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Research Article

Construction and Evaluation of Recombinant *Lactococcus lactis* Expressing the vscO Gene as a Safe Live Oral Vaccine against *Vibrio alginolyticus*

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Vibrio alginolyticus is responsible for significant economic losses in the fish industry. It is urgent to develop a vaccine against *V. alginolyticus*. In this study, a recombinant *Lactococcus lactis* expressing the VscO protein of *V. alginolyticus* (Lc-pMG36e-vscO) is constructed. The Lc-pMG36e-vscO has good hereditary stability. The immune responses in pearl gentian grouper (*Epinephelus fuscoguttatus* (♀) × *E. lanceolatu* (♂)) vaccinated with Lc-pMG36e-vscO are evaluated. The titers of specific antibody in the immunized grouper displayed an upward and then downward tendency during the total experiments and reached the peak value of 9.6 at the fourth week after booster immunization. The expression levels of IL-1β, IL-16, TNF-α, and MHC-1α gene in the Lc-pMG36e-vscO group were significantly upregulated after vaccination, indicating that the Lc-pMG36e-vscO could induce an effective and durable immune response. In vivo challenge of the Lc-pMG36e-vscO-immunized fish with *V. alginolyticus* showed a relative survival percentage of 68%. These results indicated that Lc-pMG36e-vscO could be promising as a candidate oral vaccine against *V. alginolyticus*.

1. Introduction

Vibriosis, caused by several species from the family of Vibrionaceae, negatively affects the various marine animals and impedes the sustainable development of the global aquaculture industry [1]. *Vibrio alginolyticus* is an important pathogen causing vibriosis in the marine culture. In the past several decades, some marine-cultured animals including *Lates calcarifer*, *Sparus aurata, Dicentrarchus labrax, Litopenaeus vannamei*, *Marsupenaeus japonicas*, and *Crassostrea gigas* suffered serious production losses due to infection by *V. alginolyticus* [2–7]. It has been reported that *V. alginolyticus* also infected humans and caused gastroenteritis, wound infections, and septicemia and even amputations and death [8]. Due to the harmful effect caused by *V. alginolyticus*, farmers had to combat the disease with some treatment ways, such as antibiotics, green water technique, topical disinfectants, medicinal plants, bacteriophage, probiotics, and vaccines [9]. Antibiotics have been excessively used to combat *Vibrio* spp. infections in aquaculture. Drug resistance and residues are becoming a public health issue, and more stringent requirements and regulations on the use of antibiotics will be imposed [9, 10]. Compared with antibiotics, the vaccine is more effective and safer for preventing vibriosis [9]. Therefore, it is necessary to develop a suitable vaccine against vibriosis.

Currently, most vaccines including bacterins (inactivated causative agents), live attenuated vaccines, DNA vaccines, subunit vaccines, polyvalent vaccines, and monovalent vaccines have been developed and researched, which provide some protection in fish [11]. In the current situation, vaccine antigens to protect fish against vibriosis are usually the virulence factors of *Vibrio* isolates. The recombinant subunit vaccine of the virulent factor against

V. alginolyticus is proven to be environment-friendly as an alternative to antibiotics and gives a long-lasting protective immunity to the fish [11]. The type III secretion system (T3SS) is essential for the pathogenesis of *V. alginolyticus*. VscO, a main component of T3SS, may play a similar chaperone escort role in pathogenicity and be required for normal secretion of T3SS. VscO protein was proven to have good antigenicity and could induce a high antibody titer to protect against lethal challenge with *V. alginolyticus*. Those results indicated that VscO protein could be used as a candidate antigen for developing a subunit vaccine [12].

Probiotics have been applied in aquaculture for several decades. *Lactococcus lactis*, an endogenous microbe, could exert growth performance, immune response, and disease resistance in aquaculture animals [13–15]. As an endogenous microbe, *L. lactis* could adhere to mucosal surfaces of the gut and then colonize, establish, and multiply in the fish gut [13]. The mucosal surface is an important physiological barrier and provides a portal of entry for pathogens [16]. Adequate systemic immune response protecting the host could be induced on the mucosal surface [17]. The application of probiotics delivering specific protective antigens to the mucosal surfaces may be an alternative way. In previous studies, *Lactococcus* is generally regarded as a safe probiotic and has been used to produce vaccines expressing the antigens, such as OmpAI, flagellin A protein, and OmpC of *Aeromonas veronii* [16, 18, 19] and immobilization antigen IAG-52X of *Ichthyophthirius multifiliis* [20]. Antigens expressed by *Lactococcus* are presented to the immune system in particulate form, which may be less likely to induce oral tolerance than soluble antigens. These studies have indicated that *Lactococcus* is an appropriate candidate for heterologous antigens delivery to mucosal sites.

In the present study, *L. lactis* is isolated from the intestine of healthy pearl gentian grouper (♀*Epinephelus fuscoguttatus* × ♂*E. lanceolatu*); vscO gene of *V. alginolyticus* is transferred into *L. lactis* for constructing a recombinant *L. lactis* strain. The natural antigenicity of the recombinant VscO expressed in *L. lactis* is explored. Furthermore, the potential of the recombinant *L. lactis* strain as an oral live vaccine is evaluated in pearl gentian grouper administrated orally with the strain.

2. Materials and Methods

2.1. Ethics Statement. All experimental protocols used in animal experiments were approved by the Animal Care and Use Committee of Guangdong Ocean University.

2.2. Bacterial Strains, Plasmids, and Growth Conditions. *L. lactis* was isolated from the intestine of pearl gentian grouper and cultured in De Man, Rogosa, and Sharpe medium (MRS, Huankai, China) at 30°C. Safety assays revealed that the *L. lactis* was not virulent for grouper. *V. alginolyticus* strain HY9901 isolated from the diseased maricultured fish was cultured in tryptic soy broth medium (TSB, Huankai, China) supplemented with 2% NaCl at 28°C [21]. *Escherichia coli* (DH5α) were cultured in Luria–Bertani medium (LB, Huankai, China) at 37°C. The vector pMG36e containing an encoding erm gene (Erythromycin) was purchased from BioVector NTCC Inc in China. When required, the appropriate antibiotic Erm (10 μg/mL) was added in the medium.

2.3. Construction of Recombinant L. lactis Expressing VscO Protein. The vscO gene (462 bp) of *V. alginolyticus* was amplified by PCR using the following primers with Xba I and Hind III site underlined (Table 1). The PCR product of vscO and the plasmid pMG36e were cleaved with Xba I and Hind III, respectively. Then, the processed fragment was ligated to pMG36e. Finally, the recombinant pMG36e-vscO plasmid was transformed into *L. lactis* by electroporation as previously described [22]. The strain containing pMG36e-vscO plasmid was named Lc-pMG36e-vscO. The wild *L. lactis* and PBS were used as control.

2.4. Western Blot Analysis. Western blot analysis was applied to detect the expression of VscO protein in the recombinant Lc-pMG36e-vscO as described in the previous study [23]. Briefly, the total protein of Lc-pMG36e-vscO and wild *L. lactis* was electrophoresed by SDS-PAGE on a 5% stacking gel and 15% separating gel under denaturing conditions. The protein was electrically transferred from the gel to the nitrocellulose membrane using a semidry apparatus (Bio-Rad, Hercules, CA, USA). The membrane was blocked with 5% skim milk and then incubated with mouse anti-VscO serum at a dilution of 1 : 1000 with blocking buffer (previously prepared in our laboratory) and was kept overnight at 4°C; the secondary antibody was peroxidase-conjugated goat anti-mouse IgG (Sigma, USA) used at 1 : 10000 dilutions. Finally, the blots were visualized by chemiluminescence detection with Western ECL substrate (Thermo Scientific) in an Amersham Imager 600 (GE Healthcare, UK).

2.5. Hereditary Stability of Lc-pMG36e-vscO. The hereditary stability of Lc-pMG36e-vscO was measured according to previous research [24]. The Lc-pMG36e-vscO was cultured in MRS medium with erythromycin 10 μg/mL, without shaking at 30°C. After 24 h, the plasmid was extracted from the cells and PCR was used to confirm the presence of vscO fragment in strain using specific primers vscO-R/vscO-F and universal primers pMG36e-R/pMG36e-F for vscO gene (Table 1).

2.6. Vaccine Preparation, Oral Vaccination, and Sample Collection. The Lc-pMG36e-vscO and wild strain were cultured to the logarithmic stage. The cultures were collected by centrifugation at 8000 r/min at 4°C for 10 min. Then 2.5% sodium alginate was added and mixed evenly with a commercial grouper pellet diet. The prepared diet was dried at room temperature and ventilation and stored at 4°C. The concentration of *L. lactis* is 1.0×10^9 CFU/g diet. The PBS blank control group was treated in the same way.

Healthy grouper with a mean weight of 50.0 ± 1.0 g was purchased from a fish farm in Donghai Island

Table 1: Primers used in this study.

Primer	Nucleotide sequence (5'-3')	Accession no.
vscO-R vscO-F	GCTCTAGAGATGATAGAACGTTTATTAGA (Xba I) CCCAAGCTTTTAGATAATGTCGACAGTGCG (Hind III)	JX131326.1
pMG36e-R pMG36e-F	GCCTCCTCATCCTCTTCATC AATATCGTAGCGCCGGGGTA	Universal primer
IL-1β-R IL-1β-F	ACGCTGCTGGACCTTTATCG ACACGGCTTTGTCGTCTTTC	XM_033647455.1
TNF-α-R TNF-α-F	CTTCCGTCGCTGTCCTCATGTG GCCACAGGATCTGGCGCTACTC	FJ491411.1
MHC-1α-R MHC-1α-F	TCCATCGTGGTTGGGGATGATC GCCGCCACGCTACAGGTTTCTA	FJ896112.3
IL-16-R IL-16-F	TCTGTTCTGCGGGTTTAGC TTCAGATCCTCCGTCCAAC	KP025949.1
β-actin-R β-actin-F	TCAGGATACCCCTCTTGCTCT AAATCGCCGCACTGGTTG	AY510710.2

(Guangdong Province, China). Fish were allowed to acclimatize for two weeks before vaccination. They were randomly divided into three groups with 3 replicates and 30 fishes per replicate and were fed to apparent satiation twice daily (8 : 30 and 16 : 30) for 42 d with a commercial grouper pellet diet. Fish were immunized with the prepared diets from day 1 to day 7. The booster immunizations were administered from day 21 to day 28. The strategy of oral administration of grouper and sampling is shown in Figure 1. Four fish in each group were euthanized with 100 μg/mL of tricaine methane-sulfonate (MS-222, Sigma Aldrich) at 7, 14, 21, 28, 35, and 42 d after the immunization to collect blood, spleen, kidney, and hindgut. The tissues were collected and stored in RNAlater (Thermo Fisher, China) at −80°C for RNA extraction. Sera were kept at −80°C for ELISA.

2.7. Analysis of Specific Antibody Levels. Antibody titers were determined as described previously [21]. Briefly, 96-well microtiter plates were coated with the purified VscO protein. Fish sera were added to the microplate in twofold serial dilutions (2^1 to 2^{10}). Plates in duplicate were incubated for 1 h under gentle agitation. Rabbit anti-grouper IgM was used as a secondary antibody and peroxidase-conjugated goat anti-rabbit IgG (Sigma) as tertiary antibodies. Enzyme substrate (o-phenylenediamine, Sigma) was added to the plates, and these were incubated for 10–15 min. The enzymatic reaction was stopped by adding 3 M HCl, and optical density (OD) was determined at 492 nm using an ELISA reader. Each plate included two controls, namely, wells with all antibodies and substrates except sample material (primary antibodies) as negative controls (blanks) and sera obtained from native fish. Values greater than twice the (blank) background absorbance were considered to be positive. ELISA was validated by means of the OD readings obtained from native sera, which were the same in the different plates. The experiments were replicated in triplicate.

2.8. Detection of Immune-Related Genes. The total RNA of examined tissues including the spleen, kidney, and hindgut was extracted by Trizol reagent (Invitrogen). Genomic DNA was removed using the PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara). RNA concentration was measured with a spectrophotometer (NanoDrop Technologies). RNA was reverse transcribed into cDNA using the One-Step RT-PCR Kit. The expression levels of immune-related genes (IL-1β, IL-16, TNF-α, and MHC-1α) were detected by real-time quantitative PCR (qRT-PCR) in SYBR* Green qPCR SuperMix Kit (TransGen, China). The final reaction volume of 10 μL contained 0.5 μL cDNA, 5 μL of 2 × SYBR Green qPCR SuperMix, 0.5 μL of each primer (10 μM), and 3.5 μL ddH_2O. The reaction was performed under the following conditions: 95°C, 2 min; 95°C, 30s; 57°C, 30 s, 72°C, 20 s (40 cycles). A melting curve analysis was performed to access the amplification of specific products. The β-actin gene was used as an endogenous control. The expressions of immune-related genes were normalized to β-actin using the $2^{-\Delta\Delta Ct}$ method. All samples were replicated in triplicate. The primers are shown in Table 1.

2.9. Challenge Experiments. At 42 d postimmunization, all the vaccinated fish were injected intraperitoneally with 200 μL of 1×10^6 CFU/g fish body weight (5 LD_{50} doses) of *V. alginolyticus* strain HY9901. Fish injected with 200 μL PBS were used as the negative control group. The fish challenged with *V. alginolyticus* were monitored for 15 d, and the survival rate was analyzed in all the group post challenge. The experiments were replicated in triplicate.

2.10. Statistical Analysis. Statistical analysis was performed using SPSS 19.0 software. For multiple comparisons, one-way ANOVA is followed by Tukey's test. Data are expressed as mean ± SD. In all cases, significant differences were considered at $P < 0.05$.

Figure 1: Strategy of oral administration of pearl gentian grouper and sampling.

3. Results

3.1. Lc-pMG36e-vscO Construction. The vscO gene consisting of an open reading frame of 462 bp was obtained from genomic DNA of *V. alginolyticus* by PCR amplification and cloned into the expression plasmid pMG36 generating pMG36e-vscO. The recombinant plasmid pMG36e-vscO was transferred into *L. lactis* by electroporation. The recombinant *L. lactis* containing plasmid pMG36e-vscO was named Lc-pMG36e-vscO. To determine whether the *L. lactis* is capable of producing VscO protein, whole-cell lysates of Lc-pMG36e-vscO were analyzed by western blotting after SDS-PAGE. The result showed that an immunoreactive band of about 18 kDa was detected in the recombinant strain (Figure 2), which indicated that the VscO protein was produced by Lc-pMG36e-vscO.

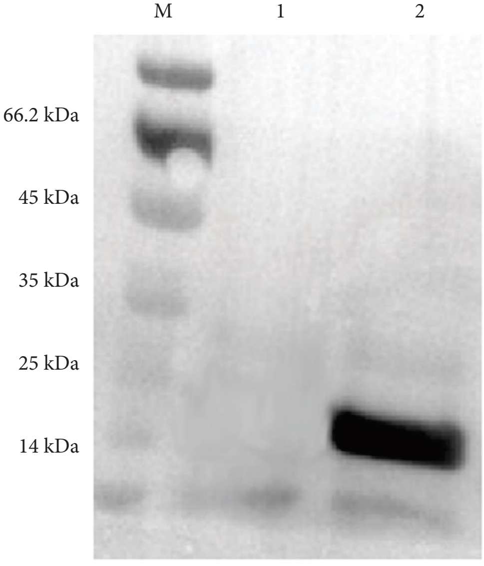


Figure 2: Analysis of VscO protein expression in *L. lactis*. M: protein marker, 1: whole protein of wild *L. lactis*; 2: whole protein of Lc-pMG36e-vscO.

3.2. Hereditary Stability of Lc-pMG36e-vscO. The Lc-pMG36e-vscO gene was screened by PCR and sequence analysis for genetic stability analysis after 50 generations. The results showed that recombinant plasmid pMG36e-vscO was stably inherited in Lc-pMG36e-vscO (Figure 3).

3.3. Analysis of Serum Antibody Titer. The titers of VscO-specific serum antibody in grouper were shown in Figure 4. The results showed that a specific antibody in the immunized grouper was detected at the first week after vaccination. The specific antibody titers in the Lc-pMG36e-vscO group were significantly higher than that in the *L. lactis* group and PBS group ($P < 0.05$). The antibody titers displayed an upward tendency from the 1st to the 4th week and then a downward tendency from the 4th week to the end of the experiments. The antibody titers reached the peak value of 9.6 at the 4th week after booster immunization (Figure 4).

3.4. The Expression Levels of Immune-Related Genes. At 7, 14, 21, 28, 35, and 42 d after oral administration, qRT-PCR was performed to analyze the expression levels of the immune-related gene in the spleen, kidney, and hindgut (Figure 5). The results showed IL-1β gene expression levels had increased after the first immunization in the Lc-pMG36e-VscO group and then had declined till the end of experiments in all detected tissues. The expression levels of the TNF-α gene did not change in spleen, increased-declined-increased-declined in the head kidney, and continuously increased in the hindgut during the immunization in the Lc-pMG36e-vscO group. The expression levels of the IL-16 gene in the Lc-pMG36e-vscO group had the trend of increasing-declining-increasing-declining during the immunization in the Lc-pMG36e-vscO group in all detected tissues. The expression levels of the MHC-1α gene displayed the trend of increasing-declining-increasing-declining during the immunization in the Lc-pMG36e-vscO group in spleen and head kidney and had no significant change in hindgut.

3.5. Challenge Experiments. Challenge experiments were performed by injecting *V. alginolyticus* virulent strain HY9901 to evaluate the protective effect of Lc-pMG36e-vscO. As shown in Figure 6, the fish immunized with Lc-pMG36e-vscO showed a relative percentage survival (RPS) of 68% after challenge with a lethal dose of *V. alginolyticus*, significantly higher than those of *L. lactis* (32.67%) and PBS group (0%).

4. Discussion

Vibrio spp is a part of normal marine flora. However, it also is a pathogen of aquaculture animals and leads to massive mortality of aquaculture animals in hatcheries and grow-out farms [1]. *V. alginolyticus* is the pathogen of vibriosis of several aquaculture animals [2–7]. Co-infection of *V. alginolyticus* and other pathogen bacteria also caused disease in fish [25, 26]. *L. calcarifer* infected with *V. alginolyticus* moved in circles on the water surface of the cage. Clinical symptoms of affected fish included congestion, haemorrhage, and vacuolation in liver [4]. In this paper, all fish in the control group died after 8 d of being challenged by *V. alginolyticus*. Vaccination is playing an increasingly important role in the control of fish diseases. Till now, many

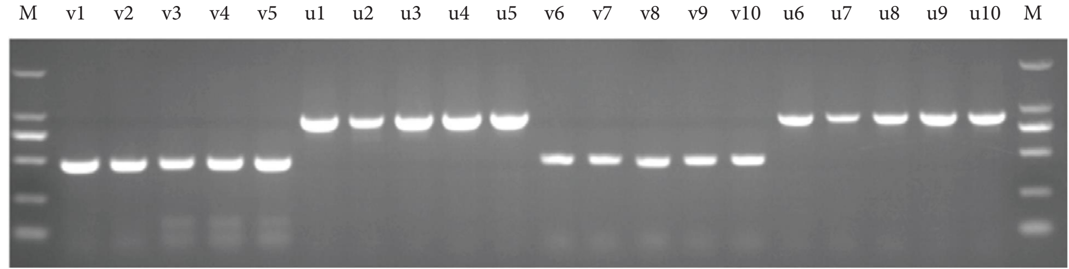


Figure 3: Hereditary stability analysis of Lc-pMG36e-vscO. M: DL2000 DNA marker; v1–v10: PCR product amplified by primers vsco–R/vsco-F; u1–u10: PCR product amplified by primers pMG36e-R/pMG36e-F. v1-10 and u1-10: 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 generations of Lc-pMG36e-vscO.

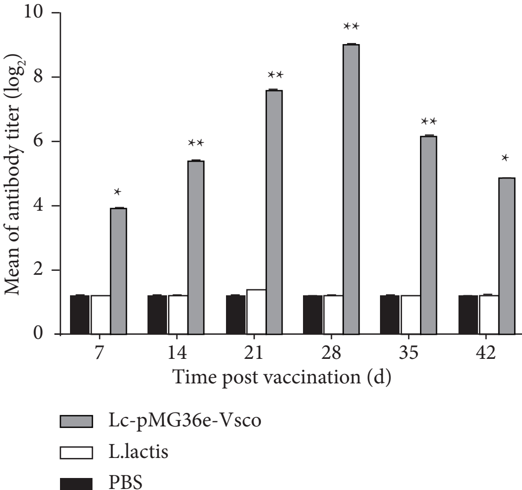
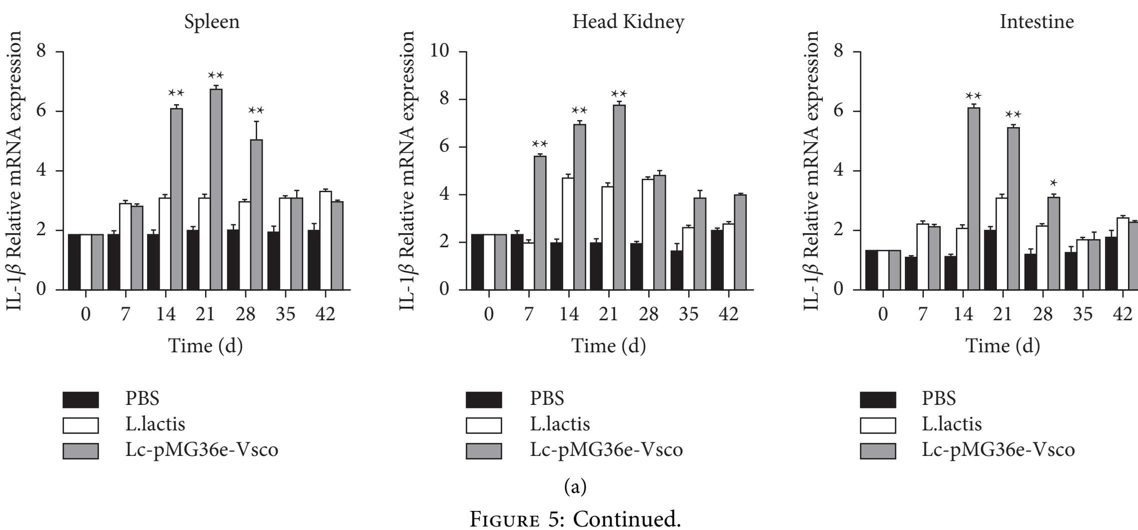


Figure 4: Antibody titers in sera of grouper immunized with PBS, *L. lactis*, and Lc-pMG36e-vscO. Sera collected at week 1–7 after vaccination. Each data column represented the mean of $\log_2$ (antibody titers) with standard error bar. The asterisks indicated significant differences ($^{*}P < 0.05$; $^{**}P < 0.01$) between the controls and immunized group.



(a)

Figure 5: Continued.

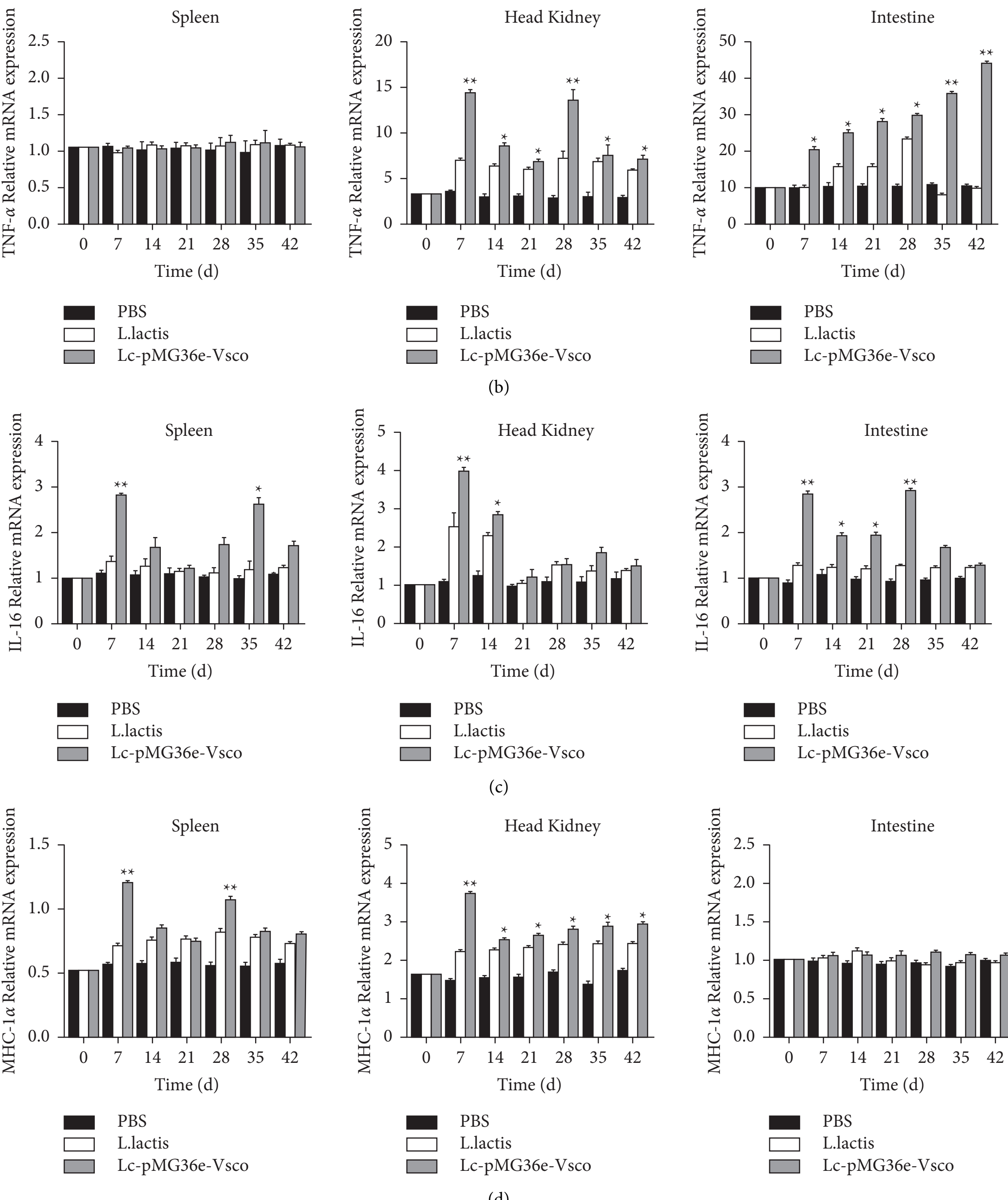


Figure 5: qRT-PCR analysis of the expression levels of immune-related genes in spleen, head kidney, and intestine of grouper ($n = 4$ fish/group) after immunization. (a) IL-1β; (b) TNF-α; (c) IL-16; (d) MHC-1α. $^{*}P < 0.05$, $^{**}P < 0.01$.

experimental vaccines against *V. alginolyticus* have been developed [12, 27, 28]. However, these vaccines have not been used on a large scale because of the stress induced during the vaccination or side effects post vaccination in practical application. Therefore, an oral vaccine with fewer side effects aroused widespread concern [11].

Many pathogenic microorganisms initiate their infectious cycle at the mucosal surfaces of fish. Appropriate mucosal stimulation can evoke the effective protection of mucosal surfaces from colonization and invasion by infectious pathogens [29]. Therefore, the vaccine antigens need to be delivered to various mucosal sites [29]. Probiotics as heterologous antigen carriers can confer protective immunity, which is a potential way to develop a mucosal vaccine [18, 24, 30]. In this study, *L. lactis* was isolated from the intestine of pearl gentian grouper and it can adhere to the

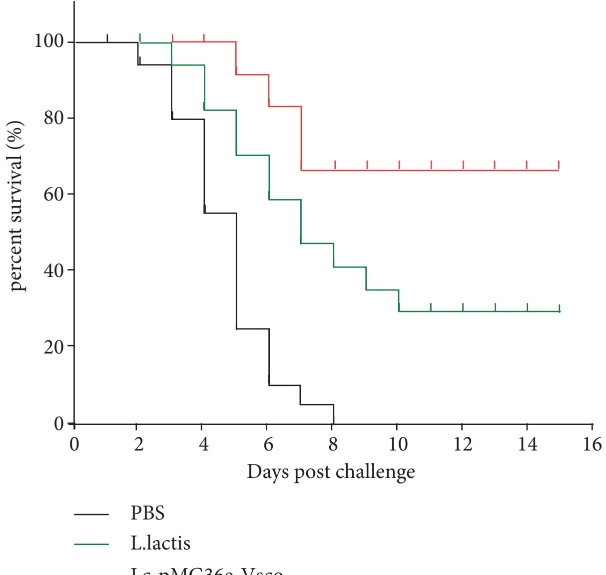


Figure 6: Survival rate of grouper after challenge with *V. alginolyticus* post immunization. 20 fish/group were used to record percent survival for 15 days. The experiments were replicated in triplicate.

mucosal surfaces of the intestine of fish, which is conducive to the delivery of the antigen protein expressed in *L. lactis* to the mucosal surfaces of the intestine. The antigen molecules used in vaccine development must have high conservation and immunogenicity and can be easily recognized by antigen-presenting cells [31, 32]. Outer membrane proteins (OmpU, OmpK, and OmpW) of *V. alginolyticus* are very antigenic and can be used as vaccine candidates [21, 32]. It is sufficiently evident that the VscO protein of *V. alginolyticus*has good immunogenicity and may be used as a candidate antigen for developing a vaccine [12]. In this study, a recombinant *L. lactis* expressing *V. alginolyticus* VscO protein is constructed. The recombinant plasmid was stably inherited, and the VscO protein was stably expressed on *L. lactis*. The result is consistent with other reports [16, 18, 19].

Oral immunization with a recombinant vaccine could induce a specific immune response at the mucosal and systemic levels [18, 20]. In the present study, the immunogenicity of Lc-pMG36e-VscO was evaluated by inoculation of grouper via oral immunization. The results showed that Lc-pMG36e-VscO strain has the capacity to induce a higher serum antibody titer when compared with the control *L. lactis* strain and PBS group. A comparison of the means of $\log_2$ (antibody titer) in both the control *L. lactis* and PBS group revealed that they were not significantly different ($P > 0.05$). Fish immunized with the rOmpC-vaccinated produced specific anti-rOmpC antibodies with a significant antibody titer [19]. The antibody level of the immunized fish with the recombinant vaccine was significantly higher than that in the control group [18, 20]. Higher antibody titers suggested that antigen induced the release of specific IgM and activated the adaptive immunity of fish.

Humoral and mucosal immune responses were demonstrated to eliminate pathogens in fish [33]. Cytokines also play a key role in the regulation of the inflammatory process and serve as an important component of innate immunity. In the present study, the expression of immune-related genes (IL-1β, IL-16, TNF-α, and MHC-1α) in the spleen, kidney, and gut of fish immunized with the Lc-pMG36e-vscO were upregulated compared with the control group. This indicates that oral immunization with Lc-pMG36e-vscO induced a higher immune response in fish. Immunized fish (*Carassius auratus*) with recombinant *L. casei* expressing AcrV protein of *A. veronii* upregulated the expression of IL-1β, TNF-α, IFN-γ, and IL-10 [34]. The expression of IL-10, IL-β, IFN-γ, and TNF-α genes in *Cyprinus carpio* immunized with recombinant *L. casei* was significantly upregulated [16]. The expression of part of immune-related genes in the main tissues of fish fed with *L. lactis* was also up-regulated. *L. casei*, as a probiotic, can stimulate the innate immune response in fish by increasing the expression of IL-1β and IFN-γ [34]. Live *L. lactis* can significantly enhance the levels of TNF-α, IL-1β, and IL-6 in *C. carpio* [15]. The cellular components of probiotics could stimulate immune-related gene expression and may be useful as adjuvants for vaccines in aquaculture [35]. These results indicated that recombinant vaccines can trigger an inflammatory response and cellular immune response, and probiotics also play an immunomodulatory role.

In the challenge experiment, the data showed that the survival rates in the Lc-pMG36e-VscO group were significantly higher than those in the *L. lactis* group and PBS group ($P < 0.05$). This result was consistent with similar studies involving recombinant OmpAI and flagellin A protein, and OmpC of *A. veronii* obtained high survival rates [16, 18, 19]. We hypothesized that Lc-pMG36e-vscO can arrive at the mucosal surfaces of the gut and produce antibodies or other inhibitory substances as a barrier against *V. alginolyticus*.

5. Conclusions

In conclusion, the Lc-pMG36e-vscO expressing VscO protein has good immunogenicity. Oral immunization with Lc-pMG36e-VscO in grouper stimulated high serum specific antibody titers and elicited immune response and conferred efficient protection against *V. alginolyticus*. These results indicated that recombinant *L. lactis* may be promising as a candidate vaccine against.*V. alginolyticus*.

Data Availability

All the data that support the findings of this study are included in this published article and available from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Shiping Yang conceptualized the study, curated the data, and wrote the original draft. Yuanzhi Wu curated the data and wrote the original draft. Yucong Huang curated and validated the data. Shaohong Ma designed the methodology.

Guangben Wei provided the resources. Jichang Jian conceptualized the study. Shuanghu Cai conceptualized the study, reviewed and edited the study.

Acknowledgments

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References

[1] N. Mohamad, M. N. A. Amal, I. S. M. Yasin et al., "Vibriosis in cultured marine fishes: a review," *Aquaculture*, vol. 512, Article ID 734289, 2019.

[2] A. Ben Kahla-Nakbi, K. Chaieb, and A. Bakhrouf, "Investigation of several virulence properties among *Vibrio alginolyticus* strains isolated from diseased cultured fish in Tunisia," *Diseases of Aquatic Organisms*, vol. 86, no. 1, pp. 21–28, 2009.

[3] K. K. Lee, S. R. Yu, T. I. Yang, P. C. Liu, and F. R. Chen, "Isolation and characterization of *Vibrio alginolyticus* isolated from diseased kuruma prawn, *Penaeus japonicus*," *Letters in Applied Microbiology*, vol. 22, no. 2, pp. 111–114, 1996.

[4] S. R. Krupesha Sharma, G. Rathore, D. K. Verma, N. Sadhu, and K. K. Philipose, "*Vibrio alginolyticus* infection in Asian seabass (*Lates calcarifer*, Bloch) reared in open sea floating cages in India," *Aquaculture Research*, vol. 44, no. 1, pp. 86–92, 2012.

[5] K. C. Lim, F. M. Yusoff, M. Shariff, and M. S. Kamarudin, "Dietary astaxanthin augments disease resistance of Asian seabass, *Lates calcarifer* (Bloch, 1790) against *Vibrio alginolyticus* infection," *Fish & Shellfish Immunology*, vol. 114, pp. 90–101, 2021.

[6] B. Yang, S. Zhai, X. Li et al., "Identification of *Vibrio alginolyticus* as a causative pathogen associated with mass summer mortality of the Pacific Oyster (*Crassostrea gigas*) in China," *Aquaculture*, vol. 535, Article ID 736363, 2021.

[7] F. Wang, L. Huang, M. Liao et al., "Integrative analysis of the miRNA–mRNA regulation network in hemocytes of *Penaeus vannamei* following *Vibrio alginolyticus* infection," *Developmental & Comparative Immunology*, vol. 131, Article ID 104390, 2022.

[8] T. Bryant, S. Ellenwood, O. Butters, and F. M. Saccoccio, "An uncommon cause of soft tissue and knee infection after penetrating injury in a non-immunocompromised adolescent male," *SAGE Open Medical Case Reports*, vol. 9, Article ID 2050313X2110346, 2021.

[9] M. Y. Ina-Salwany, N. Al-Saari, A. Mohamad et al., "Vibriosis in fish: a review on disease development and prevention," *Journal of Aquatic Animal Health*, vol. 31, no. 1, pp. 3–22, 2019.

[10] J. Dubert, C. R. Osorio, S. Prado, and J. L. Barja, "Persistence of antibiotic resistant *Vibrio* spp. in shellfish hatchery environment," *Microbial Ecology*, vol. 72, no. 4, pp. 851–860, 2016.

[11] Y. Muktar, S. Tesfaye, and B. Tesfaye, "Present status and future prospects of fish vaccination: a review," *Journal of Veterinary Science & Technology*, vol. 07, no. 2, Article ID 1000299, 2016.

[12] Z. Zhou, H. Pang, Y. Ding et al., "VscO, a putative T3SS chaperone escort of *Vibrio alginolyticus*, contributes to virulence in fish and is a target for vaccine development," *Fish & Shellfish Immunology*, vol. 35, no. 5, pp. 1523–1531, 2013.

[13] M. A. Dawood, S. Koshio, M. Ishikawa et al., "Effects of dietary supplementation of *Lactobacillus rhamnosus* or/and *Lactococcus lactis* on the growth, gut microbiota and immune responses of red sea bream, *Pagrus major*," *Fish & Shellfish Immunology*, vol. 49, pp. 275–285, 2016.

[14] S. Won, A. Hamidoghli, W. Choi et al., "Evaluation of potential probiotics *Bacillus subtilis* wb60, *Pediococcus pentosaceus*, and *Lactococcus lactis* on growth performance, immune response, gut histology and immune-related genes in whiteleg shrimp, *Litopenaeus vannamei*," *Microorganisms*, vol. 8, no. 2, p. 281, 2020.

[15] J. Wang, J. Feng, S. Liu et al., "The probiotic properties of different preparations using *Lactococcus lactis* Z-2 on intestinal tract, blood and hepatopancreas in *Cyprinus carpio*," *Aquaculture*, vol. 543, Article ID 736911, 2021.

[16] D. X. Zhang, Y. H. Kang, L. Chen et al., "Oral immunization with recombinant *Lactobacillus casei* expressing OmpAI confers protection against *Aeromonas veronii* challenge in common carp, *Cyprinus carpio*," *Fish & Shellfish Immunology*, vol. 72, pp. 552–563, 2018.

[17] J. H. W. M. Rombout, G. Yang, and V. Kiron, "Adaptive immune responses at mucosal surfaces of teleost fish," *Fish & Shellfish Immunology*, vol. 40, no. 2, pp. 634–643, 2014.

[18] J. X. Tian, Y. H. Kang, G. S. Chu et al., "Oral Administration of *Lactobacillus casei* expressing Flagellin A protein confers effective protection against *Aeromonas veronii* in common carp, *Cyprinus carpio*," *International Journal of Molecular Sciences*, vol. 21, no. 1, p. 33, 2019.

[19] S. K. Yadav, P. Dash, P. K. Sahoo, L. C. Garg, and A. Dixit, "Recombinant outer membrane protein OmpC induces protective immunity against *Aeromonas hydrophila* infection in *Labeo rohita*," *Microbial Pathogenesis*, vol. 150, Article ID 104727, 2021.

[20] J. Y. Yao, X. M. Yuan, Y. Xu et al., "Live recombinant *Lactococcus lactis* vaccine expressing immobilization antigen (i-Ag) for protection against *Ichthyophthirius multifiliis* in goldfish," *Fish & Shellfish Immunology*, vol. 58, pp. 302–308, 2016.

[21] S. H. Cai, Y. S. Lu, J. C. Jian et al., "Protection against *Vibrio alginolyticus* in crimson snapper *Lutjanus erythropterus* immunized with a DNA vaccine containing the *ompW* gene," *Diseases of Aquatic Organisms*, vol. 106, no. 1, pp. 39–47, 2013.

[22] X. L. Hou, L. Y. Yu, J. Z. Liu, and G. H. Wang, "Surface-displayed porcine epidemic diarrhea viral (PEDV) antigens on lactic acid bacteria," *Vaccine*, vol. 26, no. 1, pp. 24–31, 2007.

[23] S. Xu, D. Wang, P. Zhang et al., "Oral administration of *Lactococcus lactis*-expressed recombinant porcine epidermal growth factor stimulates the development and promotes the health of small intestines in early-weaned piglets," *Journal of Applied Microbiology*, vol. 119, no. 1, pp. 225–235, 2015.

[24] B. F. Song, L. Z. Ju, Y. J. Li, and L. J. Tang, "Chromosomal insertions in the *Lactobacillus casei* upp gene that are useful for vaccine expression," *Applied and Environmental Microbiology*, vol. 80, no. 11, pp. 3321–3326, 2014.

[25] J. Xie, L. Bu, S. Jin et al., "Outbreak of vibriosis caused by *Vibrio harveyi* and *Vibrio alginolyticus* in farmed seahorse *Hippocampus kuda* in China," *Aquaculture*, vol. 523, Article ID 735168, 2020.

[26] M. Abdelsalam, M. Ewiss, H. S. Khalefa, M. A. Mahmoud, M. Y. Elgendy, and D. A. Abdel-Moneam, "Coinfections of *Aeromonas* spp. *Enterococcus faecalis*, and *Vibrio alginolyticus* isolated from farmed Nile tilapia and African catfish in Egypt,

with an emphasis on poor water quality," *Microbial Pathogenesis*, vol. 160, Article ID 105213, 2021.

[27] J. Li, S. Ma, and N. Y. S. Woo, "Vaccination of silver sea bream (*Sparus sarba*) against *Vibrio alginolyticus*: protective evaluation of different vaccinating modalities," *International Journal of Molecular Sciences*, vol. 17, pp. 40–14, 2015.

[28] R. Nehlah, M. Firdaus-Nawi, N. Y. Nik-Haiha, M. Karim, M. Zamri-Saad, and M. Y. Ina-Salwany, "Recombinant vaccine protects juvenile hybrid grouper, *Epinephelus fuscoguttatus* × Epinephelus lanceolatus, against infection by," *Aquaculture International*, vol. 25, no. 6, pp. 2047–2059, 2017.

[29] H. Chen, "Recent advances in mucosal vaccine development," *Journal of Controlled Release*, vol. 67, no. 2-3, pp. 117–128, 2000.

[30] H. Fan, X. Wu, F. Yu, Y. Bai, and B. Long, "Oral immunization with recombinant *Lactobacillus acidophilus* expressing the adhesin Hp0410 of *Helicobacter pylori* induces mucosal and systemic immune responses," *Clinical and Vaccine Immunology*, vol. 21, no. 2, pp. 126–132, 2014.

[31] R. Khushiramani, S. K. Girisha, I. Karunasagar, and I. Karunasagar, "Cloning and expression of an outer membrane protein ompTS of *Aeromonas hydrophila* and study of immunogenicity in fish," *Protein Expression and Purification*, vol. 51, no. 2, pp. 303–307, 2007.

[32] R. Nehlah, M. Ina-Salwan, and Z. Zulperi, "Antigenicity analysis and molecular characterization of two outer membrane proteins of Vibrio alginolyticus strain VA2 as vaccine candidates in tiger grouper culture," *Journal of Biological Sciences*, vol. 16, no. 1-2, pp. 1–11, 2015.

[33] D. Gomez, J. O. Sunyer, and I. Salinas, "The mucosal immune system of fish: the evolution of tolerating commensals while fighting pathogens," *Fish & Shellfish Immunology*, vol. 35, no. 6, pp. 1729–1739, 2013.

[34] Y. Kong, M. Li, J. Tian et al., "Effects of recombinant *Lactobacillus casei* on growth performance, immune response and disease resistance in crucian carp, *Carassius auratus*," *Fish & Shellfish Immunology*, vol. 99, pp. 73–85, 2020.

[35] S. S. Giri, S. S. Sen, C. Chi et al., "Effect of cellular products of potential probiotic bacteria on the immune response of *Labeo rohita* and susceptibility to *Aeromonas hydrophila* infection," *Fish & Shellfish Immunology*, vol. 46, no. 2, pp. 716–722, 2015.