

Glyceraldehyde-3-phosphate dehydrogenase of *Edwardsiella tarda* has protective antigenicity against *Vibrio anguillarum* in Japanese flounder

Ying Liu¹, Syun-ichirou Oshima^{2,*}, Kenji Kawai¹

¹Fish Disease Laboratory, Department of Aquaculture, Faculty of Agriculture, and ²Laboratory of Cell Structure and Function, Division of Marine Bioresource Science, Graduate School of Kuroshio Science, Kochi University, Nankoku, Kochi 783-8502, Japan

ABSTRACT: *Edwardsiella tarda* glyceraldehyde-3-phosphate dehydrogenase (GAPDH) may be an effective vaccine candidate against infection by *E. tarda* in Japanese flounder *Paralichthys olivaceus*. The GAPDH of *E. tarda* is highly homologous to that of *Vibrio cholerae* (91%), and therefore *E. tarda* GAPDH may have protective antigenicity against *Vibrio* species. In this study, we immunized Japanese flounder with GAPDH of *E. tarda* and infected the fish with *V. anguillarum*. The result showed that GAPDH prepared from *E. tarda* protected Japanese flounder effectively in a challenge of *V. anguillarum*. Therefore, *E. tarda* GAPDH should be considered as a multi-purpose vaccine candidate against several kinds of pathogenic bacteria.

KEY WORDS: Glyceraldehyde-3-phosphate dehydrogenase · GAPDH · Protective antigenicity · Multi-purpose vaccine candidate · *Edwardsiella tarda* · *Vibrio anguillarum*

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

A 37 kDa outer membrane protein (OMP) among different serotype strains of *Edwardsiella tarda* is effective against infections by different serotype strains of *E. tarda* (Kawai et al. 2004). N-terminal amino acid sequence analysis showed that the 37 kDa OMP is homologous to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a cytoplasmic enzyme common in organisms (Kawai et al. 2004). To develop *E. tarda* GAPDH as a vaccine against *E. tarda*, Liu et al. (2005) obtained a sequence of the gene encoding *E. tarda* GAPDH, overexpressed the GAPDH protein, and evaluated the vaccine efficacy of expressed GAPDH against *E. tarda* infection. The GAPDH of both nucleotide and amino acid sequences are highly similar between *E. tarda* and other Gram-negative bacteria, and have especially high homologies between *E. tarda* and *Vibrio cholerae* (Liu et al. 2005).

Vibrio sp. are the etiological agents of severe diarrheal diseases in fish and humans and cause epidemic

diarrheal diseases that are feared because of their severity (Kusuda & Salati 1993, Kaper et al. 1995). The high similarities of GAPDH between *Edwardsiella tarda* and *V. cholerae* prompted us to examine the protective antigenicity of *E. tarda* GAPDH against *Vibrio* infection.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The *Edwardsiella tarda* EF-1 strain was cultured as described in Kawai et al. (2004). *Vibrio anguillarum* strain 1122 (J-O-3) isolated from ayu *Plecoglossus altivelis* was pre-cultured in brain heart infusion (BHI, Difco) broth containing 2% NaCl at 25°C for 1 d, inoculated into new BHI broth containing 2% NaCl, and then cultured at 25°C for 18 h.

Antigen. The 37 kDa OMP of *Edwardsiella tarda* EF-1 strain was extracted as described (Suzuki et al. 1994). The antigen underwent sodium dodecyl sul-

*Corresponding author. Email: s-oshima@cc.kochi-u.ac.jp

Table 1. Preliminary experiment to determine adequate bacterial (*Vibrio anguillarum*) dose for challenge test

Dose of <i>V. anguillarum</i> (CFU fish ⁻¹)	Number of fish	Mean body weight (g)	Mortality rate after 2 wk (%)
8×10^6	30	21	97.6
4×10^6	30	21	70
2×10^6	30	21	10
8×10^5	30	21	0
4×10^5	30	21	0

phate polyacrylamide gel electrophoresis (SDS-PAGE) and was visualized by Coomassie brilliant blue staining. The prepared protein also underwent Western blotting by using antiserum against the *E. tarda* 37 kDa OMP (Kawai et al. 2004) and antiserum against general (human, rabbit, and *Escherichia coli*) GAPDH (Biogenesis), respectively.

Fish and immunization. Japanese flounder *Paralichthys olivaceus* weighing about 21 g were obtained from a hatchery in Kochi Prefecture, Japan, and were divided into 2 groups: Group 1 (n = 50) was immunized with the 37 kDa GAPDH prepared from *Edwardsiella tarda* (30 µg fish⁻¹) by intraperitoneal injection, and Group 2 (n = 50) was intraperitoneally injected with phosphate-buffered saline (PBS; 100 µl fish⁻¹) as a control. The fish were reared in aquaria for 4 wk and were infected with *Vibrio anguillarum* strain 1122.

Challenge test. The appropriate dose of *Vibrio anguillarum* for a challenge test was determined in a preliminary infection experiment. A broth culture of *V. anguillarum* strain 1122 was diluted to 5 different concentrations. Thirty Japanese flounder were intraperitoneally injected with each of the 5 diluted bacterial suspensions (Table 1). The number of injected *V. anguillarum* was counted on agar plates after serial dilutions.

Based on the results of this preliminary experiment, the immunized fish in the 2 groups were challenged by a dose of 4.0×10^6 colony-forming units (CFU) fish⁻¹ with intraperitoneal injection. Mortality was recorded twice a day for 2 wk. Significant differences between the 2 groups were calculated by Fisher's exact test.

RESULTS

Fig. 1A shows a single band at the 37 kDa site (Lane 1), suggesting that the 37 kDa OMP was successfully separated from other OMPs. Fig. 1B shows that the protein reacted to the antiserum against 37 kDa OMP of *Edwardsiella tarda* (Lane 2) and antiserum against GAPDH (Lane 3). The results indicated

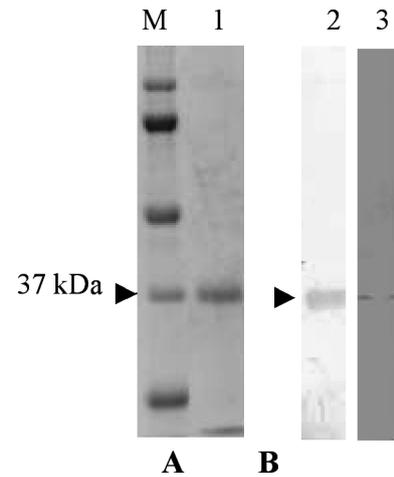


Fig. 1. Profile of 37 kDa glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in (A) SDS-PAGE gel and (B) Western blotting membrane. M: molecular weight marker; Lane 1: prepared GAPDH from *Edwardsiella tarda*; Lane 2: Western blotting using anti-37 kDa open membrane protein (OMP) of *E. tarda* serum; Lane 3: Western blotting using anti-GAPDH serum

that the prepared protein was the *E. tarda* 37 kDa GAPDH.

Fig. 2 shows the survival rate of the Japanese flounder after infection with *Vibrio anguillarum* strain 1122. From the second day after infection, fish in the control group started to die, and the survival rate decreased sharply until the fifth day after infection to a final survival rate of 42%. The final survival rate of the immunized group was 90%. Fish immunized with 37 kDa OMP of *Edwardsiella tarda* maintained a markedly higher survival rate than the unimmunized fish; the 2 groups were significantly different ($p < 0.005$). This result suggested that *E. tarda* GAPDH has protective antigenicity against infection of *V. anguillarum*.

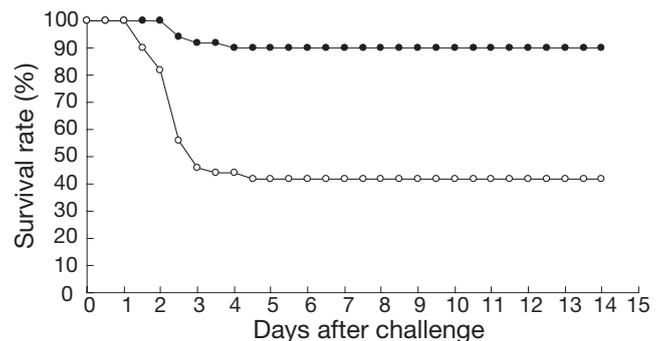


Fig. 2. *Paralichthys olivaceus*. Survival rate of Japanese flounder after a challenge with *Vibrio anguillarum* strain 1122. (●) Immunized with the 37 kDa open membrane protein (OMP) of *Edwardsiella tarda* EF-1 strain; (○) control injected with phosphate-buffered saline (PBS). A significant difference between the immunized and control groups was obtained ($p < 0.005$)

DISCUSSION

GAPDH is historically recognized as a cytoplasmic enzyme in the glycolytic pathway (Dandliker & Fox 1955, Fox & Dandliker 1956). Recent reports, however, have revealed that GAPDH binds to the cell membrane (Tsai et al. 1982, Allen et al. 1987, Modun & Williams 1999) and exists on the cell surface (Pancholi & Fischetti 1992, Hughes et al. 2002). As the surface GAPDH of *Streptococcus agalactiae* has no N-terminal signal peptide (Hughes et al. 2002), the attaching mechanism of this protein to the cell membrane is unclear. Membrane-bound GAPDH has many functions, but the signal peptide does not have the classical function of catalyzing (Tsai et al. 1982, Allen et al. 1987, Pancholi & Fischetti 1992, Modun & Williams 1999, Argiro et al. 2000a). Surface-located GAPDH of Gram-positive bacteria was reported to be a highly active receptor for host proteins and effective vaccine components (Seifert et al. 2003, Bolton et al. 2004). A 37 kDa surface GAPDH of *Schistosoma mansoni* is highly antigenic in the immune response of humans against schistosome infection (Argiro et al. 2000a), and further research has demonstrated that GAPDH of *S. mansoni* carries B-cell epitope and associates with antibody induction (Argiro et al. 2000b) in mice. Our previous study also showed that the 37 kDa OMP of *E. tarda* produced antibodies in fish and protected Japanese flounder against *Edwardsiella tarda* infection (Kawai et al. 2004).

GAPDH is widely present in Gram-positive bacteria (Iddar et al. 2005) and is a common protein of Gram-negative bacteria (Villamon et al. 2003). Alignment of the GAPDH amino acid sequences of several Gram-negative bacteria (*Escherichia coli*, *Salmonella enterica*, *Shigella flexneri*, and *Vibrio cholerae*) showed that the sequences are highly conserved and the similarities between these bacteria are $\geq 80\%$ (Liu et al. 2005) by using the DNA Data Bank of the Japan Homology Search System (www.ddbj.nig.ac.jp). The high conservation of the GAPDH among different pathogenic bacteria led to the idea that *Edwardsiella tarda* GAPDH might have protective antigenicity against other pathogenic bacteria. Thus, in the present study, Japanese flounder was immunized with the 37 kDa GAPDH of *E. tarda* and was challenged with *V. anguillarum*. An encouraging result showed that the immunized fish maintained a significantly higher survival rate than the unimmunized fish. The results were obtained with a non-adjuvanted antigen; thus, an even higher level of protection with an adjuvant might be expected. Results of the present study strongly suggest that the 37 kDa GAPDH of *E. tarda* may lead to the development of a multi-purpose vaccine against infection by different pathogenic bacteria.

GAPDH may be protective in several ways. The most reliable hypothesis is that immunization induces the production of antibodies in Japanese flounder (Kawai et al. 2004), which inhibits the function of the pathogen GAPDH. A similar observation was made in studies on parasites, where the use of drugs that inhibit the enzymatic activity of GAPDH decreased the survival of the infecting parasite (Callens & Hannaert 1995, Bourguignon et al. 1997). Anti-GAPDH antibodies produced in the immunized flounder might have inhibited the GAPDH activity of the infecting *Vibrio* in the challenge test of the present study.

Generally, our study showed that the GAPDH of *Edwardsiella tarda* has protective antigenicity against infection by *Vibrio anguillarum*, and indicates that the GAPDH of *E. tarda* can be developed as a multi-purpose vaccine against different pathogenic bacteria.

Acknowledgements. We thank Mr. K. Kurohara of the Prefectural Fisheries Experimental Station and fish pathology members for kindly providing experimental facilities and assistance.

LITERATURE CITED

- Allen RW, Trach KA, Hoch JA (1987) Identification of the 37-kDa protein displaying a variable interaction with the erythroid cell membrane as glyceraldehyde-3-phosphate dehydrogenase. *J Biol Chem* 262:649–653
- Argiro L, Kohlstädt S, Henri S, Dessein H, Matabiau V, Paris P, Bourgois A, Dessein AJ (2000a) Identification of a candidate vaccine peptide on the 37 kDa *Schistosoma mansoni* GAPDH. *Vaccine* 18:2039–2048
- Argiro L, Henri S, Dessein H, Kouriba B, Dessein AJ, Bourgois A (2000b) Induction of a protection against *S. mansoni* with a MAP containing epitopes of Sm37-GAPDH and Sm10-DLC. Effect of coadsorption with GM-CSF on alum. *Vaccine* 18:2033–2038
- Bolton A, Song XM, Willson P, Fontaine MC, Potter AA, Perez-Casal J (2004) Use of the surface proteins GapC and Mig of *Streptococcus dysgalactiae* as potential protective antigens against bovine mastitis. *Can J Microbiol* 50: 423–432
- Bourguignon SC, Alves CR, Giovanni-De-Simone S (1997) Detrimental effect of nitric oxide on *Trypanosoma cruzi* and *Leishmania major* life cells. *Acta Trop* 66:109–118
- Callens M, Hannaert V (1995) The rational design of trypanocidal drugs: selective inhibition of the glyceraldehyde-3-phosphate dehydrogenase in Trypanosomatidae. *Ann Trop Med Parasitol* 89:23–30
- Dandliker WB, Fox JB (1955) Light scattering of DL-glyceraldehyde-3-phosphate dehydrogenase. *J Biol Chem* 214: 275–283
- Fox JB, Dandliker WB (1956) A study of some of the physical properties of glyceraldehyde-3-phosphate dehydrogenase. *J Biol Chem* 218:53–57
- Hughes MJG, Moore JC, Lane JD, Wilson R and 12 others (2002) Identification of major outer surface proteins of *Streptococcus agalactiae*. *Infect Immun* 70:1254–1259
- Iddar A, Valverde F, Assobhei O, Serrano A, Soukri A (2005) Widespread occurrence of non-phosphorylating glycer-

- aldehyde-3-phosphate dehydrogenase among gram-positive bacteria. *Int Microbiol* 8:251–258
- Kaper JB, Morris Jr JG, Levine MM (1995) Cholera. *Clin Microbiol Rev* 8:48–86
- Kawai K, Liu Y, Ohnishi K, Oshima S (2004) A conserved 37 kDa outer membrane protein of *Edwardsiella tarda* is an effective vaccine candidate. *Vaccine* 22:3411–3418
- Kusuda R, Salati S (1993) Major bacterial disease affecting mariculture in Japan. *Annu Rev Fish Dis* 28:65–85
- Liu Y, Oshima S, Kurohara K, Ohnishi K, Kawai K (2005) Vaccine efficacy of recombinant GAPDH of *Edwardsiella tarda* against edwardsiellosis. *Microbiol Immunol* 49: 605–612
- Modun B, Williams P (1999) The staphylococcal transferrin-binding protein is a cell wall glyceraldehyde-3-phosphate dehydrogenase. *Infect Immun* 67:1086–1092
- Pancholi V, Fischetti VA (1992) A major surface protein on group A streptococci is a glyceraldehyde-3-phosphate-dehydrogenase with multiple binding activity. *J Exp Med* 176:415–426
- Seifert KN, McArthur WP, Bleiweis AS, Brady LJ (2003) Characterization of group B streptococcal glyceraldehyde-3-phosphate dehydrogenase: surface localization, enzymatic activity, and protein-protein interactions. *Can J Microbiol* 49:350–356
- Suzuki S, Kuroe K, Kusuda R (1994) Characteristics of porin-like major outer membrane proteins of *Listonella anguillar* serotypes J-O-1, -2 and -3. *Biochem Mol Biol Int* 32: 605–613
- Tsai IH, Murthy SNP, Steck TL (1982) Effect of red cell membrane binding on the catalytic activity of glyceraldehyde-3-phosphate dehydrogenase. *J Biol Chem* 257:1438–1442
- Villamon E, Villalba V, Noguerras MM, Tomas JM, Gozalbo D, Gil ML (2003) Glyceraldehyde-3-phosphate dehydrogenase, a glycolytic enzyme present in the periplasm of *Aeromonas hydrophila*. *Antonie Leeuwenhoek* 84:31–38

*Editorial responsibility: Jo-Ann Leong,
Kaneohe, Hawaii, USA*

*Submitted: August 8, 2005; Accepted: February 13, 2007
Proofs received from author(s): April 6, 2007*