Iranian Journal of Fisheries Sciences 20(2) 343-357 2021
DOI: 10.22092/ijfs.2021.123785

Research Article

Characterization of a high-affinity polyclonal antibody against the *Cyprinus carpio* (Linnaeus, 1758) intestinal tight junction protein occluding

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Received: July 2018 Accepted: October 2019

Abstract

Tight junction protein, occludin, plays an important role in intestinal health of fish. To study the function of occludin in the intestinal barrier at the protein level, a rabbit occludin polyclonal antibody was prepared against heterologously expressed *Cyprinus carpio* A fragment of the *occludin* gene containing antigenic determinants was first ligated into the pET-21a, which is an expression vector and transformed into *E. coli* BL21 (DE3) strain. Expression of the target fusion protein was induced by isopropyl-beta-D-thiogalactopyranoside (IPTG). The purified fusion protein was used as an antigen to immunize New Zealand long-eared rabbits (*Oryctolagus cuniculus*) through ear margin vein and subcutaneous injection to obtain rabbit anti-carp polyclonal antibodies against occludin. Enzyme-linked immunosorbent assay (ELISA) was used to evaluate the antibody titre, and the antibody was used to determine the distribution and expression of occludin in the intestine of carp after infection with *Aeromonas hydrophila*. The target fusion protein had a molecular weight of approximately 31.7 ku, the antibody titre was 2.4×10^6, and the integrity of occludin protein was lower in various intestinal segments after *A. hydrophila* infection. The results indicated that the prepared antibody had a high titre, affinity, and specificity and can be applied to study the expression and distribution of occludin in *C. carpio*. The availability of this polyclonal antibody laid the foundation for the systematic study of the intestinal barrier of *Cyprinus carpio* . Additionally, this polyclonal antibody could also be used for explorative studies of the biological function of occludin in other fishes.

Keywords: *Cyprinus carpio*, Occludin, Prokaryotic expression, Antibody titre, Immunohistochemistry

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Introduction

Intestinal epithelium is a single layer of columnar epithelial cells that are tightly bound together by intercellular junctional complexes. Function of the intestinal epithelium, as a barrier that separates the intestinal lumen from the underlying lamina propria (van der Flier and Clevers, 2009), is essential for health. There is increasing evidence that increased intestinal permeability plays a pathogenic role in diseases, such as inflammatory bowel disease (IBD) (Martini *et al.*, 2017), celiac disease (CD) (Cukrowska *et al.*, 2017), and irritable bowel syndrome (IBS) (González-Castro *et al.*, 2017). Therefore, defects in intestinal barrier function are associated with diseases of the gastrointestinal (GI) tract.

As one of junctional complexes, tight junctions (TJs) are multifunctional complexes that form a seal between adjacent epithelial cells near the apical surface (Farquhar and Palade, 1963). TJs comprise over 50 proteins, which include a series of transmembrane proteins, and form the fibrils that cross the plasma membrane and interact with other proteins in the adjoining cells (Chiba *et al.*, 2008). They seal the paracellular space between epithelial cells, thus preventing paracellular diffusion of microorganisms and other antigens across the epithelium. TJs are not static barriers but highly dynamic structures that are constantly being remodelled due to interactions with external stimuli (Otani *et al.*, 2019).

The protein, occludin, is an important structural and functional component of TJs. As a four-transmembrane protein, occludin plays a critical role in the regulation of trans-epithelial/endothelial electrical resistance (Wang *et al.*, 2012; Tan *et al.*, 2014) and actin assembly (Kuwabara *et al.*, 2001). Studies have shown that, similar to other TJ proteins, the expression and distribution of occludin are not static but are induced by the complex factors of the host intestinal environment, such as food residue and (Groh *et al.*, 2017) pathogenic (Liu *et al.*, 2011) and commensal bacteria (Ulluwishewa *et al.*, 2011; Nakata *et al.*, 2017). Occludin can regulate the entry of nutrients, ions, and water while restricting pathogen entry, thus regulating the barrier function of the epithelium. For example, western blot and immunofluorescence analyses of NCM460 epithelial cells showed that the expression level and fluorescence intensity of occludin is lower after *E. coli* infection than in the control group (Liu *et al.*, 2011; Ren *et al.*, 2017), and the same results were found at the mRNA level (Liu *et al.*, 2011). Other research findings show that porcine epidemic diarrhoea virus (PEDV) infection of epithelial cells results in disruption of the tight junctional distribution of occludin to an intracellular location (Luo *et al.*, 2017).

However, due to the lack of a corresponding antibody, most studies of occludin expression level in aquaculture are limited to the mRNA level. For instance, to investigate the effects of dietary isoleucine (Ile) on tight junctions in the intestine of juvenile

Jian carp (*Cyprinus carpio* var. Jian), the relative mRNA expression of *occludin* in the intestine showed a downward trend with increasing of dietary Ile up to a certain point (Zhao *et al.*, 2014). Further study from the same research group indicated that choline deficiency downregulated the mRNA level of the tight junction protein *occludin* in the proximal and distal intestine of juvenile Jian carp after *A. hydrophila* challenge (Wu *et al.*, 2017). In short, there have been few studies about the expression and distribution of occludin at the protein level.

In this study, the *occludin* gene of *Cyprinus carpio* was cloned and inserted into the expression vector pET-21a, the occludin fusion protein was expressed via a prokaryotic expression system and purified with 6 × His tag at the C terminus, and the purified protein was used to immunize New Zealand long-eared rabbits (*Oryctolagus cuniculus*) to obtain the corresponding polyclonal antibody. Subsequently, immunohistochemistry was used to examine the expression and distribution of occludin after *A. hydrophila* infection at the protein level to provide a basis for further studies on disease prevention in aquaculture animals.

Materials and methods

Bacterial strains, plasmids and fish

In this study, we used *Escherichia coli* strain DH5α competent cells for genetic engineering and *E. coli* strain BL21 (DE3) competent cells for protein expression. The plasmid vector for protein expression was pET-21a (Laboratory preservation). Healthy *Cyprinus carpio*, 16.5±1.0 cm in length and 55.0±1.5g in weight, were cultured at Aquaculture Training Base of the College of Fisheries, Henan Normal University.

Cloning and construction of prokaryotic expression vector

Total mRNA was extracted using an E.Z.N.A.TM Total RNA Kit II (OMEGA), and first-strand cDNA was obtained following the instructions of a cDNA Reverse Transcription Kit (Fermentas). Using the cDNA sequence of the carp *occludin* gene (GenBank accession no. KF975606), protean within the DNASTAR software was used to analyse and predict antigenic-determinant region of the amino acid sequence of Carp *occludin* protein. The primers occludin-YHBD-F (5'-CGGGATCCCACGGGAAGGACAACAT-3') and occludin-YHBD-R (5'-CCAAGCTTCTTCTTGATGTGGCTGAGT-3') were designed with restriction enzyme cutting sites, and the underlined regions indicate the *Bam*H I and *Hin*d III restriction sites, respectively. PCR was carried out under the following conditions: 3 min at 95°C for one cycle; 30s at 95°C, 30s at 50°C and 45s at 72°C for 30 cycles; and 10 min at 72°C for one cycle (Feng *et al.*, 2016).

The PCR amplification product and expression vector pET-21a were digested with *Bam*H I and *Hin*d III (52 μL of substrate, 1 μL of *Bam*H I, 1 μL of *Hin*d III, and 6 μL of 10 × Buffer at 37°C overnight). After gel extraction, the occludin and pET-21a fragments

were linked using T4 DNA ligase (1 μL of T4 DNA ligase, 2 μL of cDNA fragment, 2 μL of pET-21a vector, 1 μL of 10×Buffer, and 4 μL of water at 16°C overnight). The construct was transformed into *E. coli* (*E. coli*) DH5α competent cells. Positive clones were screened, and the plasmids were extracted according to the plasmid extraction kit manual (Sangon Biotech, China) and confirmed by restriction enzyme digestion and DNA sequencing.

Expression and purification of occludin protein

To express occludin from pET-21a-occludin in *E. coli* BL21(DE3), 100 ml of Luria–Bertani medium containing 100 μg ampicillin/mL was grown in an overnight culture (inoculated 1:100) containing the recombinant strain and grown to an optical density of 0.6 at 600 nm. Protein expression was induced with 0.2 mmol/L IPTG (isopropyl-b-D-thiogalactopyranoside) for 6h at 37°C. The bacteria were collected following centrifugation at 8,000 ×*g* for 10 min, and the pellet was resuspended in 10 mL of denaturing binding buffer (20 mmol/L Na_3PO_4, 15 mmol/L $C_3H_4N_2$, 500 mmol/L NaCl, 6 mol/L CH_4N_2O, pH 7.4). Inclusion bodies of the fusion protein were obtained by ultrasonic breaking (breaking for 3s, stopping for 3s; power: 50%; breaking time: 60 min). The fusion protein was purified on a His Trap HP column with different gradient concentrations of denaturing elution buffer (20 mmol/L Na_3PO_4, 50/75/100/125/150/175/200/225/500 mmol/L $C_3H_4N_2$, 500 mmol/L NaCl, 6 mol/L CH_4N_2O, pH 7.4). The CH_4N_2O and $C_3H_4N_2$ salt ions were removed from the fusion protein solution by dialysis, and SDS-PAGE was used to determine the level of expressed and purified fusion protein (Feng *et al.*, 2018).

Specific detection of the purified protein

To detect the specificity of the purified protein, western blotting was performed to examine the expression pattern of non-IPTG-induced proteins and IPTG-induced occludin protein. Samples were resuspended in 5 × SDS sample buffer (1.0 mol/L Tris-HCl [pH 6.8], 5.0% β-mercaptoethanol, 2.0% SDS, 1.0% bromophenol blue and 25% glycerol) and incubated at 100°C for 10 min. The samples were separated by 12% SDS-PAGE gel and transferred to a polyvinylidene difluoride (PVDF) membrane (Pall Corporation) at 400 mA for 8 min, and subsequently blocked (10% skimmed milk powder in TBST) at room temperature for 2h. Then, the membranes were incubated overnight at 4°C with an anti-his-tag polyclonal antibody in blocking solution (1: 5000, Sigma). After washing three times with TBST, the PVDF membranes were incubated with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin (1: 20 000, Sigma) at 37°C for 1h, followed by another three washes with PBST. The blots were visualized using the enhanced chemiluminescence method (ECL, Amersham Biosciences, Buckinghamshire, UK) in a

chemiluminescence imaging system (AzureC400, azure biosystem).

Preparation of polyclonal antibodies against occludin

Three female (2.5 kg) *Oryctolagus cuniculus* (Xinxiang Medical College Animal Research Center, Xinxiang, China) were used for immunization. For the first injection, each rabbit was injected subcutaneously with 2 mL of an emulsion containing 500 µg of occludin and Freund's complete adjuvant (Sigma). The rabbits were then boosted with the same volume of emulsion mixed 1:1 with incomplete Freund's adjuvant (Sigma) after 1 week, 2 weeks and 3 weeks. Rabbit antiserum was collected weekly to assess the level of immunity by ELISA. All animal experiments were performed in accordance with the guidelines of the ethics review committee for animal experiments at Henan Normal University.

Determination of polyclonal antibody activity and specificity for occludin

ELISA

Characterization of the polyclonal antibody activity against occludin was performed following the procedures described by Nie *et al.* (2012) and Wang *et al.* (2017). To prepare the solution, 100 µg of the occludin protein was dissolved in 10 mL of coating buffer (0.05 mol/L Na_2CO_3, pH 9.6). In the wells of 96-well immunological plates, 100 µL of solution (10 µg/mL) of the purified fusion protein used as the trapping antigen was added. Plates were incubated overnight at 4°C. The plates were blocked by incubating with a solution of 10% skimmed milk in PBS for 2h at 37°C. Upon three washes with PBS containing 0.05% Tween 20 (PBST), diluted rabbit hyperimmune sera (1: 10^2, 1: 10^3, 1: 10^4, 1: 3×10^4, 1: 9×10^4, 1: 2.7×10^5, 1: 8.1×10^5, and 1: 2.4×10^6) were added to wells. Pre-immunization (baseline) sera were used as negative controls. The plates were incubated for 1h at 37°C followed by three washes with PBST. Secondary antibody, anti-rabbit IgG conjugated with the horseradish peroxidase, was added at a dilution of 1: 20000. The plates were incubated for another 1h at 37°C followed by three washes with PBST. Next, 100 µL of tetramethylbenzidine solution (TMB, Sigma; 0.4 mg/mL) was added to the wells to produce a colouring reaction. The reaction was stopped after 30 min by adding 50 µL of 2M sulfuric acid per well. Optical density (OD) was read at 450 nm. Wells with dilutions of the hyperimmune sera that produced more than 2 times the OD of wells with the negative controls were considered positive, indicting the presence of specific antibodies. Antibody titres were calculated as the highest dilution of the hyperimmune sera that gave OD>2 times that of the OD of the same dilution of baseline sera.

Western blot

Western blotting was performed to examine the expression of non-IPTG-induced proteins and IPTG-induced occludin proteins using the occludin

polyclonal antibody. The detailed steps were the same as described in section 2.4.

Application of polyclonal antibodies against occludin

Isolation of intestinal tissues and toxicity assay

The common carp were sacrificed by over-anaesthetization with ethyl 3-aminobenzoate methanesulfonate (MS-222, Sigma Aldrich, USA) and bled from the caudal vein to remove as much blood as possible. Subsequently, gut tissues of healthy carp were isolated under aseptic conditions, lengths of foregut, midgut and hindgut were cut to approximately 1 cm, and the contents of the gut were repeatedly washed several times with sterile PBS buffer (8.0 g/L NaCl, 0.2 g/L KCl, 0.27 g/L KH_2PO_4, 3.58 g/L Na_2HPO_4•12H2O, pH 7.4) until they were nearly sterile. After washing two times with HBSS (8.0 g/L NaCl, 0.4 g/L KCl, 0.06 g/L KH_2PO_4, 0.0475 g/L Na_2HPO_4, 0.35 g/L $NaHCO_3$, 1.0 g/L $C_6H_{12}O_6$, pH 7.2), the different bowel tissues were subsequently added to DMEM-F12 medium containing 10% heat inactivated FBS. Subsequently, 100 μL of *A. hydrophila* (final concentration: 1×10^6 CFU/mL, positive control group) or PBS (negative control group) were added to the various tissues. The cultures were maintained at 28°C and 5% CO_2 cell incubator for 2h.

Histology and immunohistochemistry

The intestinal tissues were embedded in paraffin immediately after treatment, and 6 μm thick sections were prepared. The tissue slices were dewaxed by twice xylene baths and a series of ethanol dilutions (100%, 95%, 80%, 70%), followed by distilled water. Endogenous peroxidase was blocked using 3% H_2O_2, and the slices were heated at 95°C in sodium citrate buffer for 10 min for antigen repair. After blocking with normal serum, the tissue slides were incubated with primary anti-occludin polyclonal antibody dissolved in phosphate-buffered saline with 0.1% Triton X-100 and 1% bovine serum (1: 5000) overnight at 4°C and then washed and incubated with the HRP-tagged secondary antibody at 37°C for 1h. After washing, the slides were counterstained with haematoxylin, dehydrated, cleared with xylene and mounted. All stains were performed manually, with all individual samples processed in a single batch per staining. The expression and distribution of occludin was analysed under the microscope (de Bruyn *et al.*, 2018).

Results

Construction and identification of the recombinant expression plasmid

PCR amplification was carried out using specific primers containing *Bam*H I (occludin-YHBD-F) and *Hin*d III (occludin-YHBD-R) restriction sites, and the product was detected by agarose gel electrophoresis to obtain an expected band size of 672 bp (Fig. 1). The recombinant expression plasmid was identified by single (*Bam*H I) and double (*Bam*H I and *Hin*d III) digestion, and the digestion results were analysed

by 1.0% agarose gel electrophoresis (Fig. 2). Lanes 1, 2 and 3 show the results of recombinant plasmid, single digestion and double digestion, respectively. According to the results of digestion, pET-21a-occludin (672 bp) was a positive recombinant plasmid. Subsequently, the recombinant expression plasmid was verified by sequencing, and the sequence was more than 99% identical to the known sequence (GenBank accession no. KF975606), indicating that the inserted fragment in the constructed recombinant expression plasmid was the target fragment.

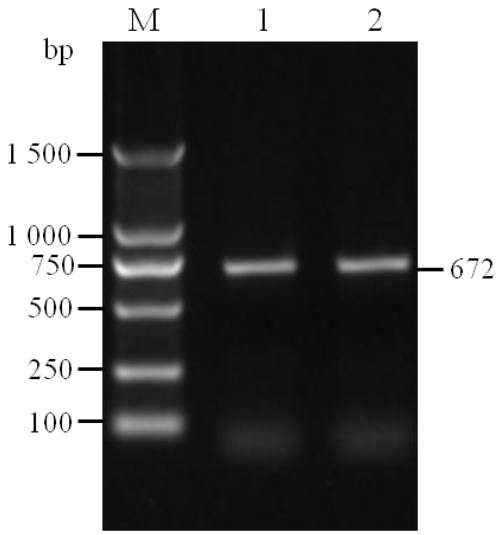

Figure 1: Gel electrophoresis pattern of occludin PCR product. M: D2000 DNA ladder; Land 1 and 2: occludin PCR products.

Expression, purification and specific detection of carp recombinant occludin

The recombinant plasmid pET-21a-occludin (672 bp) was induced by IPTG in *E. coli* BL21 (DE3). The wild strain and engineered bacteria without IPTG were used as negative control groups (Fig. 3A, lanes 1 and 2). The results showed that compared with negative control groups, IPTG-induced groups efficiently expressed a fusion protein with a molecular weight of 31.7 ku (Fig. 3A, lanes 3 and 4), consistent with the expected results.

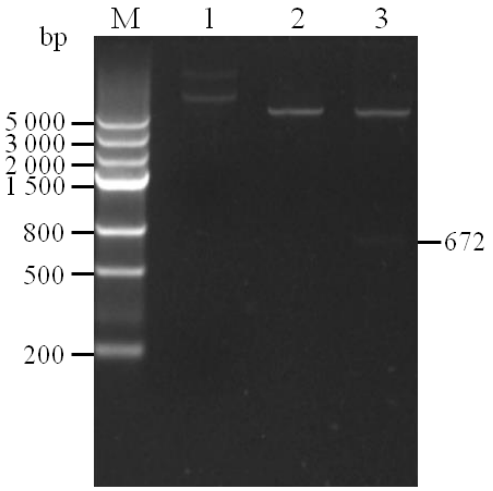

Figure 2: The single and double enzyme digestion of recombinant expression plasmid. M: D2000 plus DNA ladder; Land 1: recombinant plasmid; Land 2: single digest of recombinant plasmid; Land 3: double digest of recombinant plasmid.

Then, the fusion protein was purified on a His Trap HP column with different denaturing elution buffer concentration gradients. The amount of purified fusion protein increased with increasing concentration of imidazole, reached the maximum when imidazole concentration was 175 mmol/L (Fig. 3B, lane 6) and gradually decreased with higher concentrations. The purified fusion protein was detected by SDS-PAGE. The results showed that compared with the control groups (Fig. 3A, lanes 3 and 4), the content of

hybrid protein was significantly reduced (Fig. 3C, lanes 1 and 2).

With anti-his-tag antibody detection, the fusion protein and anti-his-tag polyclonal antibody combined to form a specific immune band (Fig. 4, lanes 2 and 3), and the negative control, without IPTG induction, group had no specific band (Fig. 4, lane 1). The results showed that the purified protein was occluding.

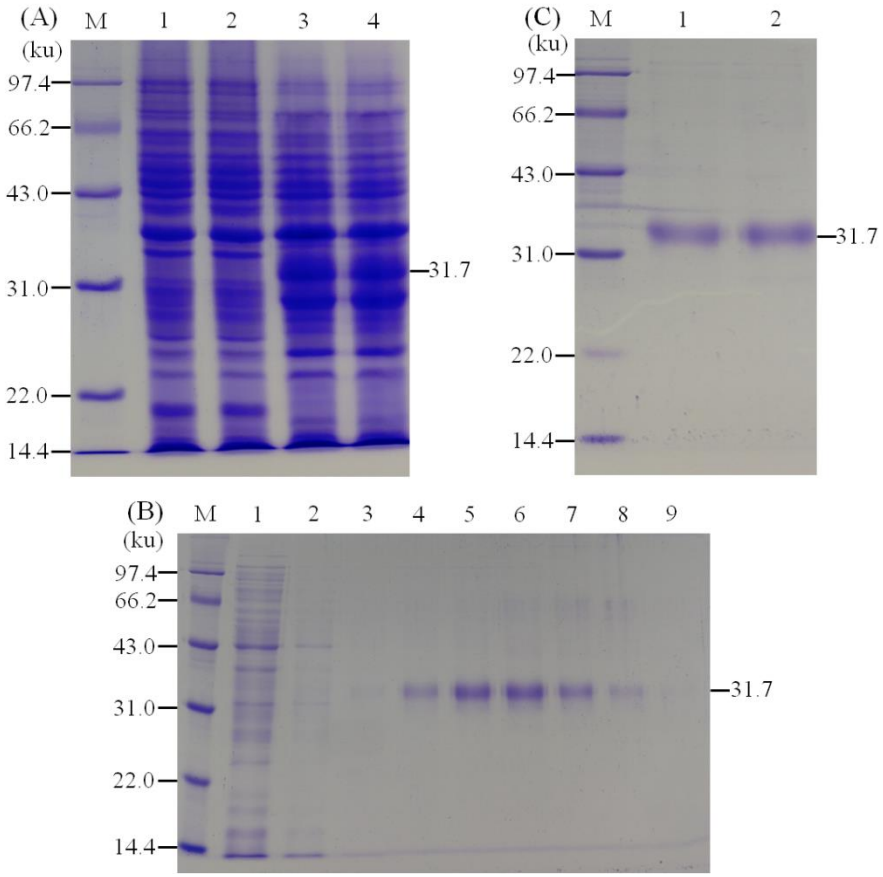

Figure 3: Expression location analysis and purification of carp occludin protein from BL21(DE3). (A) Expression of the occludin in BL21(DE3) bacteria. Lanes 1 and 2, BL21(DE3) without recombinant plasmid and engineered bacteria without IPTG induced as negative controls, respectively. Lanes 3-4, IPTG-induced groups. (B) Purification of occludin from BL21(DE3) bacteria with different concentrations of imidazole. Lanes 1-9, elution sample by elution buffer with 50, 75, 100, 125, 150, 175, 200, 225, 500 mmol/L imidazole, respectively. (C) Purified recombinant carp occludin protein.

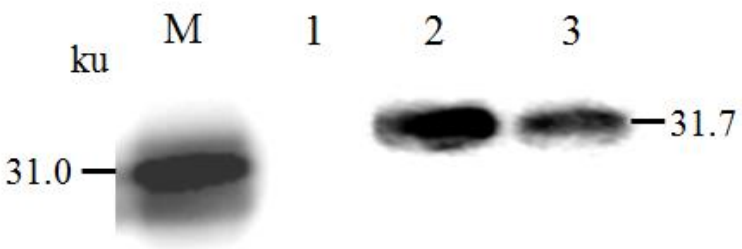

Figure 4: Specific detection of the purified protein with anti-his-tag polyclonal antibody (Western blot analysis). M. Protein marker; 1. Proteins expressed by engineered bacteria without IPTG induction; 2-3. Proteins expressed by bacteria with IPTG.

Activity analysis of the polyclonal antibody

Titre of the polyclonal antibody in the collected sera was determined by ELISA, and ratio of OD_{450} absorbance values of the treated group compared to the negative control group was calculated. As shown in Table 1, when the polyclonal antibodies against carp occludin were diluted to 2.4×10^6, the ratio was still higher than 2. Therefore, the results showed that the antibody had a high titre, which indicated that it can be used for the detection of indicators in subsequent experiments.

Table 1: Occludin polyclonal antibody titre by ELISA.

Item	Order number							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dilution	1×10^2	1×10^3	1×10^4	3×10^4	9×10^4	2.7×10^5	8.1×10^5	2.4×10^6
Treatment	1.047	0.607	0.595	0.581	0.556	0.515	0.246	0.127
Negative control	0.326	0.122	0.073	0.072	0.069	0.065	0.064	0.063
Blank control	0.060	0.059	0.061	0.058	0.060	0.058	0.059	0.060
Treatment/negative control	3.212	4.975	8.151	8.069	8.058	7.923	3.844	2.016

After western blot detection with occludin antibody, the fusion protein and polyclonal antibody combined to form a specific band (Fig. 5, lane 2), and the group without IPTG induction had no such specific band (Fig. 5, lane 1). The results showed that the prepared polyclonal antibody specifically binds carp occludin.

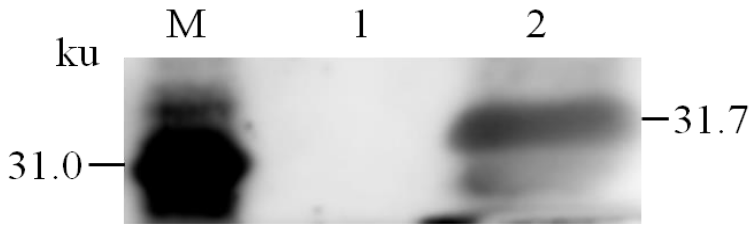

Figure 5: Specific combination of the polyclonal antibody with recombinant occludin (western blot analysis). M. Protein marker; 1. Proteins expressed by bacteria without IPTG induction; 2. Proteins expressed by bacteria with IPTG induction.

Application of occludin antibody in immunohistochemistry

To assess the expression and distribution of occludin protein in foregut, midgut and hindgut that were cultured in the presence or absence of *A. hydrophila*, the intensity of occludin protein distribution throughout the intestine was examined. This analysis demonstrated a uniform distribution of occludin protein in untreated control groups (Fig. 6A, B, C), however, *A. hydrophila* treatment of foregut, midgut and hindgut resulted in a decrease in occludin protein integrity compared to control groups (Fig. 6D, E, F). The immunohistochemistry results (Fig. 6) showed that the antibody had high specificity and sensitivity and could detect the distribution and expression of occludin protein in intestine of common carp.

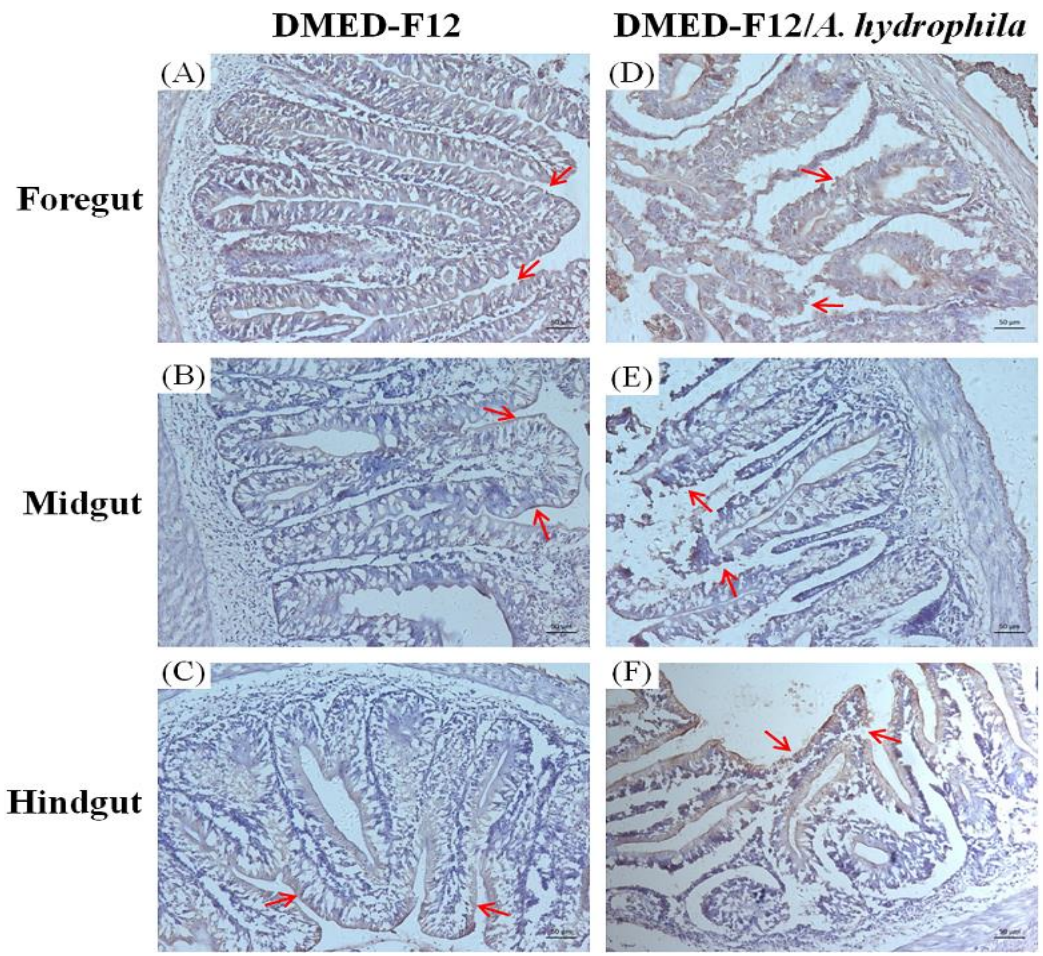

Figure 6: Immunohistochemistry staining of occludin protein. Column 1, expression and distribution of occludin protein in foregut (A), midgut (B) and hindgut (C) tissues under normal conditions; column 2, the expression and distribution of occludin protein in foregut (D), midgut (E) and hindgut (F) tissues after infection with *A. hydrophila* for 2h. The position of occludin protein was indicated by red arrow. Magnification 200X.

Discussion

In this study, polyclonal antibody against the intestinal tight junction protein occludin of common carp with high titer and affinity were prepared by prokaryotic expression and immunization of rabbits, and could be used to detect specificity of the expression and distribution of occludin in the intestinal tract.

Tight junction (TJ) complexes are important junctional complexes in many types of epithelial and endothelial cells. Much attention has been devoted to understanding how these proteins contribute to the barrier function, i.e. regulating the paracellular flux or permeability between adjacent cells (Runkle and Mu, 2013). According to location, TJs can be sub-divided into integral membrane and cytoplasmic fractions. A tetra-spanning membrane protein, occludin, is the first discovered TJ protein (Furuse *et al.*, 1993). In addition, studies of occludin have shown that silencing occludin in vitro increases the permeability of divalent organic cations and small molecules

under hydrostatic pressure (Yu *et al.*, 2005). Furthermore, chronic unpredictable mild stress (CUMS)-induced disruption of mucosal barrier integrity was associated with a reduction in expression of the tight junction protein, occludin 1, and an inhibition in mucosal layer functioning via reductions in goblet cells (Wei *et al.*, 2019). BPA increased the colonic permeability in mice by reducing the expression of tight junction proteins (ZO-1, occludin, and claudin-1), and this effect was closely related to disruption of intestinal chemistry and physical and biological barrier functions (Feng *et al.*, 2019). However, relatively little research on occludin in aquaculture has been done; therefore, the function of occludin in aquaculture is not completely understood.

In this study, antigenic determinant region of the intestinal tight junction protein occludin in carp was analysed and predicted by Protean in DNASTAR software (Feng *et al.*, 2018). Due to intermittent distribution of the epitopes in the amino acid sequences, success rate of full-length synthesized protein has been extremely low in previous experiments. Therefore, in this study, only a partial region of the ORF of the *occludin* gene was selected for protein expression (Nie *et al.*, 2012; Wang *et al.*, 2015; Feng *et al.*, 2016; Yan *et al.*, 2016).

The expressed fusion protein was eluted and purified on a His Trap HP (GE) column, using different concentrations of imidazole (Feng *et al.*, 2018). Advantages of this method were that we could recover large amounts of the fusion protein and effectively remove other hybrid proteins expressed by *E. coli*. Then, the purified fusion protein was used as an antigen to immunize New Zealand long-eared rabbits to obtain the polyclonal antibody against occludin. As detected by indirect ELISA, the serum antibody titre reached 2.4×10^6, which was similar to the polyclonal antibody titres of nutrient transporters (Nie *et al.*, 2012; Yan *et al.*, 2016) and cytokines (Feng *et al.*, 2018) in common carp.

The polyclonal antibody prepared in this study was used as a tool to detect the distribution and expression of occludin in intestinal tract of the common carp after infection with *A. hydrophila* by immunohistochemistry. Results showed that after infection, integrity of the tight junction protein occludin was significantly disrupted at the protein level by *A. hydrophila* treatment compared to control groups ($p<0.05$; Fig. 5). This indicated that permeability of the intestinal barrier and the expression and distribution of tight junction protein occludin could be altered by *A. hydrophila* infection (Kong *et al.*, 2017). These results are similar to the findings in other aquaculture animals, such as juvenile Jian carp, *Cyprinus carpio* var. Jian, (Zhao *et al.*, 2014) and olive flounder, *Paralichthys olivaceus*, (Bo *et al.*, 2016) at the transcriptome level and at the protein level in mammals (Liu *et al.*, 2011; Luo *et al.*, 2017; Ren *et al.*, 2017). Studies have shown that polyclonal antibodies against the intestinal tight

junction protein occludin produced in this study had high affinity and specificity.

In conclusion, a polyclonal antibody against occludin of carp intestinal protein with high affinity, specificity and titer was prepared in this study, and it can be used to determine relative protein levels. The results of this study lay a foundation for further research on intestinal barrier function in aquaculture animals.

Acknowledgement

This study was supported by the Innovation Scientists and Technicians Troop Construction Projects of Henan Province (CXTD2016043), Doctoral Fund of Henan Normal University (qd16155), Key Technology Research Project of Henan Province (212102110106, 172102110195, 182102110022); Key Research Projects of Henan Higher Education Institutions (17B240002); Youth Fund of Henan Normal University (2016QK23).

Ethical approval

This study conformed to the guidance of animal ethical treatment for the care and use of experimental animals, and was approved by the Institutional Animal Care and Use Committee of Henan Normal University. The fishes were anesthetized with diluted MS-222 before being euthanized, and all efforts were made to minimize suffering.

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