) links with immune response in gills under different stimuli. Fish Shellfish Immunol 71(October):275–285. https://doi.org/10.1016/j.fsi.2017.10.014

44. Verhelst J, Hulpiau P, Saelens X (2013) Mx proteins: antiviral gatekeepers that restrain the uninvited. Microbiol Mol Biol Rev 77(4):551–566. https://doi.org/10.1128/MMBR.00024-13

45. Wang T, Liu F, Tian G, Sebembs CJ, Wang T (2019) Lineage/ species-specific expansion of the Mx gene family in teleosts: differential expression and modulation of nine Mx genes in rainbow trout Oncorhynchus mykiss. Fish Shellfish Immunol 90(Feb uary):413–430. https://doi.org/10.1016/j.fsi.2019.04.303

46. Wang W, Sun J, Liu C, Xue Z (2016) Application of immunostimulants in aquaculture: current knowledge and future perspectives. Aquac Res 48(1):1–23. https://doi.org/10.1111/are.13161

47. Wu XM, Zhang J, Li PW, Hu YW, Cao L, Ouyang S, Bi YH, Nie P, Chang MX (2020) NOD1 promotes antiviral signaling by binding viral RNA and regulating the interaction of MDA5 and MAVS. J Immunol 204(8):2216–2231. https://doi.org/10.4049/jimmunol.1900667

48. Zeng W, Wang Q, Wang Y, Zhao C, Li Y, Shi C, Wu S, Song X, Huang Q, Li S (2016) Immunogenicity of a cell culture-derived inactivated vaccine against a common virulent isolate of grass
carp reovirus. Fish Shellfish Immunol 54:473–480. https://doi.org/10.1016/j.fsi.2016.04.133

49. Zou PF, Chang MX, Xue NN, Liu XQ, Li JH, Fu JP, Chen SN, Nie P (2014) Melanoma differentiation-associated gene 5 in zebrafish provoking higher interferon-promoter activity through signalling enhancing of its shorter splicing variant. Immunology 141(2):192–202. https://doi.org/10.1111/imm.12179

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