Oral immunization with surface immunogenic protein from *Streptococcus agalactiae* expressed in *Lactococcus lactis* induces protective immuneresponses of tilapia (*Oreochromis niloticus*)

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ABSTRACT

Streptococcus agalactiae infection has become a serious bacterial disease of tilapia for its damage. In order to effectively prevent the outbreak of streptococcicosis, a recombinant *Lactococcus lactis* expressing surface immunogenicity protein (Sip) of *S. agalactiae* was constructed as probiotic vector oral vaccine in this study. The immunogenicity of the recombinant *L. lactis* was evaluated through detecting the level of specific serum IgM antibody, expression level of immune-related genes and relative percent survival (RPS). The results showed that the recombinant *L. lactis* could express a 45.5 kDa protein consistent with the expected size of Sip. The recombinant Sip mainly expressed in the form of inclusion body and the concentration of purified Sip could reach 7.65 mg / mL. Nile tilapia were orally immunized with different concentrations (2.24×10^9, 2.24×10^{10} and 2.24×10^{11} CFU / mL) of recombinant *L. lactis* (NZ9000-pNZ8148-sip). The analysis results of specific serum antibody showed that the level of antibody of tilapia which were vaccinated with medium and high concentration of recombinant *L. lactis* (2.24×1010 CFU / mL and 2.24×1011 CFU / mL) were significantly higher than that of control group. The relative percentage of survival (RPS) of tilapia vaccinated with medium concentration of NZ9000-pNZ8148-sip reached 61.6% at 21 days post-immunization. Quantitative real-time PCR (qRT-PCR) results revealed that the immune-related genes of *IgT*, *IgM*, *CD8a* and *C3* were significantly upregulated expression in thymus, liver, spleen and hindgut. In conclusion, the recombinant *L. lactis* vaccine induced both humoral and cellular immunity of tilapia. This study demonstrates the potential of using *L. lactis* as a delivery system to develop an oral vaccine against *S. agalactiae*.

BACKGROUND & OBJECTIVES

* Tilapia is an internationally farmed freshwater fish recommended by the Food and Agriculture Organization, which is farmed in 135 countries of the world (Wang et al., 2016).

* With the expansion of breeding scale and the deterioration of aquaculture environment, streptococcicosis has become an important disease threatening the tilapia industry. *Streptococcus agalactiae* is the main pathogen of streptococcicosis in tilapia (Liu et al., 2016; Lu et al., 2010; Prettogiordano et al., 2010).

* The surface proteins of *S. agalactiae* are frequently used as candidate antigens for subunit vaccine. Surface immunogenicity protein (Sip) exists in all the serotypes of *S. agalactiae*, and has a strong immunogenicity in a variety of animal models (Brodeur et al., 2000; Tanaka et al., 2007; Manning et al., 2006).

* The traditional vaccines are commonly immunization by injection, which is time-consuming and difficult to administer to small fishes (Caipang et al., 2014). Oral vaccination is easy to operate without causing any stress to the fish, which is the ideal method of vaccine delivery to fish.

* Oral recombinant *L. lactis* vaccine has the advantages of low production cost, simple preparation, without separation or purification and suitable for large-scale population immunity (Szatraj et al., 2017; Maryam et al., 2016). Oral recombinant lactobacillus vaccines have shown a good immune protective effect in the previous studies (Min et al. 2012; Anuradha et al. 2012).

* In this study, a recombination *L. lactis* expressing Sip protein of *S. agalactiae* was constructed. The immunogenicity of the recombinant *L. lactis* was evaluated by detecting the RPS, the level of specific serum antibody and the expression of immune-related genes.

MATERIALS & METHODS

1. Construction of recombinant plasmid NZ9000-pNZ8148-sip

Recombinant pMD19-T-sip vector and pNZ8148 vector (REBIO, China) were digested by KpnI and HindIII, and then they were ligated together. The structure of recombinant plasmid pNZ8148-sip was shown in Fig. 1. The pNZ8148-sip plasmid was electro-transformed into *L. lactis* NZ9000 competent cells. The conditions of electroporation were 2000 V, 200 Ω and electric shock 4.5 ~ 5 ms.

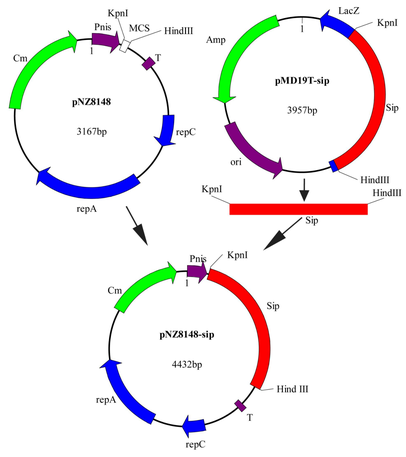
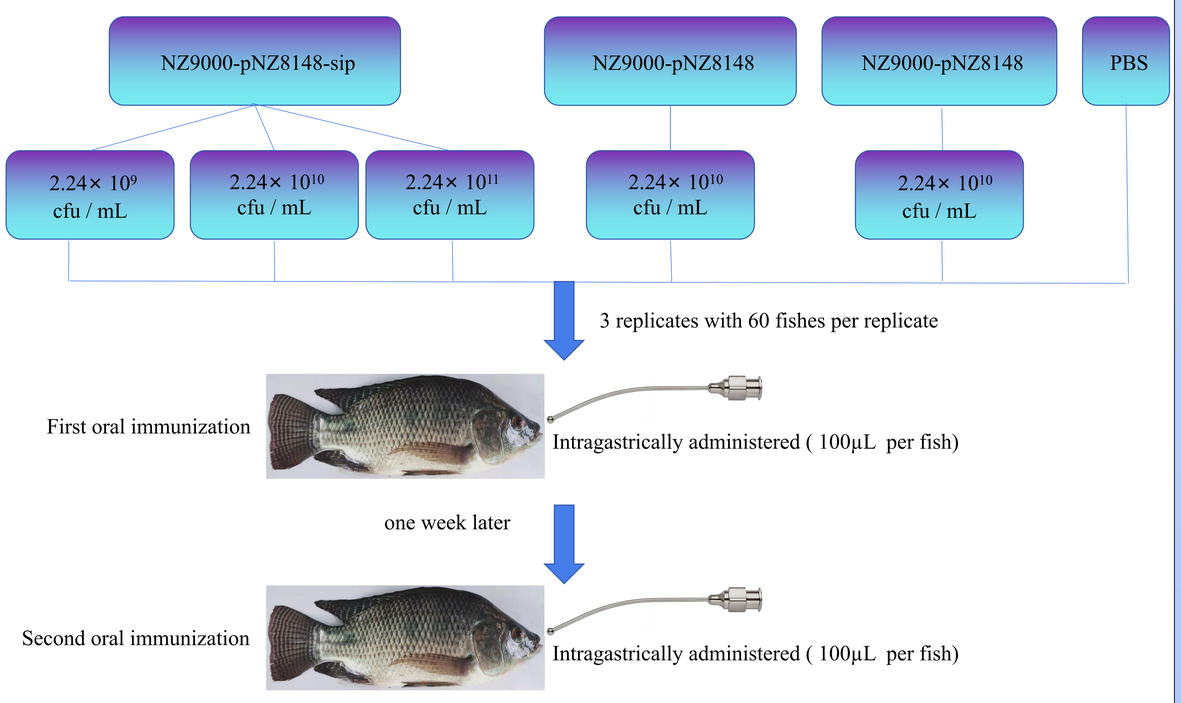


Fig. 1 Schematic drawing of the construction of recombinant vector pNZ8148-sip

2.. Prokaryotic expression, protein purification, concentration detection and western blot

Purification of recombinant Sip protein → Detection of protein concentration by BCA method → Western blotting

3. Immunization and challenge



Three fishes were randomly sampled from each group at 1 day, 2 days, 4 days, 8 days, 16 days and 21 days after the first immunization,. Sera were collected for antibody detection and tissue samples (thymus, liver, spleen and hindgut) were sampled for detecting the expression levels of immune-related genes (*IgT*, *IgM*, *CD8a* and *C3*). At the 21 day after the first immunization, 30 fishes from each groups were challenged with 100μL of *S. agalactiae* (2.25×10^7 CFU / mL, LD_{50}). All the fishes were monitored for mortality for 14 days post-challenge. The relative percentage of survival (RPS) was calculated according to the following formula: RPS = {1 - (mortality in vaccinated fish/ mortality in control fish)} × 100%.

RESULTS

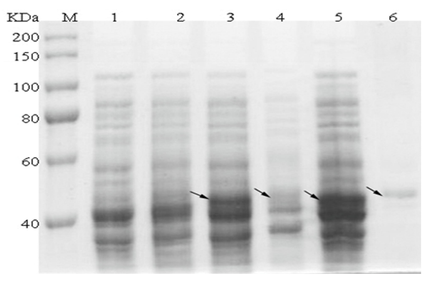


Fig.2 SDS-PAGE analysis the distribution of induced expression products of recombinant *L. lactis* (NZ9000-pNZ8148-sip). M. protein Marker; 1. supernatant protein of uninduced NZ9000-pNZ8148-sip lysate; 2. total protein of uninduced NZ9000-pNZ8148 -sip lysate; 3. total protein of induced NZ9000-pNZ8148-sip lysate; 4. supernatant protein of induced NZ9000-pNZ8148-sip lysate; 5. precipitated protein of induced NZ9000-pNZ8148-sip lysate; 6. purified recombinant protein from supernatant of NZ9000- pNZ8148-sip lysate

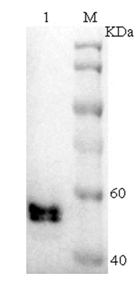


Fig. 3 Western blot analysis of the induced recombinant Sip of NZ9000-pNZ8148-sip

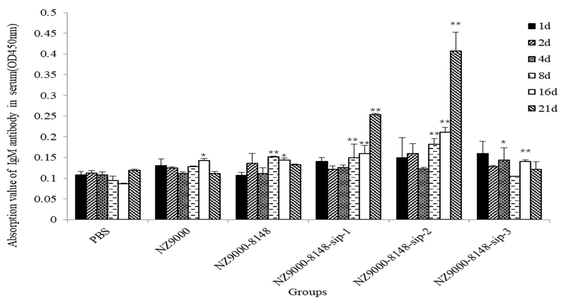


Fig. 4 The change of serum antibody level of tilapia oral immunized by recombinant *L. lactis* (NZ9000-pNZ8148-sip). * represents it was significantly different compared with PBS control group at the same time point ($P<0.05$), ** represent extremely significant ($P<0.01$), NZ9000-8148-sip-1 represents the group immunized with high concentration of recombinant bacteria (2.24×10^{11} CFU/mL), NZ9000-8148-sip-2 represents the group immunized with medium concentration of recombinant bacteria (2.24×10^{10} CFU/mL), NZ9000-8148-sip-3 represents the group immunized with low concentration of recombinant bacteria (2.24×10^{9} CFU/mL)

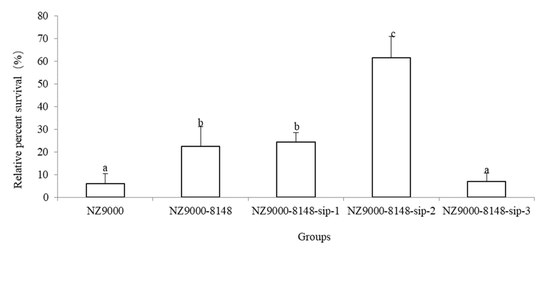


Fig. 3 The RPS of tilapia oral immunized with recombinant *L. lactis* (NZ9000-pNZ8148-sip). Data were shown as mean ± SD; the different letters above columns represent significant difference, $P < 0.05$; NZ9000-8148-sip-1 represents the group immunized with high concentration of recombinant bacteria (2.24×10^{11} CFU/mL), NZ9000-8148-sip-2 represents the group immunized with medium concentration of recombinant bacteria (2.24×10^{10} CFU/mL), NZ9000-8148-sip-3 represents the group immunized with low concentration of recombinant bacteria (2.24×10^{9} CFU/mL)

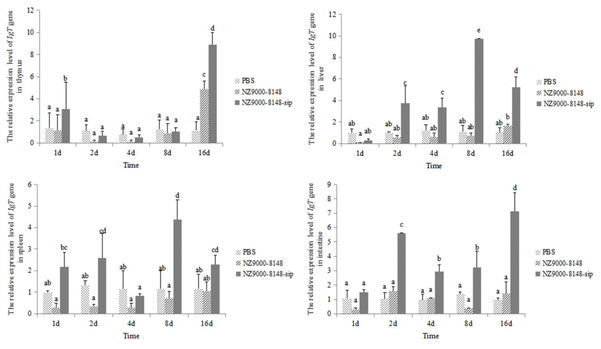


Fig. 4 The relative expression of *IgT* gene in different tissues of tilapia after oral immunization with recombinant *L. lactis* (NZ9000-pNZ8148-sip). Data were shown as mean ± SD; the different letters above columns represent significant difference, $P < 0.05$

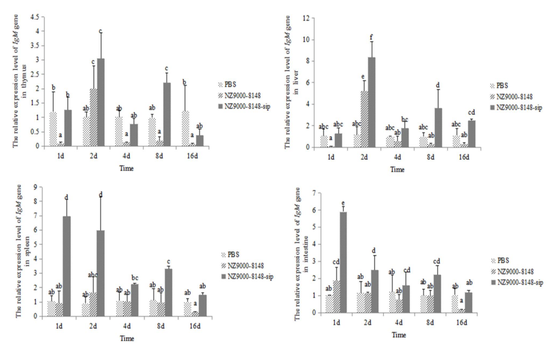


Fig. 5 The relative expression of *IgM* gene in different tissues of tilapia after oral immunization with recombinant *L. lactis* (NZ9000-pNZ8148-sip). Data were shown as mean ± SD; the different letters above columns represent significant difference, $P < 0.05$

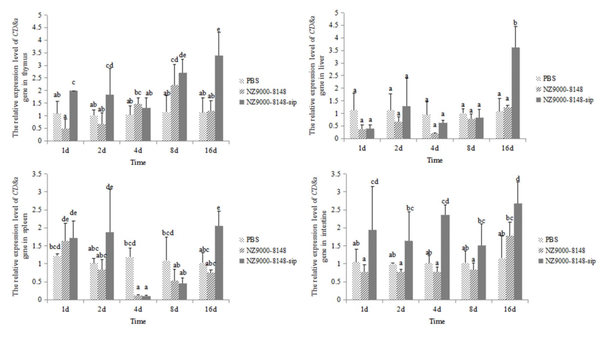


Fig. 6 The relative expression of *CD8a* gene in different tissues of tilapia after oral immunization with recombinant *L. lactis* (NZ9000-pNZ8148-sip). Data were shown as mean ± SD; the different letters above columns represent significant difference, $P < 0.05$

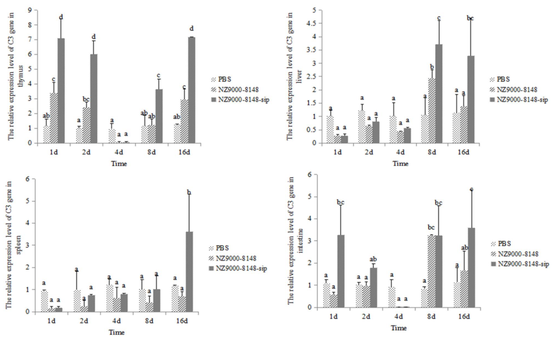


Fig. 7 The relative expression of *C3* gene in different tissues of tilapia after oral immunization with recombinant *L. lactis* (NZ9000-pNZ8148-sip). Data were shown as mean ± SD; the different letters above columns represent significant difference, $P < 0.05$

DISCUSSION & CONCLUSION

1. We constructed a recombinant *L. lactis* NZ9000-pNZ8148-sip expressing Sip protein of *S. agalactiae*. The medium doses of NZ9000-pNZ8148-sip (2.24×10^{10} cfu / mL) induced a high level of serum antibody and the highest RPS (61.1%) of tilapia in this study. The results indicated that the *L. lactis* oral vaccine was an effective strategy for the prevention and control of tilapia infection with *S. agalactiae*.

2. The expression of *IgT* and *IgM* gene were incrased after the first and second immunization, while the exprssion of *CD8a* and *C3* gene were increased all the time after initial immunization. The up-regulated expression of *IgT*, *IgM*, *CD8a*, and *C3* genes indicated that both non-specific and specific immune responses of tilapia were induced by oral immunization with recombinant *L. lactis*.

3. The serum antibody level and RPS of tilapia immunized with high concentration of NZ9000-pNZ8148-sip (2.24×10^{11} cfu / mL) was lower than that of other concentration immune groups. This may be related to the generation of immune tolerance, which plays an important role in reducing the inflammatory reactions of intestinal tissue (Strobel et al., 1998).

4. Live bacteria can be killed and digested by a variety of bactericidal substances and hydrolases in lysosome of macrophages, and then the intracellular antigens were presented to lymphocyte (Martin et al., 2012). There are a large number of T cells and B cells in fish intestine, which indicated that antigenic stimulation in the intestine could induce the cellular immunity and humoral immunity of fish (Takizawa et al., 2011; Martin et al., 2012).