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RESEARCH

The immunoadjuvant effects of interleukin-8 on *Aeromonas veronii* inactivated vaccine in largemouth bass (*Micropterus salmoides*)

Shun Yang[1,2] · Qian-Rong Liang[3] · Dao-Le Liu[5] · Ze-Sheng Zhang[2] · Yu-Hong Yang[1] · Xiao-Fei Yao[4] · Hui Fei[2]

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Abstract
Largemouth bass (*Micropterus salmoides*) is an economically important fish, yet it is plagued by frequent diseases caused by *Aeromonas veronii* infection in its aquaculture. Vaccination is a pivotal strategy in the prevention and management of infectious diseases, and adjuvants play a crucial role in augmenting the effectiveness of vaccines. Interleukin-8 (IL-8), one of the CXC chemokines, has been identified as having adjuvant potential in various species, although its efficacy in largemouth bass remains uncertain. In the present study, recombinant largemouth bass IL-8 (rLbIL-8) was prepared to explore its adjuvant potential in the *A. veronii* inactivated vaccine. Challenge experiment results revealed that the relative percent survival (RPS) of largemouth bass in the inactivated *A. veronii* + rLbIL-8 vaccinated group was calculated to be 70% following challenges with live *A. veronii*, higher than that of the inactivated *A. veronii* vaccinated group (56.67%). Furthermore, co-inoculation of rLbIL-8 and inactivated *A. veronii* markedly elevated serum SOD levels and lysozyme activities in largemouth bass. Indirect ELISA analysis found that rLbIL-8 enhanced the inactivated *A. veronii* vaccine's ability to induce serum-specific antibodies against *A. veronii*. qRT-PCR analysis showed that rLbIL-8 enhanced the upregulation of IL-1β, TNF-α, CD4, MHC II, and IgM expression induced by inactivated *A. veronii*, with peak expression occurring on days 1 and 5 post-vaccination, respectively. Collectively, these findings attest to the potential of rLbIL-8 as a vaccine adjuvant for largemouth bass, advancing the progress and implementation of vaccines in aquaculture.

Keywords *Micropterus salmoides* · Adjuvant · Interleukin-8 · *Aeromonas veronii* · Inactivated vaccine

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Shun Yang and Qian-Rong Liang contributed equally to this study.

Extended author information available on the last page of the article

Introduction

Aeromonas veronii is a Gram-negative pathogen with a broad spectrum of hosts capable of infecting humans, livestock, and aquatic animals (Ling et al. 2017; Liu et al. 2022; Wang et al. 2023). In aquaculture, numerous studies have documented *A. veronii* infections in a variety of teleosts, including European seabass (*Dicentrarchus labrax*), Nile tilapia (*Oreochromis niloticus*), and Channel catfish (*Ictalurus punctatus*), leading to outbreaks of bacterial diseases (Hoai et al. 2019; Raj et al. 2019; Karatas et al. 2023). The infection of *A. veronii* can result in multiple tissue and organ hemorrhaging and ulcerative lesions in afflicted fish, causing a substantial number of fatalities in aquaculture. To prevent and control its spread, numerous investigations have been undertaken to develop vaccines against *A. veronii* (Song et al. 2018; Kong et al. 2019; Zhang et al. 2020a, b; Youssef et al. 2022). Adjuvants play a crucial role in enhancing the efficacy of vaccines. However, there is currently limited research in aquaculture focusing on the development of adjuvants for *Aeromonas* vaccines. Largemouth bass (*Micropterus salmoides*) is a significant commercially farmed fish species, and its cultivation scale continues to expand in Asia due to its distinctive flavor (Fei et al. 2022; Che et al. 2023). Unfortunately, recent studies have reported that *A. veronii* infections have resulted in frequent diseases in largemouth bass farming, leading to substantial economic losses (Pei et al. 2021; Zhu et al. 2022). To ensure the healthy development of the largemouth bass farming industry, many studies have also focused on the development of *A. veronii* vaccines for largemouth bass (Wu et al. 2021; Chi et al. 2023), while the adjuvant for *A. veronii* vaccines is still lacking.

The adjuvants have the capacity to augment the immune response induced by vaccines, which is a crucial mechanism for enhancing vaccine efficacy (Lin et al. 2023; Nie et al. 2023). Cytokines, as pivotal immune regulatory molecules, actively participate in the host cell immune response and play an indispensable role. Consequently, numerous cytokines hold the potential to be harnessed as vaccine adjuvants due to their distinctive functions (Kobari et al. 2020; Bekele et al. 2021; Guo and Li. 2021). Interleukin 8 (IL-8), a CXC chemokine, assumes a pivotal role in the inflammatory and other immune responses. In mammals, extensive studies have verified the potential of IL-8 to evolve into a vaccine adjuvant (Kim et al. 2000; Min et al. 2001), with only limited reports in fish. Notably, in channel catfish, it has been reported that pcDNA3.1/IL-8, when co-injected with DNA vaccine, can enhance the immune response and protection against *Streptococcus iniae* infection (Wang et al. 2016a). Similar results have been observed in the co-injection of pcDNA3.1/IL-8 and DNA vaccines against infectious hematopoietic necrosis virus (IHNV) infection in rainbow trout (*Oncorhynchus mykiss*) (Cao et al. 2017). Furthermore, it has been documented that recombinant IL-8 (rIL-8) as an adjuvant can augment the immune response and increase the relative percent survival (RPS) induced by a subunit vaccine in channel catfish and flounder (*Paralichthys olivaceus*) (Wang et al. 2016b; Guo et al. 2018a). While the adjuvant potential of IL-8 in many animals has been established, it remains unknown whether largemouth bass IL-8 (LbIL-8) possesses adjuvant potential due to a lack of study.

In the current study, recombinant LbIL-8 (rLbIL-8) was prepared in order to explore its adjuvant capacity on the *A. veronii* inactivated vaccine. The simultaneous administration of rLbIL-8 and inactivated *A. veronii* was carried out, followed by the analysis of the alterations in the immune response of largemouth bass. Subsequently, largemouth bass post-vaccination was exposed to live *A. veronii* to monitor the discrepancy in RPS. This study will elucidate the adjuvant potential of LbIL-8 and foster the robust development of the largemouth bass aquaculture industry.

Materials and methods

Fish and bacteria

Largemouth bass (21 ± 2 g) were acquired from a fish farm in Huzhou, Zhejiang Province, China, and subsequently raised for one week in aerated filtered fresh water at 25 ± 0.5 °C before the commencement of the experiments. The rearing conditions remained consistent throughout the duration of the experiment. Additionally, all fish were anesthetized with ethyl 3-amino-benzoate-methanesulfonic acid (MS-222) prior to being sacrificed and sampled.

Aeromonas veronii HZ012 was obtained from diseased largemouth bass in Huzhou, Zhejiang Province, PR China, and cultivated in Luria–Bertani (LB) agar medium at 28 °C for the present study. The *A. veronii* was rendered inactive using 0.5% formalin at 4 ℃ for 72 h, and the inactivation effect was confirmed prior to vaccination.

Preparation of recombinant IL-8

The LbIL-8 sequence was obtained from the NCBI database (accession number: XM_038704088). Subsequently, LbIL-8 was synthesized by Genscript company in China and incorporated into the pET-32a vector to create the pET-32a-IL-8 vector. The resulting recombinant vector was transformed into *Escherichia coli* (DE3), and the positive transformant was identified through colony PCR and sequencing analysis. The positive transformant was then cultured in LB medium at 37 ℃ until the OD_{600} value reached 0.6 and then induced with isopropyl β-D-1-thiogalactopyranoside (IPTG 0.1 mM) at 16 ℃ for 20 h. Following this, rLbIL-8 was purified using HisTrap™ HP Ni-Agarose (GE Healthcare, USA) in accordance with the manufacturer's instructions. The purified rLbIL-8 was subjected to gradual dialysis for refolding and subsequently treated with Triton X-114 to eliminate endotoxin. The concentration of rLbIL-8 was determined using the BCA method. Meanwhile, the purity of rLbIL-8 was assessed using SDS-PAGE.

Vaccination and sampling

For the purpose of vaccination, four hundred robust largemouth bass were randomly allocated into four equal cohorts following temporary rearing. The inactivated *A. veronii* and rLbIL-8 were adjusted to 1×10^8 CFU/mL and 1 mg/mL, respectively. In the group vaccinated with inactivated *A. veronii*, the fish were inoculated with 200 μL of inactivated *A. veronii* and 10 μL PBS. In the rLbIL-8 vaccinated group, the fish were inoculated with 200 μL of PBS and 10 μL rLbIL-8. In the group vaccinated with inactivated *A. veronii* + rIL-8, the fish were inoculated with 200 μL of inactivated *A. veronii* and 10 μL rLbIL-8. The control group received 210 μL of PBS. All inoculations in this study were carried out by intraperitoneal injection.

The spleen was gathered at 0 d, 1 d, 3 d, and 5 d post-vaccination. The collected spleen was frozen in liquid nitrogen and stored at − 80 ℃ for quantitative real-time PCR (qRT-PCR). Additionally, blood was isolated from the caudal vein at weeks 4 and 5 post-vaccination. Subsequently, the serum was prepared by allowing the collected blood to stand at 4 ℃ overnight and then undergoing centrifugation at 3000 g for 15 min at

4 °C. The prepared serum was stored at − 80 ℃ for the detection of antibodies and non-specific enzyme activities. All tissues and serum were collected from fifteen largemouth bass to form three individual samples at each time point in each group.

Detection of non-specific enzyme activities

The serum superoxide dismutase (SOD) and lysozyme activities of largemouth bass were assessed post-vaccination. The SOD activities were evaluated utilizing water-soluble tetrazolium salt-1 (WST-1) with a commercial kit (Nanjing Jiancheng Bioengineering Institute, A001-3). Briefly, the serum was added to the enzyme working solution and substrate solution in accordance with the manufacturer's guidelines and incubated at 37 °C for 20 min. Subsequently, the absorbance at 450 nm was quantified using an ELISA reader to calculate SOD activities as per the prescribed formula.

The serum lysozyme activities were measured using a commercial kit (Nanjing Jiancheng Bioengineering Institute, A050-1–1). Briefly, the serum was added to the standard bacterial solution in accordance with the manufacturer's guidelines and allowed to incubate at 37 °C for 15 min. Subsequently, the mixture was transferred to an ice water bath maintained below 0 °C for 3 min to halt the enzymatic reaction. Finally, the absorbance at 530 nm was determined using an ELISA reader to calculate lysozyme activities as per the formula provided in the instructions.

Indirect ELISA

For indirect ELISA, inactivated *A. veronii* in carbonate-bicarbonate buffer (50 mM, pH = 9.6) was adjusted to 1×10^8 CFU/mL. Following this, 100 µL of inactivated *A. veronii* was coated into the wells of microplates (96 wells, Costar) and left overnight at 4 °C. Subsequently, 200 µL of 5% BSA was introduced into the wells and obstructed for 1 h at 37 °C after rinsing with PBST. Next, 100 µL of serum from largemouth bass (1:200) was placed into the wells and incubated for 3 h at 28 °C. After another round of rinsing, 100 µL of polyclonal antibodies against largemouth bass IgM that was produced in a prior study was introduced into the wells and incubated for 1 h at 37 °C (Yang et al. 2020). Following this, 100 µL of goat anti-rabbit Ig-horseradish peroxidase conjugate (GAM-HRP, 1:5000) was added into the wells and incubated at 37 °C for 1 h. Finally, 100 µL of TMB substrate solution (Beyotime, China) was applied into the wells and incubated for 15 min at room temperature in the absence of light. The reaction was halted using 100 µL of 1 M HCl per well, and then absorbance at 450 nm was measured using an ELISA reader to analyze specific serum antibodies against *A. veronii*.

Additionally, to identify total serum antibodies, 100 µL of serum from largemouth bass was applied to the wells of microplates overnight at 4 °C. After the application of 5% BSA, 100 µL polyclonal antibodies against largemouth bass IgM and goat anti-rabbit Ig-horseradish peroxidase conjugate were introduced into the wells and incubated sequentially as primary and secondary antibodies. Finally, total serum antibodies were determined by measuring absorbance at 450 nm using an ELISA reader after the reaction with TMB substrate solution.

Quantitative real-time PCR

Total RNA was extracted using an RNA extraction kit (Tiangen, China) from the spleen of largemouth bass in accordance with the manufacturer's instructions. Subsequently, the extracted RNA served as a template for synthesizing first-strand cDNA using the FastKing RT kit (Tiangen, China). The resulting first-strand cDNA was quantified and adjusted to a concentration of 100 ng/μl. The qRT-PCR was then performed in an ABI Prism® 7500 real-time system (Applied Biosystems, USA) using SYBR Green I Master Mix (Tiangen, China). The relative abundance was calculated using the β-actin gene as an internal reference with the $2^{-\Delta\Delta Ct}$ method (Yang et al. 2022). The primers for the immune-related genes (MHC II, CD4, IgM, IL-1β, and TNF-α) were designed and are presented in Table 1.

Challenge test

For the experimental challenge, thirty largemouth bass were randomly selected from each group at week 5 after vaccination. The live *A. veronii* was cultured and adjusted to 1×10^9 CFU/mL. Subsequently, all fish were intraperitoneally injected with 200 μL of *A. veronii* for the challenge. The dosage of live *A. veronii* chosen for the challenge test was 10 times the median lethal dose (LD50) for largemouth bass. Mortalities were monitored within 14 days after the challenge, and then the relative percentage survival (RPS) was calculated as previously outlined (Yang et al. 2022). The log-rank test was used for the statistical difference in the survival percentage between the groups, and differences were deemed significant at $p < 0.05$.

Statistical analysis

The data was analyzed utilizing the Statistical Product and Service Solution (SPSS) software (Version 20.0; SPSS, Inc). Statistical significance was assessed through an independent-sample t-test and one-way analysis of variance (ANOVA) with Duncan's multiple range test (DMRT), and differences were deemed significant at $p < 0.05$. All data were presented as the mean ± standard deviation.

Table 1 Sequence information of primers for qRT-PCR

Primer no	Primer name	Sequence (5' → 3')
1	CD4-F	GAAATGTATGTTCTCCTTTGGCG
2	CD4-R	ATTGGGGCAGGTTGGGT
3	MHC II-F	GAAAGGCACAGTGAAAGGCAA
4	MHC II-R	TTCCCTGTGCGATGAACTCTC
5	IgM-F	CCACCTGAACAATGCGAAGG
6	IgM-R	CGACAGATGGCTTGTTTGCC
7	IL-1β-F	TTTGAATCCTGCTGCTTTGACA
8	IL-1β-R	TCACACTGTTGGCACGGATG
9	TNF-α-F	ACAGGACGGTGGTTTTGGTAG
10	TNF-α-R	ATTCGGCTCAGCGTGTAGTG
11	β-actin-F	TGGAAGGGACCTCACAGACTAC
12	β-actin-R	GGGCAACGGAACETCTCAT

Results

Inducible expression and purification of rLbIL-8

The recombinant LbIL-8 was expressed using the pET-32a vector and purified in the present study. SDS-PAGE analysis revealed a clear band at approximately 34 kDa following IPTG induction (Fig. 1). This distinct band corresponded to the molecular weight of LbIL-8 plus the tag protein from the pET-32a vector, indicating successful expression of rLbIL-8. Furthermore, SDS-PAGE analysis indicated that, following Ni^{2+} affinity chromatography, only rLbIL-8 was present, suggesting the acquisition of high-purity soluble rLbIL-8 after gradual dialysis for refolding. This purified protein could be used to inoculate largemouth bass after endotoxin removal (Fig. 1).

Enhanced effect of LbIL-8 on inactivated A. veronii-induced immunoprotection

After inoculation, no fatalities were observed in all groups of largemouth bass. Subsequently, largemouth bass were exposed to live *A. veronii* at week 5 post-inoculation. As depicted in Fig. 2, a substantial number of deaths began to occur following exposure, and the mortality of largemouth bass in the control group reached 100%. The mortality of largemouth bass in the inactivated *A. veronii* vaccinated group was significantly lower than that in the control group ($p < 0.05$), at only 43.33% with an RPS of 56.67%. In the rLbIL-8 vaccinated group, 86.33% of largemouth bass mortality was observed after exposure, with an RPS of 13.67%. The lowest mortality of largemouth bass was found in the inactivated *A. veronii* + rLbIL-8 vaccinated group, at only 30% ($p < 0.05$). The RPS of the inactivated *A. veronii* + rLbIL-8 vaccinated group was calculated to be 70%, which was higher than that of the inactivated *A. veronii* vaccinated group.

Effect of rLbIL-8 on serum non-specific enzyme activities

The serum levels of superoxide dismutase (SOD) and lysozyme activities were observed post-vaccination. The findings indicated a significant increase in serum SOD levels following vaccination with *A. veronii* and rLbIL-8, both individually and in combination

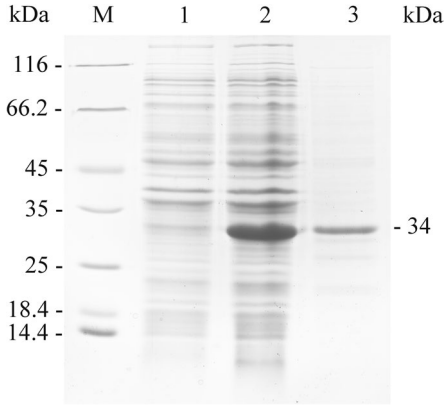

Fig. 1 SDS-PAGE analysis of expression and purification of rLbIL-8 protein. Lane M: molecular mass marker; lane 1: transformated *E. coli* without IPTG induction; lane 2: transformated *E. coli* induced with IPTG; lane 3: purified rLbIL-8

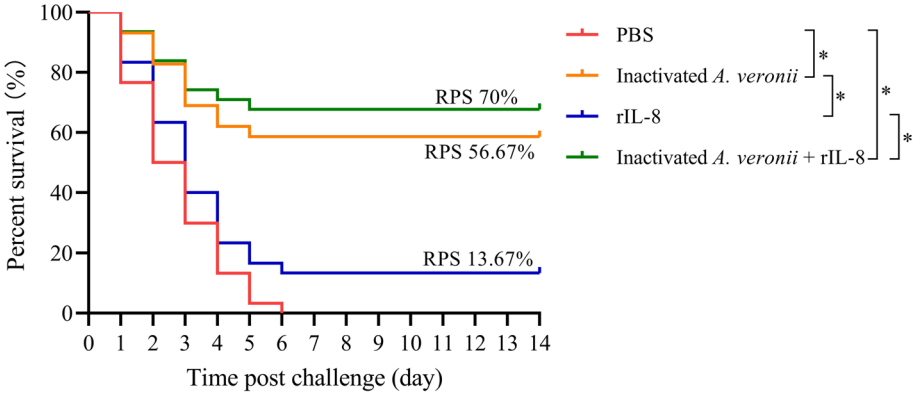

Fig. 2 Cumulative mortality of largemouth bass from control and vaccinated groups following challenged with live *A. veronii*. The asterisk indicates the statistical significance of the survival percentage between two groups ($p < 0.05$)

(Fig. 3A). The highest serum SOD level was detected in the group vaccinated with inactivated *A. veronii* + rLbIL-8 at week 4 after vaccination ($p < 0.05$). Furthermore, serum lysozyme activities in the group vaccinated with inactivated *A. veronii* + rLbIL-8 and the group vaccinated with rLbIL-8 were higher compared to the control group ($p < 0.05$), with the highest lysozyme activities observed in the rLbIL-8 vaccinated group at week 4 after vaccination (Fig. 3B). There were no notable differences in serum lysozyme activities between the group vaccinated with inactivated *A. veronii* and the control group following vaccination ($p > 0.05$).

Effect of rLbIL-8 on inactivated A. veronii-induced serum-specific antibodies

The indirect ELISA analysis revealed no significant disparity in the total serum antibodies of largemouth bass across all groups post-vaccination (Fig. 4A). Following vaccination, the specific serum antibodies against *A. veronii* in largemouth bass were also detected through

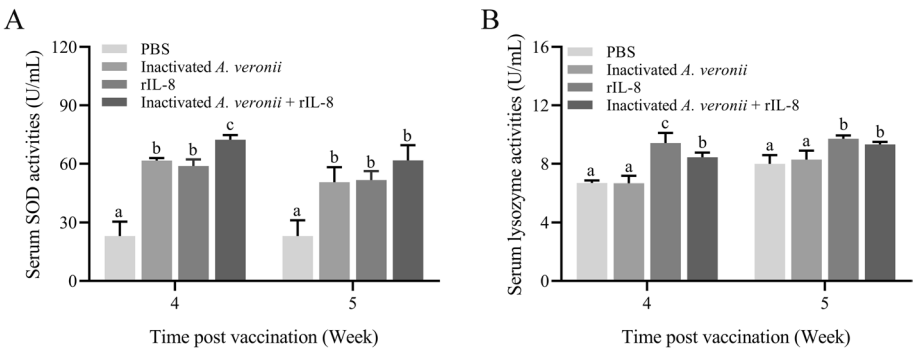

Fig. 3 Analysis of serum SOD and lysozyme activities of largemouth bass following vaccination. (**A**) Largemouth bass serum SOD activities. (**B**) Largemouth bass serum lysozyme activities. Different letters on the bars indicate statistically significant differences among different groups at the same time point ($p < 0.05$)

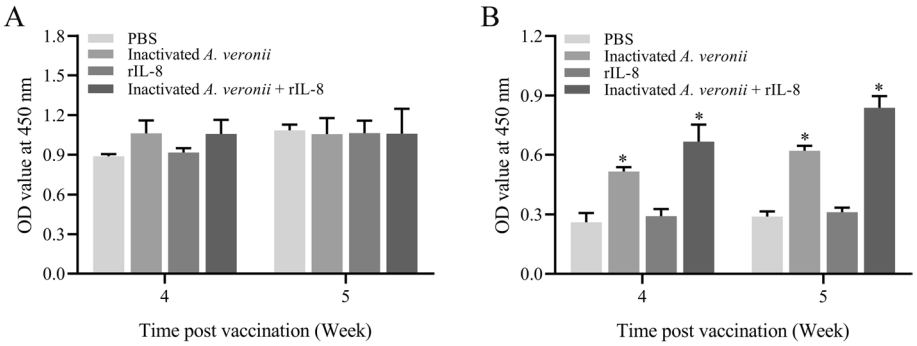

Fig. 4 Analysis of serum antibodies of largemouth bass following vaccination. (**A**) Indirect ELISA analysis of total serum antibodies of largemouth bass. (**B**) Indirect ELISA analysis of specific serum antibodies against *A. veronii*. Asterisks on the bars indicate statistically significant differences between the vaccination and control groups at the same time point ($p < 0.05$)

indirect ELISA, and the findings indicated that the specific serum antibodies in the inactivated *A. veronii* vaccinated group were notably higher than those in the control group at weeks 4 and 5 post-vaccination ($p < 0.05$) (Fig. 4B). In the rLbIL-8 vaccinated group, there was no significant contrast in specific serum antibodies observed compared to the control group post-vaccination ($p > 0.05$). Furthermore, the specific serum antibodies in the inactivated *A. veronii* + rLbIL-8 vaccinated group were also found to significantly increase post-vaccination ($p < 0.05$), surpassing those in the inactivated *A. veronii* vaccinated group.

mRNA expression of immune-related genes

The CD4, MHC II, IgM, IL-1β, and TNF-α expressions of largemouth bass were scrutinized via qRT-PCR subsequent to vaccination. All genes examined in this investigation exhibited significant upregulation subsequent to vaccination (Fig. 5). The levels of CD4, MHC II, and IgM in the inactivated *A. veronii* + rLbIL-8 vaccinated group were notably higher compared to those in the inactivated *A. veronii* vaccinated group ($p < 0.05$), with peak expression occurring on day 5 post-vaccination at all time points under observation (Fig. 5A–C). In the case of IL-1β and TNF-α, heightened levels of expression were similarly noted in the inactivated *A. veronii* + rLbIL-8 vaccinated group as compared to other groups ($p < 0.05$). The zenith of IL-1β and TNF-α expression was observed in the inactivated *A. veronii* + rLbIL-8 vaccinated group on day 5 following vaccination (Fig. 5D,E).

Discussion

Vaccination stands as a pivotal measure in controlling and preventing the outbreak of diseases in aquaculture (Irshath et al. 2023; Xiong et al. 2023). The advancement of adjuvants plays a crucial role in augmenting the efficacy and commercial application of vaccines (Guo and Li. 2021). IL-8, a noteworthy CXC chemokine, has been recognized for its potential as a vaccine adjuvant across various species. This study unveiled that rLbIL-8 possesses adjuvant potential, as it bolstered the protective impact of the *A. veronii* inactivated vaccine in largemouth bass. Compared with the inoculation of the *A. veronii* inactivated vaccine alone, co-inoculation of rLbIL-8 and *A. veronii* inactivated vaccine not only

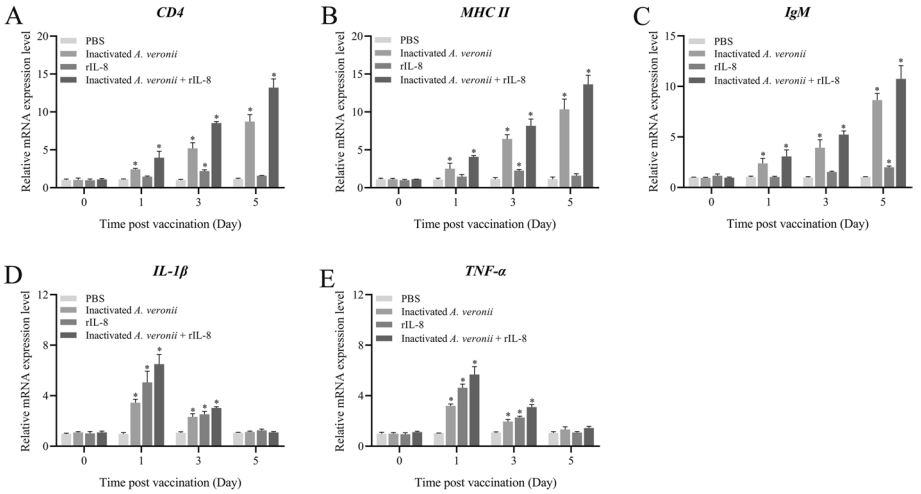

Fig. 5 The qRT-PCR analysis of expression of largemouth bass immune-related genes following vaccination. (**A**) The expression of CD4 gene. (**B**) The expression of the MHC II gene. (**C**) The expression of IgM gene. (**D**) The expression of IL-β gene. (**E**) The expression of TNF-α gene. The mRNA levels of detected genes were determined by normalized against the β-actin gene. The asterisk on the bars indicates the statistical significance of gene expressions compared to the control group ($p < 0.05$)

reduced largemouth bass mortality by 13.33% post *A. veronii* challenge but also elevated the RPS of the vaccine from 56.67 to 70%. Similarly, in channel catfish, studies reported that rIL-8 as an adjuvant substantially enhanced the RPS induced by the subunit vaccine from 53.33 to 73.33% (Wang et al. 2016b). The flounder rIL-8 was also reported to elevate the RPS triggered by the *Edwardsiella tarda* subunit vaccine from 40 to 68% (Guo et al. 2018a). The varying degrees of enhancement in vaccine protection efficacy by rIL-8 across different species, despite their shared adjuvant potential, suggest a relationship with species and vaccine distinctions. Additionally, inoculation of rLbIL-8 alone was observed to fortify the resistance of largemouth bass against *A. veronii*, albeit yielding a low RPS of 13.67%. While the effect of rIL-8 alone on flounder was not explored, it was identified in channel catfish that rIL-8 alone could induce a low RPS of 7.14% against *S. iniae* challenge (Wang et al. 2016b; Guo et al. 2018a). Apart from rIL-8, the pcDNA3.1/IL-8 plasmid was also examined for its adjuvant effect in numerous species. Results indicated that the pcDNA3.1/IL-8 plasmid could augment the protective impact of the vaccine (36 to 65% in flounder, 53.33 to 73.33% in channel catfish), with the increase in RPS within the same species showing no significant difference from that of rIL-8 (Wang et al. 2016a; Guo et al. 2018b), thereby suggesting that the manner of employing IL-8 as an adjuvant does not notably affect its amplified vaccine immune protection.

The alterations in serum antibodies were assessed post-vaccination in this investigation. Analysis using indirect ELISA revealed that inactivated *A. veronii* vaccine could stimulate largemouth bass to generate serum-specific antibodies against *A. veronii*. The co-inoculation of rLbIL-8 and the inactivated *A. veronii* vaccine elicited higher levels of serum-specific antibodies compared to inoculation with the inactivated *A. veronii* vaccine alone. These specific antibodies are crucial effector molecules for eradicating pathogenic infections and play a protective role in vaccines (Easterhoff et al. 2020; de Souza-Silva et al. 2023). The use of IL-8 in channel catfish and flounder as an adjuvant

to augment the protective efficacy of vaccines has been documented to enhance the vaccine's ability to induce specific antibodies (Wang et al. 2016a and 2016b; Guo et al. 2018a and 2018b). Therefore, the enhancement of rLbIL-8 on the induction of serum-specific antibodies by the inactivated *A. veronii* vaccine is a significant factor in boosting the vaccine's protective efficacy. Numerous studies have demonstrated that vaccines up-regulate the expression of genes related to humoral immunity (Yang et al. 2022; Guo et al. 2018a). The upregulation of CD4, MHC II, and IgM expressions was also observed in this study following inoculation with the inactivated *A. veronii* vaccine, and the expression patterns were consistent with those seen after inoculation with the AHA_3793 subunit vaccine in largemouth bass (Yang et al. 2022). Co-inoculation with rLbIL-8 and the inactivated *A. veronii* vaccine was also found to enhance the upregulation of CD4, MHC II, and IgM expressions compared to inoculation with the inactivated *A. veronii* vaccine alone, in line with the enhanced secretion of specific antibodies by rLbIL-8. In flounder, IL-8 was noted to enhance the upregulation of CD4, MHC I, MHC II, IgM, and CD8 expressions induced by the subunit vaccine (Guo et al. 2018a). Similarly, the augmentation of the upregulation of CD4, MHC I, MHC II, and CD8 expressions induced by the subunit vaccine by IL-8 was also reported in channel catfish (Wang et al. 2016b).

In addition, it was also discovered that rLbIL-8 upregulated the expressions of genes related to inflammatory response (IL-1β and TNF-α) in the current study, reaching the zenith earlier than genes related to humoral immunity. IL-8 plays a pivotal role in the regulation of neutrophils and the host's inflammatory response (de Oliveira et al. 2013; Kaiser et al. 2021). Numerous studies have demonstrated that IL-8 can trigger the secretion of various cytokines, including IL-1β and TNF-α (Yan et al. 2021; Zhao et al. 2021). The inflammatory response is crucial for the host's defense against pathogen invasion, while an excessive inflammatory response can lead to host damage and even mortality (Patel et al. 2016; Dong et al. 2018). A prior study has established that an exaggerated inflammatory response is among the primary causes of largemouth bass mortality induced by *Aeromonas* (Yuan et al. 2021). Hence, the upregulation of inflammatory cytokines is accompanied by an elevation in anti-inflammatory molecules to forestall an excessive inflammatory response. Superoxide dismutase (SOD) is deemed a pivotal anti-oxidative stress molecule that plays a critical role in non-specific immunity and anti-inflammatory responses (Krzystek-Korpacka et al. 2021; Yao et al. 2022). In the present study, it was observed that rLbIL-8 heightened serum SOD activities in largemouth bass following inoculation with the inactivated *A. veronii* vaccine. This elevation in serum SOD activities bolstered the non-specific immunity of largemouth bass and averted an excessive inflammatory response. Numerous studies have also noted that the expression of IL-8 and SOD activities exhibit a co-upregulated pattern upon stimulation (Hoseinifar et al. 2020; Liang et al. 2020; Yao et al. 2022). Furthermore, rLbIL-8 significantly boosted serum lysozyme activities in largemouth bass, indicating that rLbIL-8 as an adjuvant could enhance serum lysozyme activities to enhance the non-specific immunity of largemouth bass. In other studies, IL-8 has been shown to enhance host lysozyme activities (Lee et al. 2009). Moreover, the administration of rLbIL-8 alone substantially increased the expression of genes related to inflammatory response, serum SOD, and lysozyme activities. These findings suggest that the administration of rLbIL-8 alone could amplify the non-specific immunity of largemouth bass, potentially linked to the low RPS induced by rLbIL-8 alone.

In conclusion, the inclusion of rLbIL-8 not only enhanced the protective efficacy of the *A. veronii* inactivated vaccine but also elevated the levels of specific antibodies and non-specific immunity in largemouth bass. These findings showcase the potential of rLbIL-8 as

a vaccine adjuvant for largemouth bass, thereby facilitating the advancement and utilization of vaccines in aquaculture.

Author contribution All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Shun Yang, Qian-Rong Liang, and Ze-Sheng Zhang. Shun Yang, Yu-Hong Yang, and Hui Fei contributed reagents/materials/analysis tools. Conceptualization, formal analysis, methodology, project administration, resources, supervision, visualization, and writing—original draft and editing by Shun Yang, Qian-Rong Liang, Dao-Le Liu, and Xiao-Fei Yao. All authors read and approved the final manuscript.

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Data availability Data will be made available from the corresponding author upon reasonable request.

Declarations

Ethics approval The animal experiments in the present study were in strict accordance with the relevant provisions of the welfare ethics and protection of experimental animals in the state and Zhejiang Province. The animal-related experiments were approved and supervised by the Committee of Ethics on Animal Care and Experiments at the Zhejiang Sci-Tech University.

Competing interests The authors declare no competing interests.

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Authors and Affiliations

Shun Yang[1,2] · Qian-Rong Liang[3] · Dao-Le Liu[5] · Ze-Sheng Zhang[2] · Yu-Hong Yang[1] · Xiao-Fei Yao[4] · Hui Fei[2]

✉ Yu-Hong Yang
yuhongyang@neau.edu.cn

✉ Xiao-Fei Yao
13758232946@139.com

✉ Hui Fei
feihui@zstu.edu.cn

[1] College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, People's Republic of China

[2] College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou 310018, China

[3] Zhejiang Fisheries Test and Aquatic Disease Prevention Center, Zhejiang Fisheries Technical Extension Center, Hangzhou 310023, China

[4] Zhejiang Huadi Pharmaceutical Group Co., Ltd, Hangzhou 313300, China

[5] Huanggang Modern Agriculture Exhibition and Information Center, Huanggang 438021, China