Fish and Shellfish Immunology 87 (2019) 772–777

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Phenotypic characterization, virulence, and immunogenicity of *Pseudomonas plecoglossicida rpoE* knock-down strain

Lixing Huang[a,*], Youyu Zhang[b,1], Rongchao He[a], Zhenghong Zuo[c], Zhuhua Luo[d], Wei Xu[d], Qingpi Yan[a,**]

[a] Fisheries College, Key Laboratory of Healthy Mariculture for the East China Sea, Ministry of Agriculture, Jimei University, Xiamen, Fujian, PR China
[b] Institute of Electromagnetics and Acoustics, School of Electronic Science and Engineering, Xiamen University, Xiamen, Fujian, PR China
[c] School of Life Sciences, Xiamen University, Xiamen, Fujian, PR China
[d] Third Institute of Oceanography, State Oceanic Administration, Xiamen, 361005, PR China

ARTICLE INFO

Keywords:
Pseudomonas plecoglossicida
rpoE
Virulence
Live attenuated vaccine
Epinephelus coioides

ABSTRACT

Pseudomonas plecoglossicida, a temperature dependent bacterial pathogen in fish, expresses *rpoE* gene that is sensitive to temperature and probably critical for pathogen virulence and disease development. In this study, the *rpoE* silence strain *rpoE*-RNAi-1 was constructed by gene knock-down. The *rpoE*-RNAi-1 displayed significant changes in biofilm formation, swarming motility, adhesion and virulence. Meanwhile, vaccination of grouper with *rpoE*-RNAi-1 led to a relative percent survival (RPS) value of 85% after challenged with the wild-type *P. plecoglossicida*. qRT-PCR assays showed that vaccination with *rpoE*-RNAi-1 enhanced the expression of immune-related genes, including *MHC-I*, *MHC-II*, *IgM*, and *IL-1β*, indicating that it was able to induce humoral and cell-mediated immune response in grouper. These results validated the possibility of *rpoE* as a potential target for constructing *P. plecoglossicida* live attenuated vaccine.

1. Introduction

Pseudomonas plecoglossicida, first isolated from ayu (*Plecoglossus altivelis*), is the cause of bacterial hemorrhagic ascites in freshwater fish. To date, infection of ayu [1], large yellow croaker (*Pseudosciaena crocea*) [2–4], orange spotted grouper (*Epinephelus coioides*) [5,6], and rainbow trout (*Oncorhynchus mykiss*) [7] with *P. plecoglossicida* has been reported. Outbreaks of *P. plecoglossicida* infection in cage-farmed *P. crocea*, which is characterized by white nodules in the internal organs of infected fish (including kidney, spleen and liver) and causes high mortality, have led to severe economic losses in the Fujian and Zhejiang provinces of China [8]. Therefore, it is of great significance to study the factors affecting the virulence of *P. plecoglossicida*.

Outbreaks of *P. plecoglossicida* infection are mainly recorded when the seawater temperature was between 15 °C and 20 °C [9]. Our previous research further proved that *P. plecoglossicida* was a temperature-dependent facultative pathogen by sequencing the transcriptome of *P. plecoglossicida* incubated under virulent (18 °C) and avirulent (28 °C) temperatures, and showed that *rpoE* was significantly high expressed under 18 °C [10].

RpoE is a kind of extracytoplasmic function (ECF) sigma factor, which could regulate gene expression in response to environmental stimuli, such as temperature [11]. RpoE is found to be involved in the regulation of virulence at the transcriptional level in some bacterial pathogens. For example, Dou et al. [12] have confirmed that RpoE plays an important role in the virulence regulatory network of *Porphyromonas gingivalis*, including the regulation of type IX secretion system genes and several virulence genes. Zhang et al. [13] have proved that RpoE could promote invasion and intracellular survival by regulating the expression of SPI-1 and SPI-2 in *Salmonella enterica* serovar Typhi. Barchinger et al. [14] have revealed that RpoE could facilitate survival of membrane perturbations and is required for virulence in *Bordetella bronchiseptica*. Hanawa et al. [15] have also reported that RpoE influences alleviation of membrane stress in *Bordetella pertussis*. Together with our previous results of RNA-seq, these indicated that RpoE probably play an important role in the pathogenesis of *P. plecoglossicida*, but the specific mechanism is not clear. However, no research on function of RpoE in *P. plecoglossicida* has been reported.

For better understanding of the function of RpoE in *P. plecoglossicida*, we constructed an *rpoE* silence strain via gene knock-down, then

* Corresponding author. Fisheries College, Jimei University, Yindou Street 43, Xiamen, Fujian, 361021, PR China.
** Corresponding author. Fisheries College, Jimei University, Yindou Street 43, Xiamen, Fujian, 361021, PR China.
E-mail addresses: lixinghuang@outlook.com (L. Huang), yanqp@jmu.edu.cn (Q. Yan).
[1] Co-first author.

https://doi.org/10.1016/j.fsi.2019.02.028
Received 5 December 2018; Received in revised form 13 February 2019; Accepted 15 February 2019
Available online 16 February 2019
1050-4648/

investigated the physiology and pathogenicity of the *rpoE*-RNAi-1 strain. Moreover, we evaluated the immunoprotective potential of *rpoE*-RNAi-1 strain, and revealed that *rpoE* possess the potential of being a target to construct live attenuated vaccine to combat *P. plecoglossicida* in *E. coioides*.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The pathogenic *P. plecoglossicida* strain (NZBD9) was isolated from the spleen of naturally infected large yellow croaker with white-spot disease [16]. The NZBD9 strain was routinely grown in LB (Luria Bertani) medium at 18 or 28 °C with shaking at 220 rpm. *E. coli* DH5α was obtained from TransGen Biotech (Beijing, China), which was grown in LB medium (37 °C, 220 rpm).

2.2. Construction of rpoE knock down strain

RNAi strain was constructed according to methods described before [5]. Five short hairpin RNA sequences targeting *rpoE* were designed respectively and synthetized by Shanghai Generay Biotech Co., Ltd. (Shanghai, China) (Table S1). After linearizing pCM130/tac vectors with the restriction enzymes *Nsi*I and *Bsr*GI (New England Biolabs, U.S.A), the oligonucleotides were annealed and ligated to the linearized pCM130/tac vectors using T4 DNA ligase (New England Biolabs) following the manufacturer's recommendations. The recombinant pCM130/tac vectors were transformed into the competent *E. coli* DH5a cells by heat shock and then were extracted and electroporated into *P. plecoglossicida*. Finally, the expression level of *rpoE* of each RNAi strain was detected by quantitative real-time PCR (qRT-PCR).

2.3. Characterization of the silence strain of rpoE

The *rpoE* silence strain was characterized by biofilm formation, motility, and *in vitro* adhesion. The biofilm assay for *P. plecoglossicida* was performed using the crystal violet stain method as described by Huang et al. [10], and then quantitated by measuring OD_{590} nm, while six independent biological replicates were performed for each data point. For the assay of motility of *P. plecoglossicida* strains, the soft agar method was adapted [17], while the diameters of the colonies were measured at 24 h (n = 6). *In vitro* adhesion was measured according to a procedure reported by Kong et al. [18], while the stained adherent bacteria cells were counted by microscopy (× 1000) (n = 6).

2.4. Artificial infection and sampling

All animal experiments were carried out strictly under the recommendations in the 'Guide for the Care and Use of Laboratory Animals' set by the National Institutes of Health. The animal protocols were approved by the Animal Ethics Committee of Jimei University (Acceptance NO JMULAC201159).

Healthy *E. coioides* (average weight 17.4 ± 1.2 g) were obtained from Zhangzhou (Fujian, China) and acclimatized at 18 °C for one week under specific pathogen-free laboratory conditions. For virulence comparison through survival assays, twenty *E. coioides* were injected intraperitoneally with 100 μL 10^4-10^9 cfu/mL wild-type or *rpoE*-RNAi-1 strain suspended in sterile phosphate buffered saline (PBS). *E. coioides* intrapleurally injected with PBS were used as a negative control. The fish were monitored for 14 days at 18 ± 1 °C and observed for morbidity and mortality daily.

For detection of *rpoE* during the infection process, the spleens of six weight-matched *E. coioides* infected with 10^3 cfu/g wild type strain *P. plecoglossicida* were sampled at each time point including 1, 3, 6, 12, 24, 36, 48, 60, 72, 84, and 96 hpi (hours post injection).

2.5. E. coioides vaccination

One hundred *E. coioides* (average weight 17.4 ± 1.2 g) were randomly divided into two groups and intraperitoneally injected with 100 μL 10^5 cfu/mL *rpoE*-RNAi-1 strain or equal volume of PBS. All the fish were maintained at 18 °C. Six *rpoE*-RNAi-1 strain infected *E. coioides* spleens were sampled at 48 hpi for qRT-PCR analysis of the expression of immune-related genes. Three *rpoE*-RNAi-1 and PBS injected *E. coioides* spleens were aseptically collected from day 1 to day 7. All the samples were weighed and homogenized in 1 ml PBS. The homogenates were serially diluted and plated in triplicate onto LB plates (10 μg/ml Tetracycline) and incubated at 28 °C for 18 h. The bacteria counts were calculated by dividing the weights of the tissues and from the mean of three samples.

Four weeks after vaccination, 30 *E. coioides* per group were intraperitoneally injected with 100 μL 1 × 10^8 cfu/mL of wild-type strain. The water temperature during infection was 18 ± 1 °C. The daily mortality of infected *E. coioides* was recorded.

2.6. RNA isolation

TRIzol reagent (Invitrogen, USA) was used for total RNA extraction from bacterial cells, as directed by the manufacturer. Reverse transcription was carried out with A Reverse Aid Mu-MLV cDNA synthesis kit (TransGen Biotech, China) from 2.0 mg total RNA, as instructed by the manufacturer [19,20].

2.7. qRT-PCR

qRT-PCR was carried out using a QuantStudio 6 Flex real-time PCR system (Life Technologies, U.S.A). All primer sequences are provided in Table S2. The expression of bacterial genes was normalized using *16s rRNA*. In *E. coioides*, the expression of genes was normalized to *β-actin*. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative level of gene expression [21,22].

2.8. Statistical analysis

Data were presented as mean ± standard deviation (SD) and assessed with SPSS 18.0. Differences were determined by analysis of one-way ANOVA followed by the Dunnett's multiple comparison test. $P < 0.05$ indicated statistical significance.

3. Results

3.1. Validation of rpoE expression under virulent and avirulent temperatures, and during the infection process

Expression levels of *rpoE* under virulent (18 °C) and avirulent (28 °C) temperatures were assessed by qRT-PCR. The results showed that the expression level of *rpoE* under 18 °C was much higher than that under 28 °C, which reinforced the reliability of the previous sequencing data (Fig. 1). In addition, we used qRT-PCR to detect the expression of *rpoE* gene with the progressing of infection. The expression level of *rpoE* increased with the progressing of infection and reached its peak at 48 h after infection, then gradually decreased (Fig. 2). These results indicated that *rpoE* was closely related to virulence regulation and environment response of *P. plecoglossicida*.

3.2. Construction of the rpoE-RNAi strains

Five *rpoE* stable silence strains were constructed, and the expression level of *rpoE* was detected. The results showed that the expression level of *rpoE* in the silence strains decreased significantly, indicating that the silence strains were successfully constructed (Fig. 3). Among them, the *rpoE*-RNAi-1 strain exhibited the best efficiency of gene silencing, so it

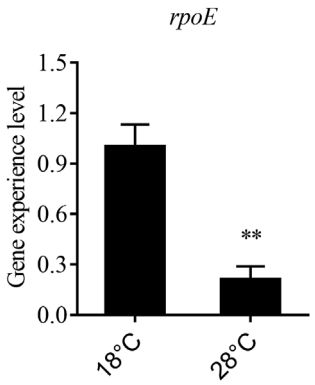

Fig. 1. qRT-PCR analysis of the expression of *rpoE* under different temperatures. The data are presented as the means ± S.D. (n = 6). The means of the treatments not sharing a common letter are significantly different at $P < 0.05$.

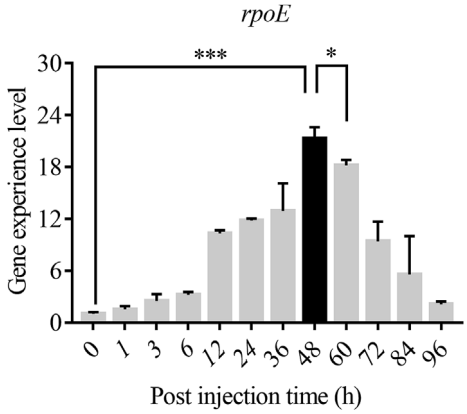

Fig. 2. qRT-PCR analysis of the expression level of *rpoE* in the wild type *P. plecoglossicida* infected *E. coioides* spleen at different stages of infection. Data are presented as mean ± S.D. (n = 3). *$P < 0.05$, ***$P < 0.001$.

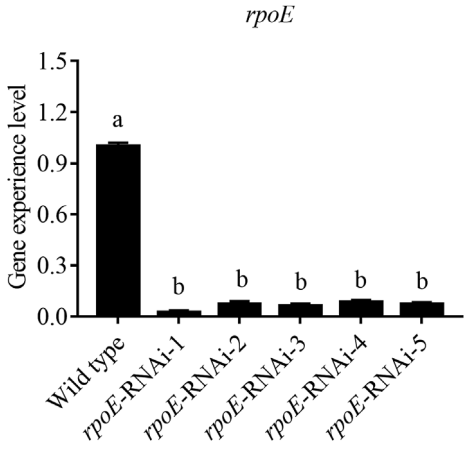

Fig. 3. Construction of *rpoE*-RNAi strains of *P. plecoglossicida*. The *rpoE* expression levels of 5 *rpoE*-RNAi silence strains are shown as means ± SD from three independent biological replicates. Means of treatments not sharing a common letter are significantly different at $P < 0.05$.

was selected as the object of further research.

3.3. Biofilm formation, motility, adhesion and virulence of rpoE silence strain

Comparison of biofilm formation between wild-type and *rpoE*-RNAi-1 strain showed that, the biofilm production of *rpoE*-RNAi-1 was 6.75 folds lower than that of the wild-type (Table 1). So, *rpoE* probably play a role in the biofilm development of *P. plecoglossicida*. Meanwhile, *rpoE*-RNAi-1 displayed a significantly decreased motility and adhesion ability compared with the wild-type (Table 1), which indicated that *rpoE* took part in the regulation of motility and adhesion. Furthermore, the virulence of *rpoE*-RNAi-1 was significantly decreased (Fig. 4). No clinical symptoms or mortalities were detected within 2 weeks following challenge of the fish with doses less than 10^5 cfu/mL of *rpoE*-RNAi-1 strain. These results indicated that the *rpoE* gene contributes to the pathogenesis of *P. plecoglossicida*.

Table 1
Characteristics of *rpoE*-RNAi-1 strain.

Characteristics	Wild type	*rpoE*-RNAi-1
Biofilm formation (OD_{570})	0.81 ± 0.12	0.12 ± 0.03**
Motility (mm)	7.2 ± 0.15	1.3 ± 0.11**
Adhesion (cells per field of view)	1322 ± 59	217 ± 11**

$P < 0.01$.

3.4. Gene expression in rpoE knock-down strain

qRT-PCR was carried out to analyze the transcription levels of virulence genes including *rpoA*, *aspA*, *ompW* and *fliA*, which were proved to be regulated by *rpoE* in other bacteria [13,23]. The results showed that all four genes were significantly reduced in *rpoE*-RNAi-1 strain (Fig. 5), which might be an explanation for the decreased biofilm formation, motility, adhesion and virulence of *rpoE* silence strain.

3.5. Investigation of the livability of rpoE-RNAi-1 strain in vivo

rpoE-RNAi-1 strain was able to disseminate into but survive transiently in fish spleen then was gradually eliminated from the host body (Fig. 6). The highest bacterial number was detected in spleen on day 3.

3.6. Expressing of immune-related genes in E. coioides after vaccination with rpoE-RNAi-1 strain

qRT-PCR was carried out to analyze the transcription levels of genes encoding *MHC-I*, *MHC-II*, *IgM* and *IL-1β* in the spleen of *E. coioides*. The results showed that all four genes were significantly induced by *rpoE*-RNAi-1 strain (Fig. 7). The results suggested that *rpoE*-RNAi-1 strain can effectively elicit protective immune responses in *E. coioides*.

3.7. Immune protective effects of rpoE-RNAi-1 strain in E. coioides

The immune protection effect of *rpoE*-RNAi-1 was evaluated by injecting wild-type *P. plecoglossicida* into *E. coioides* which survived after injected with PBS and *rpoE*-RNAi-1 strain, respectively. The results indicated that most of the *E. coioides* with *rpoE*-RNAi-1 inoculation survived after injection with *P. plecoglossicida* wild-type, and the immune protection rate was as high as 85%, whereas mortality in the group administered PBS was 100% (Fig. 8). The results suggested that *rpoE* may be a potential target for constructing *P. plecoglossicida* live attenuated vaccine.

4. Discussion

As an enigmatic protein, RpoE has been studied for more than 30 years for various organisms, but its function is still not well understood [24]. Zhang et al. [13] performed proteomics analysis in the *rpoE* mutant *S. typhi* and observed that many of the known virulence factors were affected, including DNA-directed RNA polymerase subunit α (RpoA), aminoacyl-histidine dipeptidase precursor (PepD), D-mannonate oxidoreductase (UxuB), aspartate ammonia-lyase (AspA), outer

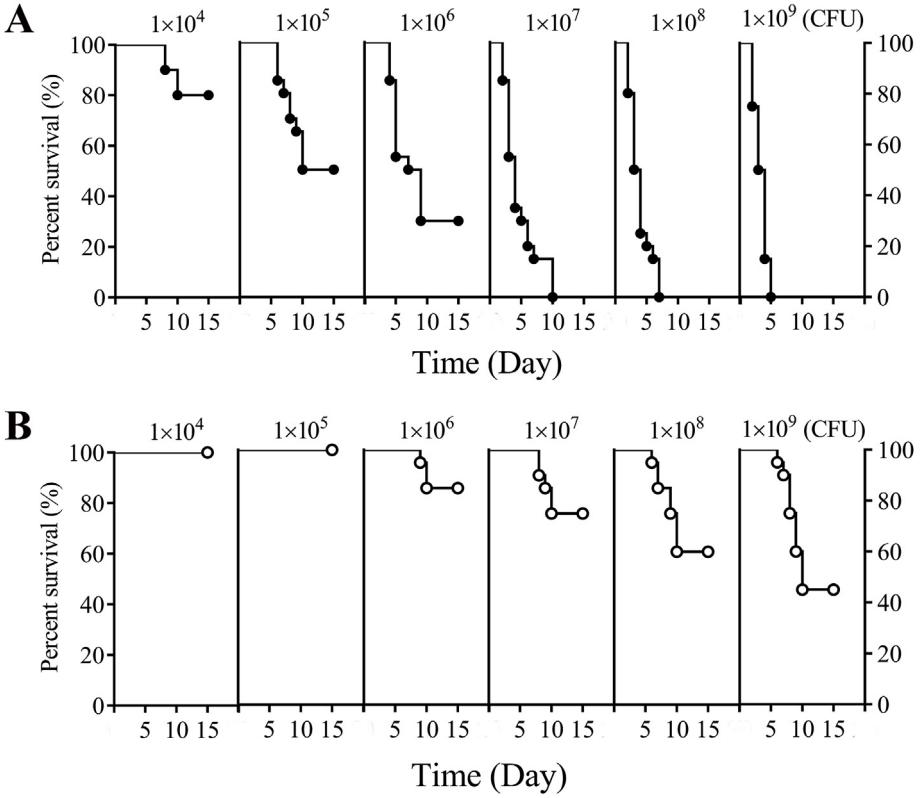

Fig. 4. Survival curves of *E. coioides* challenged with *P. plecoglossicida* wild-type **(A)** and *rpoE*-RNAi-1 **(B)**. *E. coioides* were challenged with bacteria at indicated doses and monitored daily for 14 days as described in the section of Materials and methods.

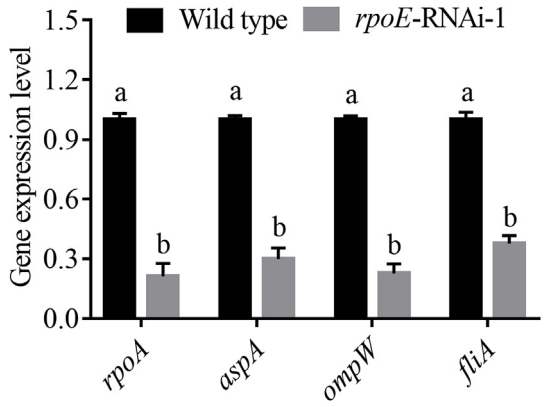

Fig. 5. qRT-PCR analysis of the expression of *rpoE*-regulated virulence genes in *P. plecoglossicida* wild-type and *rpoE*-RNAi-1. The data are presented as the means ± S.D. (n = 6). The means of the treatments not sharing a common letter are significantly different at $P < 0.05$.

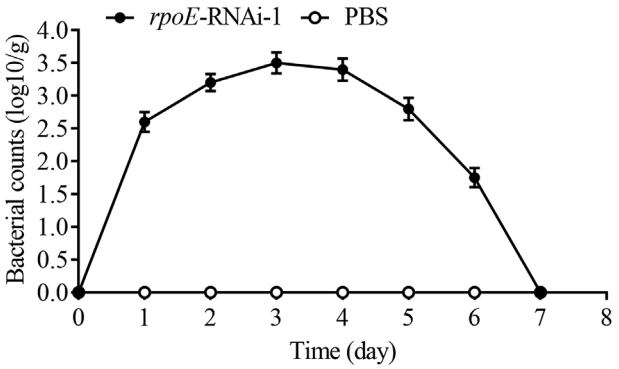

Fig. 6. Propagation of *rpoE*-RNAi-1 in *E. coioides* spleen after injection. Control fish were injected with equal volume of sterile PBS. The number of viable bacteria was shown as the mean ± standard of three samples.

membrane protein W (OmpW), outer membrane protein X (OmpX), and ATP-dependent Clp protease proteolytic subunit (ClpP). Among these virulence factors, our genomic analysis suggested that *P. plecoglossicida* possesses RpoA, AspA, and OmpW [9], all of which were proved to be associated with biofilm production and adhesion [25–30]. Zhang et al. [23] proved that RpoE could initiate *fliA* expression and promote the motility of *S. typhi*, while our genomic analysis suggested that *P. plecoglossicida* also possesses *fliA*. As we know, bacterial adhesion to host surfaces is one of the initial steps in the infection process, which has a close connection with the motility [31,32]. Host mucus is abundantly found on the surface of the skin, gills, and gut lining; therefore, it is the first site of interaction between the pathogen and its host [33]. After adhesion to its host, bacteria are possible to start the invasion, besides, they should try to protect themselves against the host immune system during this process. Therefore various ways were developed, such as biofilm production [34–36]. Our results showed that there were significant differences between wild-type and *rpoE*-RNAi-1 strain in motility, adhesion and biofilm production. Meanwhile, our results indicated that *P. plecoglossicida* with knock down of *rpoE* displayed decreased virulence in *E. coioides*, which was consistent with reports on the virulence regulation by *rpoE* in other pathogenic bacteria [12]. Therefore, our results revealed that *rpoE* contributed in multistep of *P. plecoglossicida* pathogenesis, which was probably mediated by these *rpoE*-regulated virulence genes including *rpoA*, *aspA*, *ompW* and *fliA*. Therefore, qRT-PCR was carried out to analyze the transcription levels of *rpoA*, *aspA*, *ompW* and *fliA*. The results showed that all four genes were significantly reduced in *rpoE*-RNAi-1 strain, which might be an explanation for the decreased biofilm formation, motility, adhesion and virulence of *rpoE* silence strain.

Live attenuated vaccines is found to induce a more robust cell-

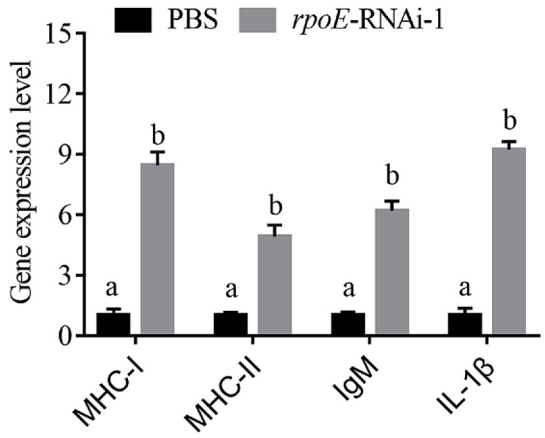

Fig. 7. qRT-PCR analysis of the expression of immune-related genes in *rpoE*-RNAi-1 strain infected *E. coioides* spleen at 48 hpi. The data are presented as the means ± S.D. (n = 6). The means of the treatments not sharing a common letter are significantly different at $P < 0.05$.

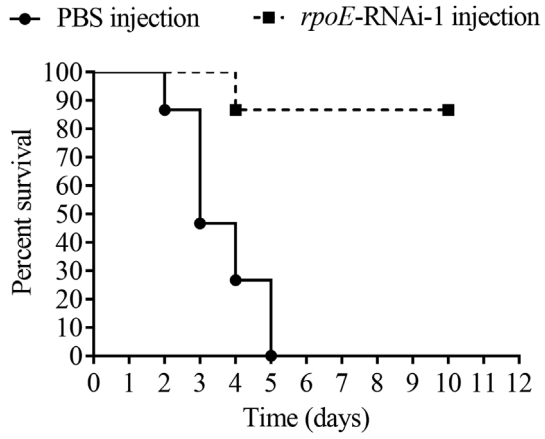

Fig. 8. Survival in *E. coioides* vaccinated with *rpoE*-RNAi-1 strain and PBS following challenge with wide-type *P. plecoglossicida*.

mediated and humoral immune response than inactivated vaccine [37]. MHC I and II are found in the jawed vertebrates which can display antigen from within the cell to cytotoxic T cells and thus trigger an immediate response of the immune system [38]. IgM is the largest antibody, which appears firstly in the response to antigen exposure [39]. IL-1β, the first interleukin to be characterized, is crucial to the initiation and regulation of immune and inflammatory responses in many economically important teleost [40]. Furthermore, via dual RNA-seq, *MHC-I*, *MHC-II*, *IgM*, and *IL-1β* were proved to play key roles in the host-pathogen interaction during *P. plecoglossicida* infection of *E. coioides* [5,6]. In this study, the elevated expression of these immune-related genes (*MHC-I*, *MHC-II*, *IgM*, and *IL-1β*) confirmed the stimulation of innate and acquired immune responses in *E. coioides* after vaccination with *rpoE*-RNAi-1 strain. In addition, we evaluated the efficacy of *rpoE*-RNAi-1 as a live attenuated vaccine by injection route in an *E. coioides* model, resulting in a RPS of 85% post vaccination.

Taken together, with gene silencing, we revealed that *rpoE* contributed in multistep of *P. plecoglossicida* pathogenesis, which was probably mediated by *rpoE*-regulated virulence genes including *rpoA*, *aspA*, *ompW* and *fliA*. Meanwhile, *rpoE*-RNAi-1 strain exhibited a considerable protection effect against *P. plecoglossicida* challenge, and could elicit both cell mediated and humoral immune responses in *E. coioides*. These results provided further evidence for the importance of *rpoE* in *P. plecoglossicida* and serve as a reference for further investigation on this virulence factor.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

This work was supported by grants from the National Key R&D Program of China under Contract No. 2017YFC1404500; the Open Research Fund of State Key Laboratory of Cellular Stress Biology, Xiamen University under Contract No. SKLCSB2018KF012; and the Key laboratory of marine biogenetic resources, under Contract No. HY201804.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.02.028.

References

[1] E. Nishimori, K. Kita-Tsukamoto, H. Wakabayashi, *Pseudomonas plecoglossicida* sp. nov., the causative agent of bacterial hemorrhagic ascites of ayu, Plecoglossus altivelis, Int. J. Syst. Evol. Microbiol. 50 (2000) 83–89.
[2] Z.J. Mao, M.F. Li, J.G. Chen, Draft genome sequence of *Pseudomonas plecoglossicida* strain NB2011, the causative agent of white nodules in large yellow croaker (larimichthys crocea), Genome Announc. 1 (4) (2013) e00586-13.
[3] L. Huang, Y. Zuo, Q. Jiang, Y. Su, Y. Qin, X. Xu, L. Zhao, Q. Yan, A metabolomic investigation into the temperature-dependent virulence of *Pseudomonas plecoglossicida* from large yellow croaker (*Pseudosciaena crocea*), J. Fish. Dis. (2019), https://doi.org/10.1111/jfd.12957 [Epub ahead of print].
[4] J.T. Zhang, S.M. Zhou, S.W. An, L. Chen, G.L. Wang, Visceral granulomas in farmed large yellow croaker, Larimichthys crocea (Richardson), caused by a bacterial pathogen, Pseudomonas plecoglossicida, J. Fish. Dis. 37 (2) (2014) 113–121.
[5] Y.J. Sun, G. Luo, L.M. Zhao, L.X. Huang, Y.X. Qin, Y.Q. Su, Q.P. Yan, Integration of RNAi and RNA-seq reveals the immune responses of Epinephelus coioides to sigX gene of Pseudomonas plecoglossicida, Front. Immunol. 9 (2018) 1624.
[6] B.B. Zhang, G. Luo, L.M. Zhao, L.X. Huang, Y.X. Qin, Y.Q. Su, Q.P. Yan, Integration of RNAi and RNA-seq uncovers the immune responses of Epinephelus coioides to L321_RS19110 gene of Pseudomonas plecoglossicida, Fish Shellfish Immunol. 81 (2018) 121–129.
[7] T. Akayli, O. Canak, B. Basaran, A new Pseudomonas species observed in cultured young rainbow trout (Oncorhynchus mykiss Walbaum, 1792), BİBAD (Res. J. Biol. Sci.) 4 (2011) 107–111.
[8] Z. Tao, T. Zhou, S. Zhou, G. Wang, Temperature-regulated expression of type VI secretion systems in fish pathogen *Pseudomonas plecoglossicida* revealed by comparative secretome analysis, FEMS Microbiol. Lett. (22) (2016) 363.
[9] L.X. Huang, L.M. Zhao, Y.Q. Su, Q.P. Yan, Genome sequence of *Pseudomonas plecoglossicida* strain NZBD9, Genome Announc. 6 (4) (2018) e01412–e01417.
[10] L.X. Huang, W.J. Liu, Q.L. Jiang, Y.F. Zuo, Y.Q. Su, L.M. Zhao, Y.X. Qin, Q.P. Yan, Integration of transcriptomic and proteomic approaches reveals the temperature-dependent virulence of Pseudomonas plecoglossicida, Front.Cell. Infect.Microbiol. 8 (2018) 207.
[11] D. Gu, M. Guo, M. Yang, Y. Zhang, X. Zhou, Q. Wang, A σE-mediated temperature gauge controls a switch from LuxR-mediated virulence gene expression to thermal stress adaptation in Vibrio alginolyticus, PLoS Pathog. 12 (6) (2016) e1005645.
[12] Y. Dou, H. Rutanhira, X. Chen, A. Mishra, C. Wang, H.M. Fletcher, Role of extracytoplasmic function sigma factor PG 1660 (RpoE) in the oxidative stress resistance regulatory network of Porphyromonas gingivalis, Mol. Oral Microbiol. 33 (1) (2018) 89–104.
[13] H.F. Zhang, Y.W. Jia, X.F. Xie, M. Wang, Y. Zheng, S.G. Xu, W. Zhang, Q. Wang, H.H. Wang, H. Du, RpoE promotes invasion and intracellular survival by regulating SPI-1 and SPI-2 in Salmonella enterica serovar typhi, Future Microbiol. 11 (8) (2016) 1011–1024.
[14] S.E. Barchinger, X. Zhang, S.E. Hester, M.E. Rodriguez, E.T. Harvill, S.E. Ades, sigE facilitates the adaptation of Bordetella bronchiseptica to stress conditions and lethal infection in immunocompromised mice, BMC Microbiol. 12 (1) (2012) 179.
[15] T. Hanawa, H. Yonezawa, H. Kawakami, S. Kamiya, S.K. Armstrong, Role of Bordetella pertussis Rsea in the cell envelope stress response and adenylate cyclase toxin release, FEMS Immunol. Med. Microbiol. 69 (1) (2013) 7–20.
[16] J. Hu, F. Zhang, X. Xu, Y.Q. Su, Y.X. Q, Y. Ma, et al., Isolation, identification and virulence of the pathogen of white-spots disease in internal organs of Pseudosciaena crocea, Oceanol. Limnol. Sinica 45 (2) (2014) 409–417.
[17] G. Luo, L.X. Huang, Y. Su, Y.X. Qin, X. Xu, L.M. Zhao, Q.P. Yan, *flrA, flrB* and *flrC* regulate adhesion by controlling the expression of critical virulence genes in *Vibrio alginolyticus*, Emerg. Microb. Infect. 5 (8) (2016) e85.
[18] W.D. Kong, L.X. Huang, Y.Q. Su, Y.X. Qin, Y. Ma, J.X. Xu, et al., Investigation of possible molecular mechanisms underlying the regulation of adhesion in Vibrio

alginolyticus with comparative transcriptome analysis, Antonie Leeuwenhoek 107 (2015) 1197–1206, https://doi.org/10.1007/s10482-015-0411-9.
[19] Y. Zhang, L. Huang, Y. Zhao, T. Hu, Musk xylene induces malignant transformation of human liver cell line L02 via repressing the TGF-β signaling pathway, Chemosphere 168 (2017) 1506–1514.
[20] L. Huang, Z. Xi, C. Wang, Y. Zhang, Z. Yang, S. Zhang, Z. Zuo, Phenanthrene exposure induces cardiac hypertrophy via reducing miR-133a expression by DNA methylation, Sci. Rep. 6 (2016) 20105.
[21] L. Huang, Z. Zuo, Y. Zhang, C. Wang, Toxicogenomic analysis in the combined effect of tributyltin and benzo [a] pyrene on the development of zebrafish embryos, Aquat. Toxicol. 158 (2015) 157–164.
[22] L. Huang, D. Gao, Y. Zhang, C. Wang, Z. Zuo, Exposure to low dose benzo [a] pyrene during early life stages causes symptoms similar to cardiac hypertrophy in adult zebrafish, J. Hazard Mater. 276 (2014) 377–382.
[23] Q. Zhang, Y. Zhang, X. Zhang, L. Zhan, X. Zhao, S. Xu, X. Huang, The novel cis-encoded antisense RNA AsrC positively regulates the expression of rpoE-rseABC operon and thus enhances the motility of Salmonella enterica serovar Typhi, Front. Microbiol. 6 (2015) 990.
[24] A. Weiss, J.A. Ibarra, J. Paoletti, R.K. Carroll, L.N. Shaw, The δ subunit of RNA polymerase guides promoter selectivity and virulence in Staphylococcus aureus, Infect. Immun. 82 (4) (2014) 1424–1435.
[25] R. Anupama, S.S. Lulu, A. Mukherjee, S. Babu, Cross-regulatory network in Pseudomonas aeruginosa biofilm genes and TiO2 anatase induced molecular perturbations in key proteins unraveled by a systems biology approach, Gene 647 (2018) 289–296.
[26] A.C. Archer, P.M. Halami, Probiotic attributes of Lactobacillus fermentum isolated from human feces and dairy products, Appl. Microbiol. Biotechnol. 99 (19) (2015) 8113–8123.
[27] L. Franklin, A.H. Nobbs, L. Bricio-Moreno, C.J. Wright, S.E. Maddocks, J.S. Sahota, A. Kadioglu, Correction: the AgI/II family adhesin AspA is required for respiratory infection by Streptococcus pyogenes, PLoS One 8 (9) (2013).
[28] S.E. Maddocks, C.J. Wright, A.H. Nobbs, J.L. Brittan, L. Franklin, N. Strömberg, H.F. Jenkinson, Streptococcus pyogenes antigen I/II-family polypeptide AspA shows differential ligand-binding properties and mediates biofilm formation, Mol. Microbiol. 81 (4) (2011) 1034–1049.
[29] Y. Ye, N. Ling, J. Gao, X. Zhang, M. Zhang, L. Tong, Q. Wu, Roles of outer membrane protein W (OmpW) on survival, morphology, and biofilm formation under NaCl stresses in Cronobacter sakazakii, J. Dairy Sci. 101 (5) (2018) 3844–3850.
[30] S. McClean, M.E. Healy, C. Collins, S. Carberry, L. O'Shaughnessy, R. Dennehy, L.A. Cahill, Linocin and OmpW are involved in attachment of the cystic fibrosis-associated pathogen Burkholderia cepacia complex to lung epithelial cells and protect mice against infection, Infect. Immun. 84 (5) (2016) 1424–1437.
[31] L.X. Huang, J. Hu, Y.Q. Su, Y.X. Qin, W.D. Kong, Y. M, X.J. Xu, M. Lin, Q.P. Yan, Identification and characterization of three Vibrio alginolyticus non-coding RNAs involved in adhesion, chemotaxis, and motility processes, Front.Cell. Infect.Microbiol. 5 (2015) 56.
[32] L. Guo, L. Huang, Y. Su, Y. Qin, L. Zhao, Q. Yan, *secA, secD, secF, yajC*, and *yidC* contribute to the adhesion regulation of *Vibrio alginolyticus*, MicrobiologyOpen 7 (2) (2018) e00551.
[33] L. Huang, L. Wang, X. Lin, Y. Su, Y. Qin, W. Kong, Q. Yan, *mcp, aer, cheB*, and *cheV* contribute to the regulation of *Vibrio alginolyticus* (ND-01) adhesion under gradients of environmental factors, MicrobiologyOpen 6 (6) (2017) e00517.
[34] W. Liu, L. Huang, Y. Su, Y. Qin, L. Zhao, Q. Yan, Contributions of the oligopeptide permeases in multistep of *Vibrio alginolyticus* pathogenesis, MicrobiologyOpen 6 (5) (2017) e00511.
[35] Y. Qin, G. Lin, W. Chen, X. Xu, Q. Yan, Flagellar motility is necessary for Aeromonas hydrophila adhesion, Microb. Pathog. 98 (2016) 160–166.
[36] L. Huang, L. Huang, Q. Yan, Y. Qin, Y. Ma, M. Lin, J. Zheng, The TCA pathway is an important player in the regulatory network governing *Vibrio alginolyticus* adhesion under adversity, Front. Microbiol. 7 (2016) 40.
[37] K.P. Killeen, V.J. DiRita, Live attenuated bacterial vaccines, N.Vaccine Technol. (2001) 151.
[38] E.W. Hewitt, The MHC class I antigen presentation pathway: strategies for viral immune evasion, Immunology 110 (2) (2003) 163–169.
[39] S. Deshmukh, P.W. Kania, J.K. Chettri, J. Skov, A.M. Bojesen, I. Dalsgaard, K. Buchmann, Insight from molecular, pathological, and immunohistochemical studies on cellular and humoral mechanisms responsible for vaccine-induced protection of rainbow trout against Yersinia ruckeri, Clin. Vaccine Immunol. 20 (10) (2013) 1623–1641.
[40] J. Wu, Y.H. Shi, X.H. Zhang, C.H. Li, M.Y. Li, J. Chen, Molecular characterization of an IL-1β gene from the large yellow croaker (Larimichthys crocea) and its effect on fish defense against Vibrio alginolyticus infection, Zool. Res. 36 (3) (2015) 133.