MOLECULAR CLONING AND VACCINE EFFICACY OF OUTER MEMBRANE PROTEIN FROM *EDWARSIELLA ICTALURI* AGAINST *EDWARDSIELLA TARDA* IN TILAPIA

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INTRODUTION

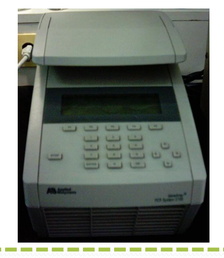
Edwardsiella tarda causes edwardsiellosis in several kinds of fish, especially tilapia, eel, carp and flounder in the world. Besides, enteric septicemia of catfish (ESC), caused by the bacterial *Edwardsiella ictalu*ri, is one of the most important infectious disease problems in catfish and other freshwater fish in the United States of America (USA), Vietnam and other Asian countries. For these reasons, the developing an effective vaccination strategy to edwardsiellosis and ESC in fish is a necessary. Recent studies indicate that outer membrane protein like glyceraldehydes- 3-phosphate dehydrogenase (GAPDH) from *E. tarda* can be an effective vaccine candidate against *E. tarda* or *Vibrio anguillarum* in fish. In this study, the gene encoding 37 kDa GAPDH of *E. ictaluri* was determined and overexpressed by using the *Escherichia coli* expression system. On the other hand, tilapias were intraperitoneally immunized with formalin-killed *E. ictaluri* whole cell, recombinant GAPDH (30μg fish^{-1}) from *E. ictaluri* and both. ISA 763A was as adjuvant for vaccine and phosphate buffered saline as control. Post-immunized 3 months, fish were challenged with live *E. tarda* to assess the vaccine efficacy.

MATERIALS & METHODS

Bacteria

Edwardsiella ictaluri strain OT9606S isolated from diseased Chinese catfish, *Parasilius asotus* in Taiwan was used.

PCR amplification of the GAPDH gene



The primers, gapdhF (5'-caccATGACTATCAAAGT-AGGTATC-3') and gapdhR (5'-TTATTTGGAGAT-GTGCGC-3',) targeted a region glyceraldehydes- 3-phosphate dehydrogenase gene of *E. ictaluri* and produced a 996 bp amplicon.

Cloning, sequencing and express of GAPDH gene

Commercial pET 151D/TOPO linearized vector (Invitrogen, USA) was used in this study.

SDS–PAGE **Western blot**

Table 1. Immunization groups for tilapia.

Groups	Adjuvant	Vaccine dose[a] (0.2ml fish^{-1})
FKWC[b]	ISA 763A	10^8CFU fish^{-1}
GAPDH	ISA 763A	30μg fish^{-1}
FKWC+GAPDH	ISA 763A	10^8CFU + 30μg fish^{-1}
PBS	—	—

a: Fishes (101.4 ±17.3g) were injected intraperitioneally with the vaccine or phosphate buffer saline (PBS) and held at 25°C for 3 months.
b: Formalin killed whole cell of *E. ictaluri* strain OT9606S

Challenge experiment

CONCLUSIONS

- **Thirty- seven kDa GAPDH of *E. ictaluri* showed a similarity to *E. tarda* with DNA and amino acid sequence .**
- **The GAPDH of *E. ictaluri* only has a protective antigenicity against *E. tarda* infection in tilapia.**
- **The formalin-killed whole cell and GAPDH of *E. ictaluri* with ISA 763A showed the higher protective than formalin-killed whole cell with ISA 763A alone, suggest that GAPDH can enhance protective for *E. ictaluri* whole cell vaccine against *E. tarda* infection in tilapia.**

RESULTS

Percent Identity

	1	2	3	4	5	6	7	8	9	10	
1		100.0	95.5	84.2	84.3	82.9	77.5	75.0	74.7	74.1	1
2	0.0		95.5	84.2	84.3	82.9	77.5	75.0	74.7	74.1	2
3	4.7	4.7		85.5	85.4	83.9	78.2	76.3	76.0	75.1	3
4	18.1	18.1	16.4		99.8	94.3	80.7	79.3	78.9	78.7	4
5	17.9	17.9	16.5	0.2		94.3	80.6	79.2	78.8	78.6	5
6	19.6	19.6	18.4	6.0	6.0		79.2	77.5	77.0	77.2	6
7	27.0	27.0	26.0	22.4	22.5	24.5		88.5	88.2	88.6	7
8	30.8	30.8	28.8	24.4	24.5	26.9	12.5		94.3	97.7	8
9	31.3	31.3	29.3	24.9	25.1	27.6	12.9	5.9		94.5	9
10	32.2	32.2	30.7	25.2	25.3	27.3	12.4	2.3	5.7		10
	1	2	3	4	5	6	7	8	9	10	

Divergence

OT9606S
Edwardsiella ictaluri CP001600
Edwardshella tarda FJ605131
Escherichia coli EU899899
Shigella_flexneri AE005674
Salmonella enteric serovar Typhi AL6272
Vibrio cholera CP001485
Vibrio parahaemolyticus BA000031
Vibrio vulnificus AEC16795
Vibrio harveyi DQ184650

Fig. 1 Percentage similarities and divergences of the glyceraldehydes- 3-phosphate dehydrogenase nucleotide sequence in *E. ictaluri* strain and reference strains using the clustal program of the DNASTAR software package.

Percent Identity

	1	2	3	4	5	6	7	8	9	10	
1		100.0	98.8	89.5	89.5	88.6	85.0	85.0	84.1	84.1	1
2	0.0		98.8	89.5	89.5	88.6	85.0	85.0	84.1	84.1	2
3	1.2	1.2		90.1	90.1	89.2	85.6	84.7	83.8	83.8	3
4	11.4	11.4	10.7		100.0	98.8	85.3	84.7	84.1	84.4	4
5	11.4	11.4	10.7	0.0		98.8	85.3	84.7	84.1	84.4	5
6	12.4	12.4	11.7	1.2	1.2		84.7	84.7	83.5	83.8	6
7	16.8	16.8	16.1	16.4	16.4	17.2		91.9	90.7	91.9	7
8	16.8	16.8	17.2	17.2	17.2	17.2	8.6		97.0	98.2	8
9	17.9	17.9	18.3	17.9	17.9	18.7	10.0	3.1		97.6	9
10	17.9	17.9	18.3	17.6	17.6	18.3	8.6	1.8	2.4		10
	1	2	3	4	5	6	7	8	9	10	

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Fig. 2 Percentage similarities and divergences of the glyceraldehydes- 3-phosphate dehydrogenase amino acid sequence in *E. ictaluri* strain and reference strains using the clustal program of the DNASTAR software package.

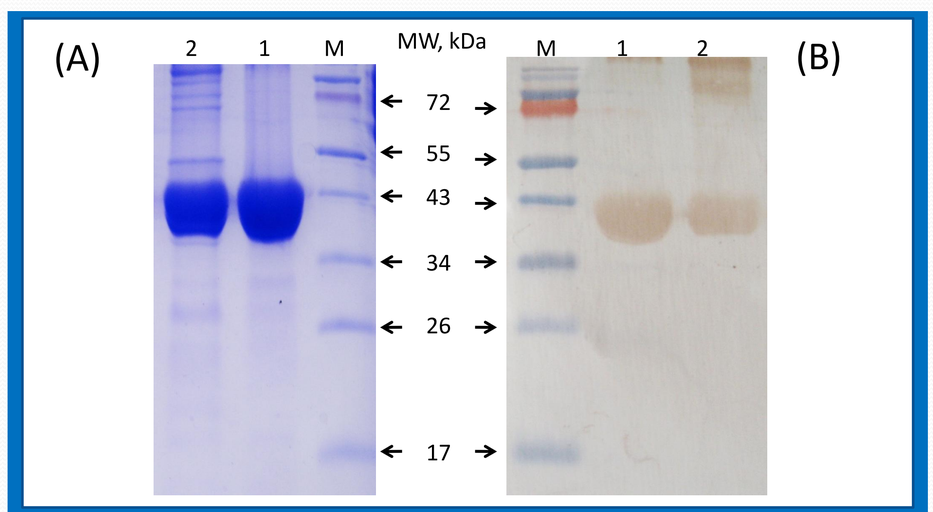


Fig. 3 SDS-PAGE and Western blot with rabbit antibody injected with recombinant *E.ictaluri* GAPDH protein antigen. (A) SDS-PAGE (12% gel) with Coomassie brilliant blue staining. (B) Western blot assay Lane 1: purified GAPDH protein. Lane: 2: GAPDH protein. Lane M: marker.

Table 2. Fish cumulative mortality and relative percent survival (RPS) of tilapia challenged at 3 months after immunization.

Groups	Mortality		RPS	
	7D[b]	14D	7D	14D
FKWC[a]+ ISA 763A	12.5%	62.5%	85.7%	28.6%
GAPDH+ ISA 763A	25%	50%	71.4%	42.9%
FKM+GAPDH+ ISA 763A	12.5%	25%	85.7%	71.4%
PBS	87.5%	87.5%	—	—

a: Formalin killed whole cell of *E. ictaluri* strain OT9606S
b: Days post-challenge, fish were injected intraperitioneally with the *E. tarda* (Challenge dose: 1.65x10^7 CFU fish^{-1}) isolated from diseased tilapia.

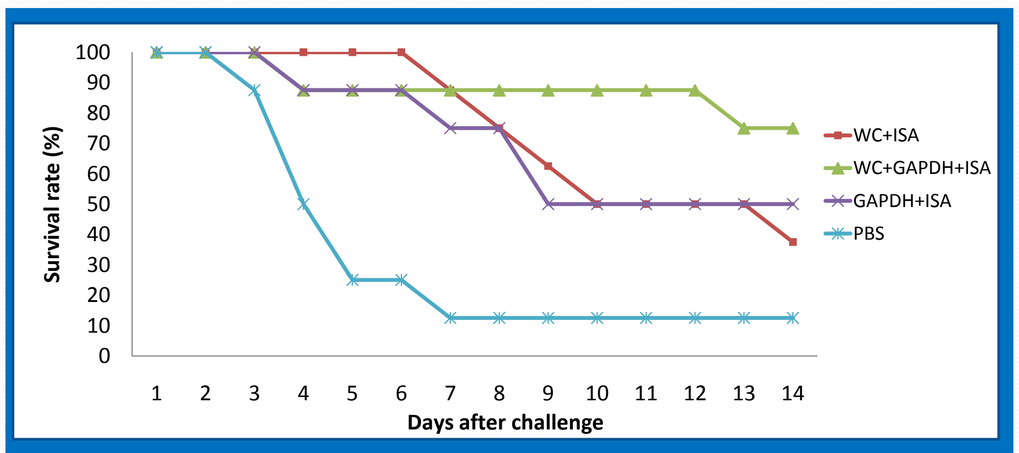
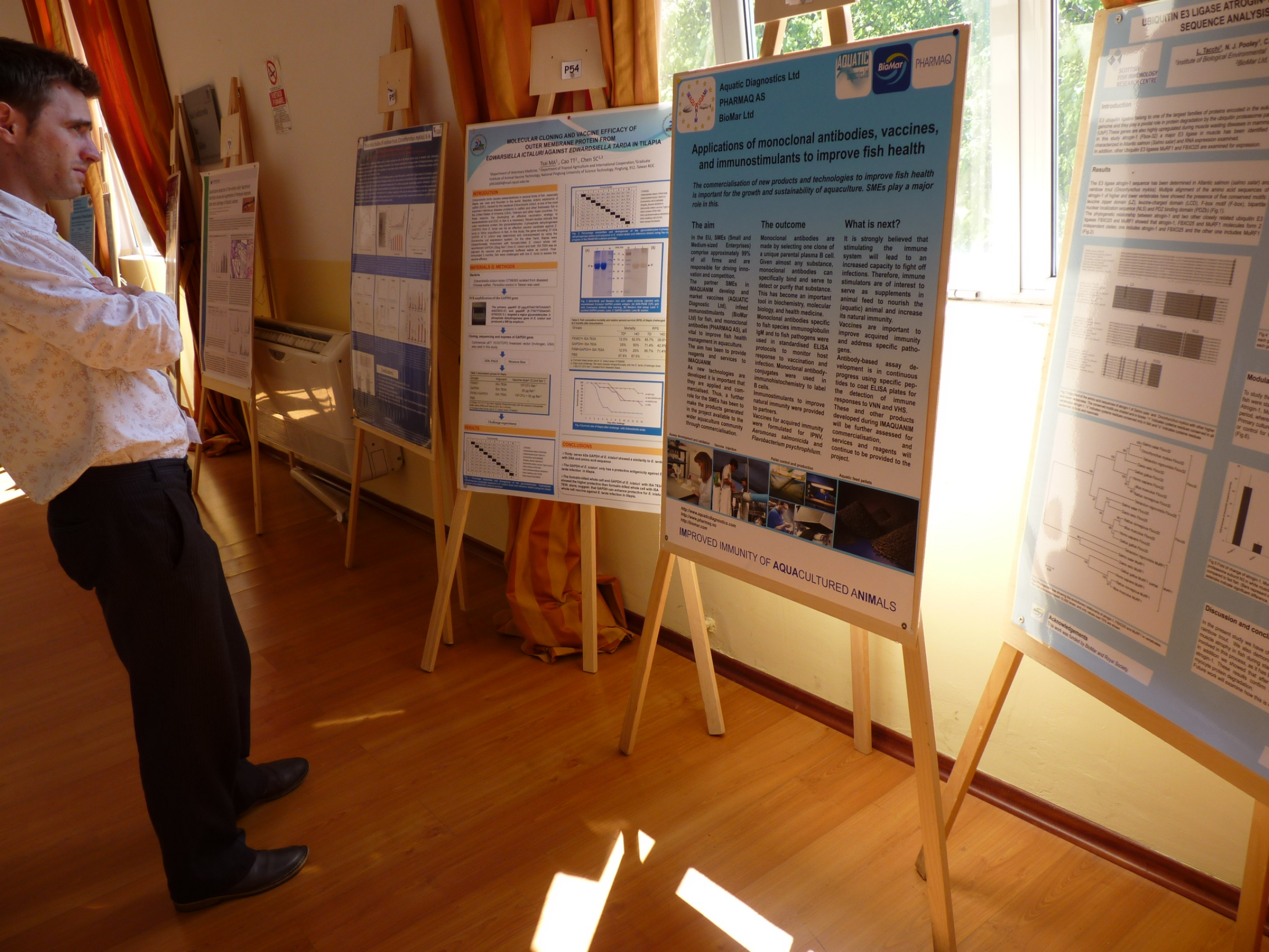


Figure 4. Survival rate of tilapia after challenge with *Edwardsiella tarda*.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Aquatic Diagnostics Ltd
PHARMAQ AS
BioMar Ltd
Applications of monoclonal antibodies, vaccines, and immunostimulants to improve fish health
The aim
The outcome
What is next?
IMPROVED IMMUNITY OF AQUACULTURED ANIMALS
UBIQUITIN E3 LIGASE ATROGIN-1 SEQUENCE ANALYSIS