UNDP/GEF Project "Reducing Environmental Stress in the Yellow Sea Large Marine Ecosystem"

Regional Mariculture Conference

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Immunity and Biological Methods of Disease Prevention and Control

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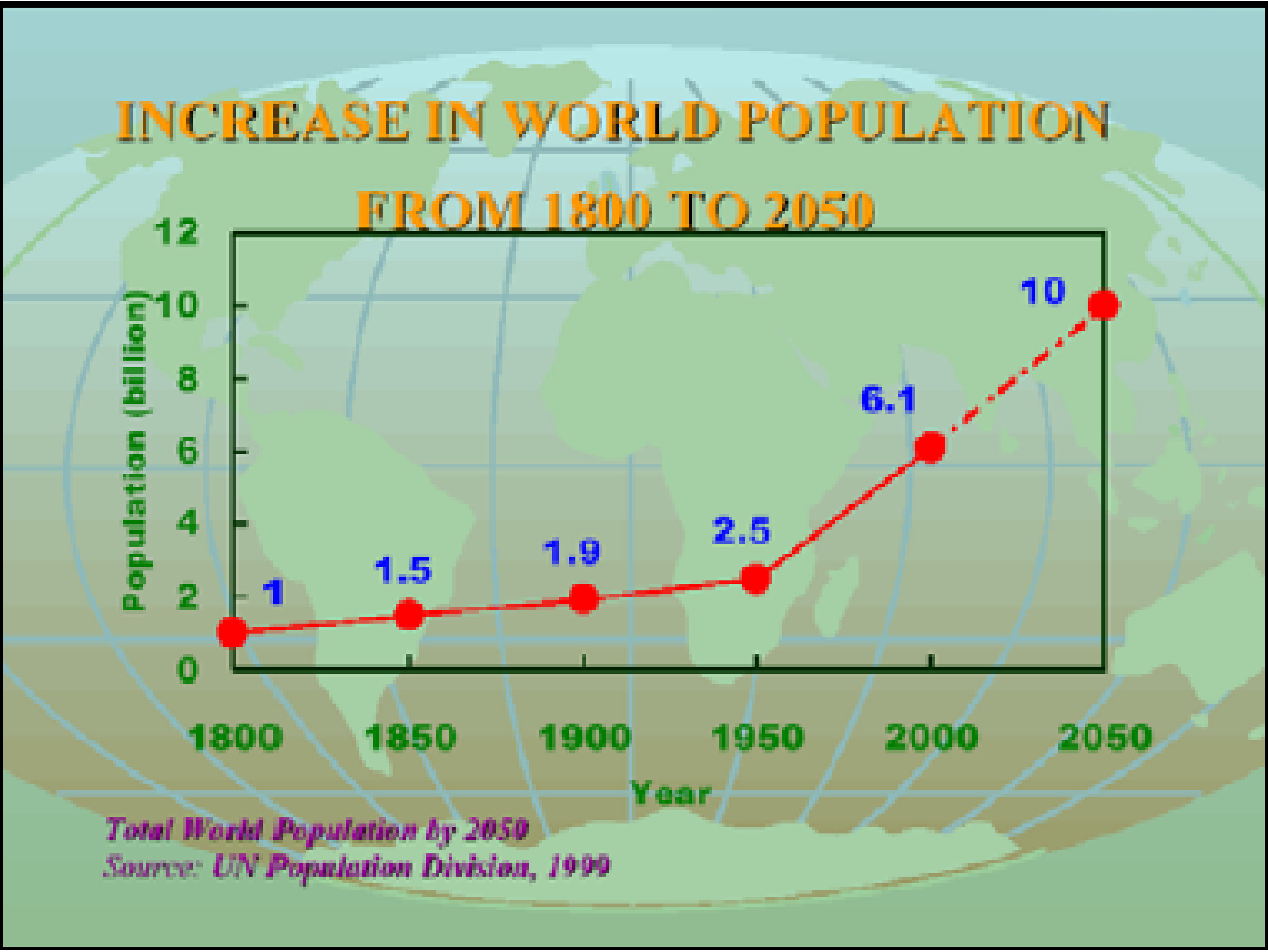
Outline

1. Aquaculture: importance and challenge.
2. A brief introduction about the immune system and its application in disease prevention and control.
3. The preliminary progress in the study of scallop immunity and the potential immunity-based approaches to control disease.

1. Aquaculture: importance and challenge


INCREASE IN WORLD POPULATION
FROM 1800 TO 2050
Population (billion)
12
10
8
6
4
2
0
1
1.5
1.9
2.5
6.1
10
1800
1850
1900
1950
2000
2050
Year
Total World Population by 2050
Source: UN Population Division, 1999

Food security

"In the next 50 years, mankind will consume as much food as we have consumed since the beginning of agriculture 10,000 years ago"

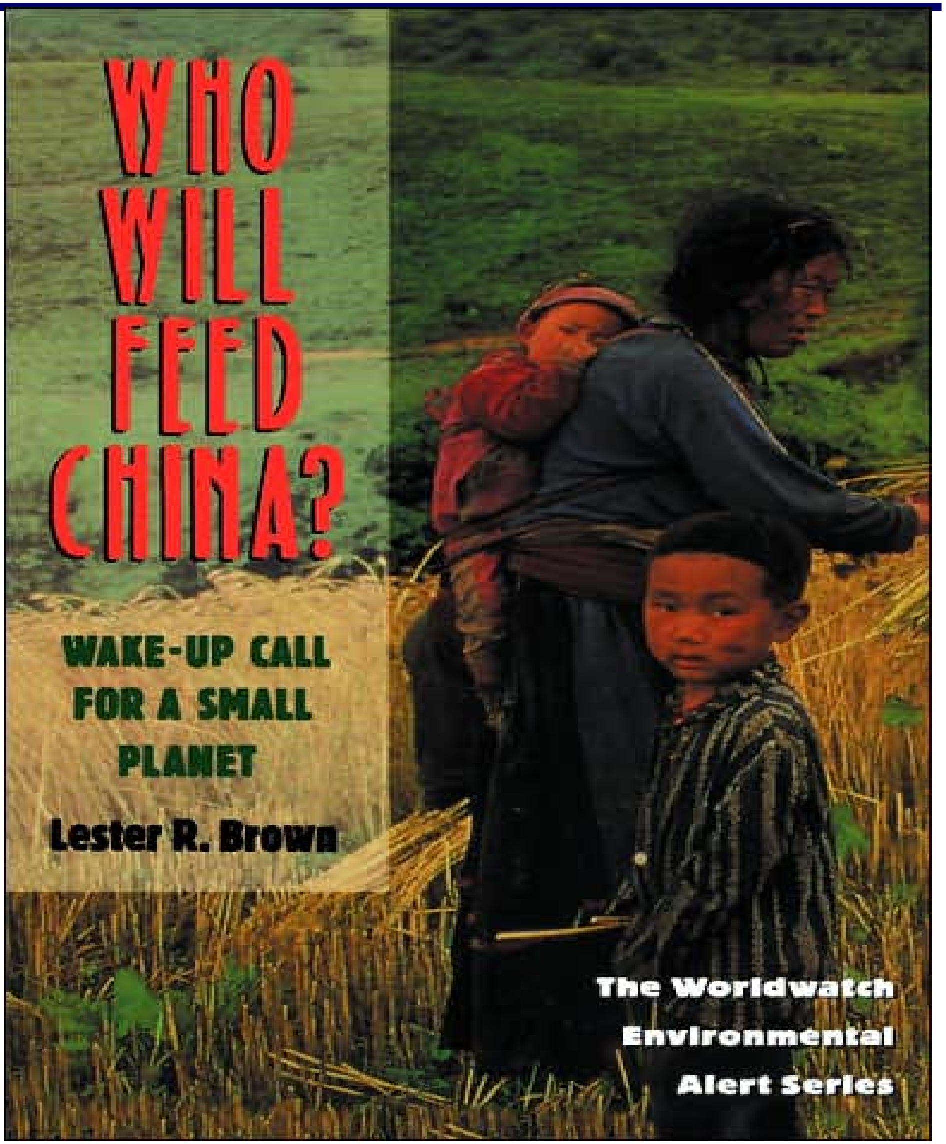
Clive James

"Without an increase in farm productivity, additional 1.6 billion hectares of arable land will be need by 2050"

(FAO)

Who will feed China?

Dr. Lester R. Brown



Increasing agricultural productivity is the key to food security in the world.

Existing problem:

- Conventional plant improvement methods are reaching their limits.
- Agriculture growth is now 1% compared to 3% in 1970s.
- Limited Water and Land.

Aquaculture is the most efficient way to produce animal protein

(1) High reproductive potential

Fish: tilapia > 2,000 babies/year. **Pig:** 12 babies/year.

Chicken: 100 babies/year. **Cattle:** 1 baby/year.

(2) Low land use for growing each animal

Fish: 0.00005 acre or less. **Pig:** 0.005 acre.

Chicken: 0.0001 acre. **Cattle:** 1 acre.

(3) High food conversion rate- (lbs. of dry feed consumed to produce each lb. of animals)

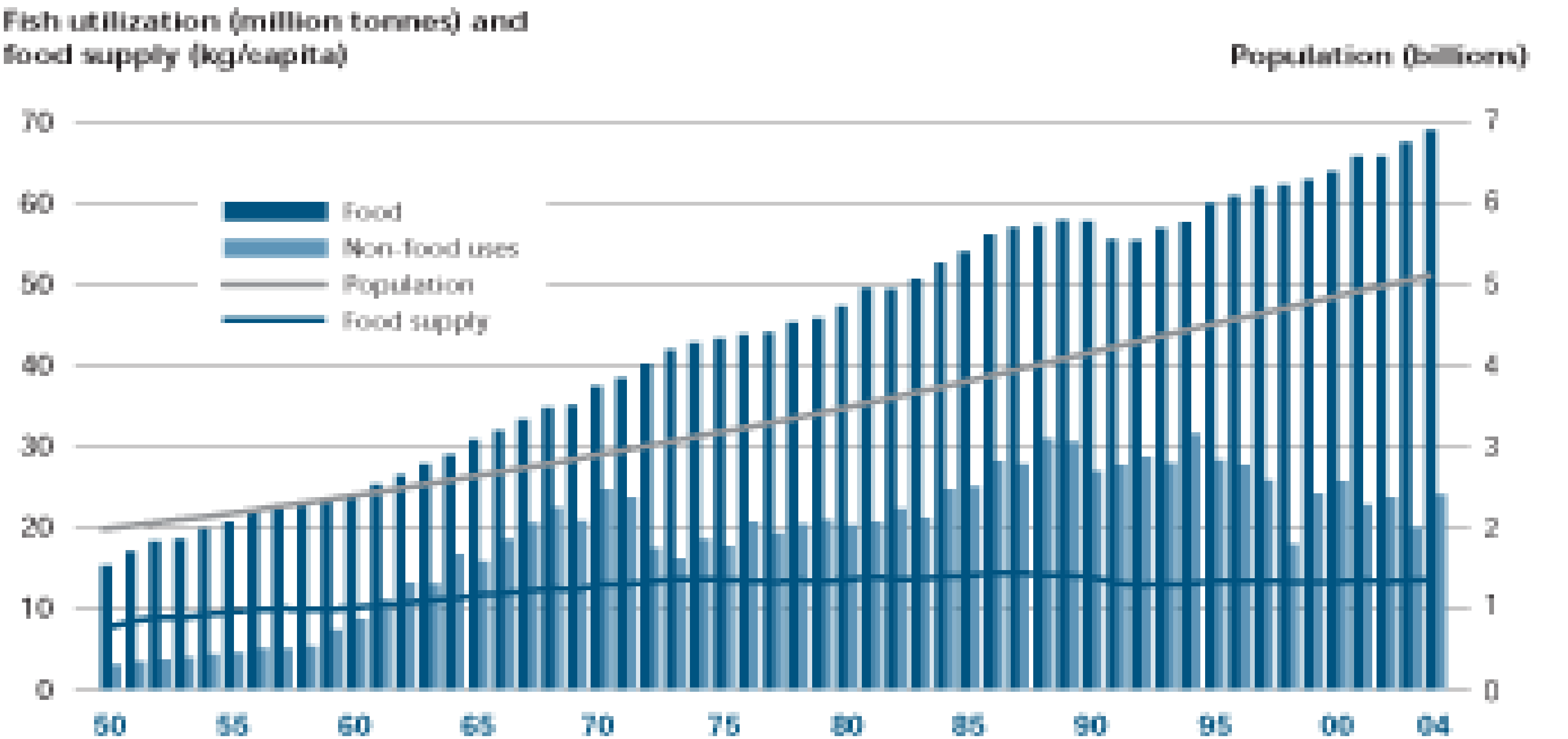
Fish : 1 to 3 lbs. **Pig :** 5 to 12 lbs.

Chicken: 3 to 6 lbs. **Cattle :** 12 to 45

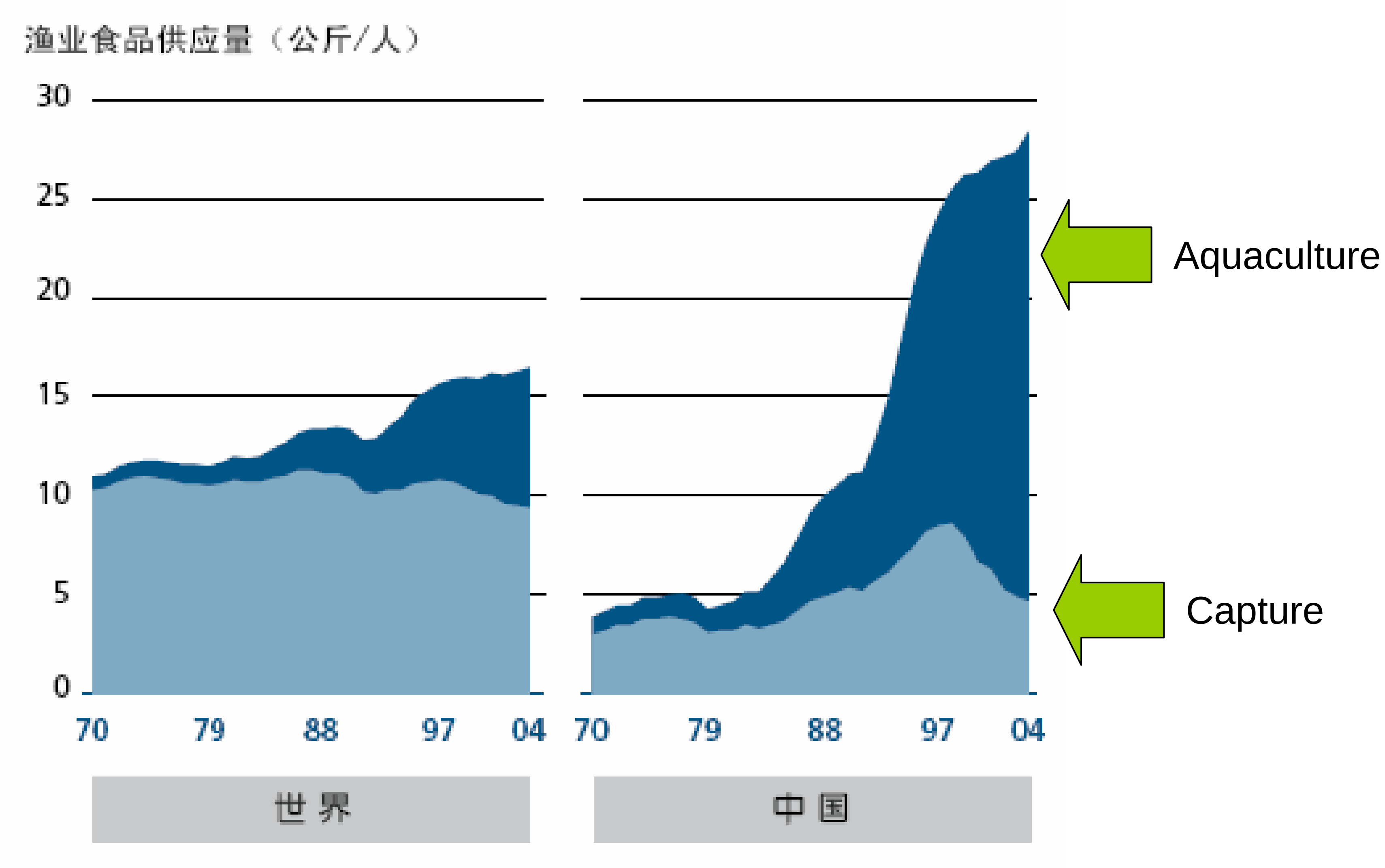
Fish provided more than 2.6 billion people with at least 20 percent of their average per capita animal protein intake. (FAO, 2006)

Figure 2

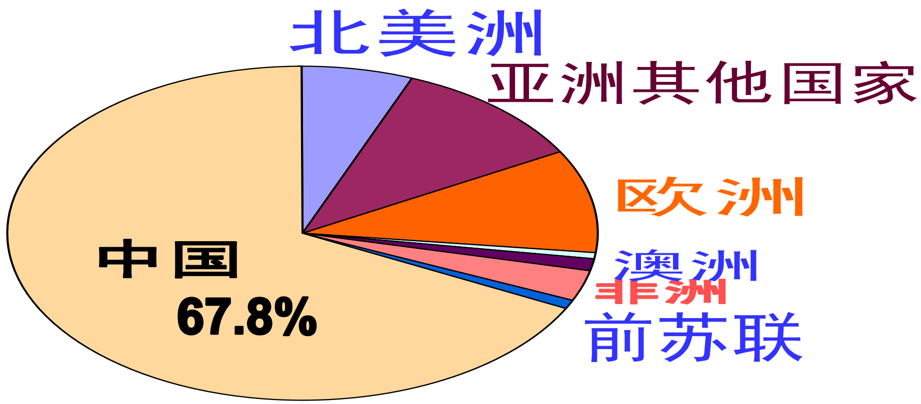
World fish utilization and supply, excluding China



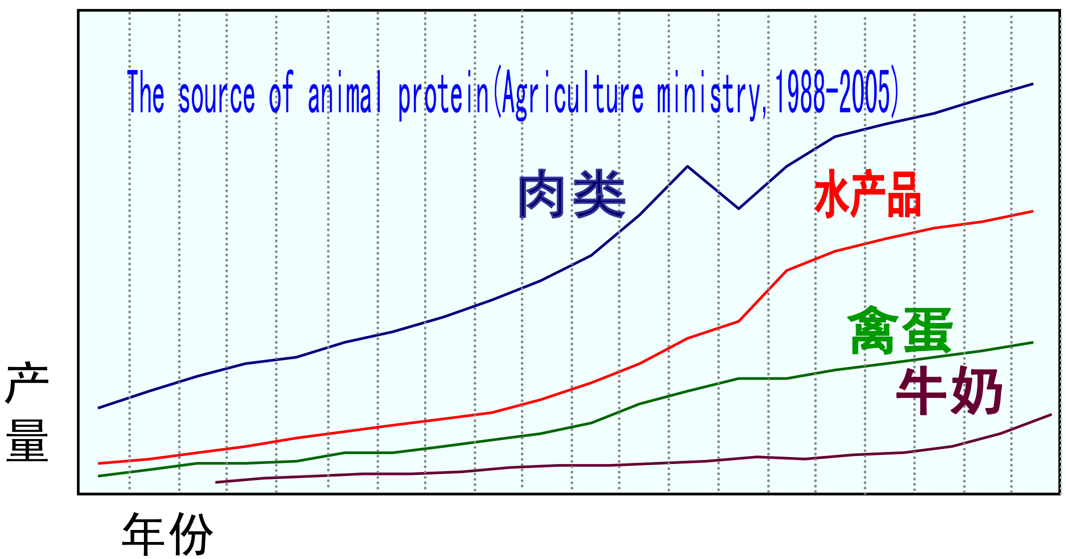
Relative contribution of aquaculture and capture fisheries to food fish consumption

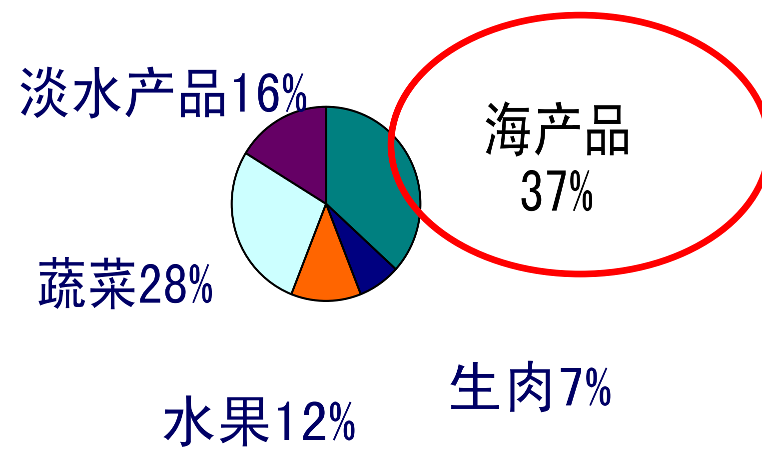


The aquaculture in China



Aquaculture production in China reached 70% of the total production in world.(FAO,2003)





Proportion of Exportation (FAO, 2003)

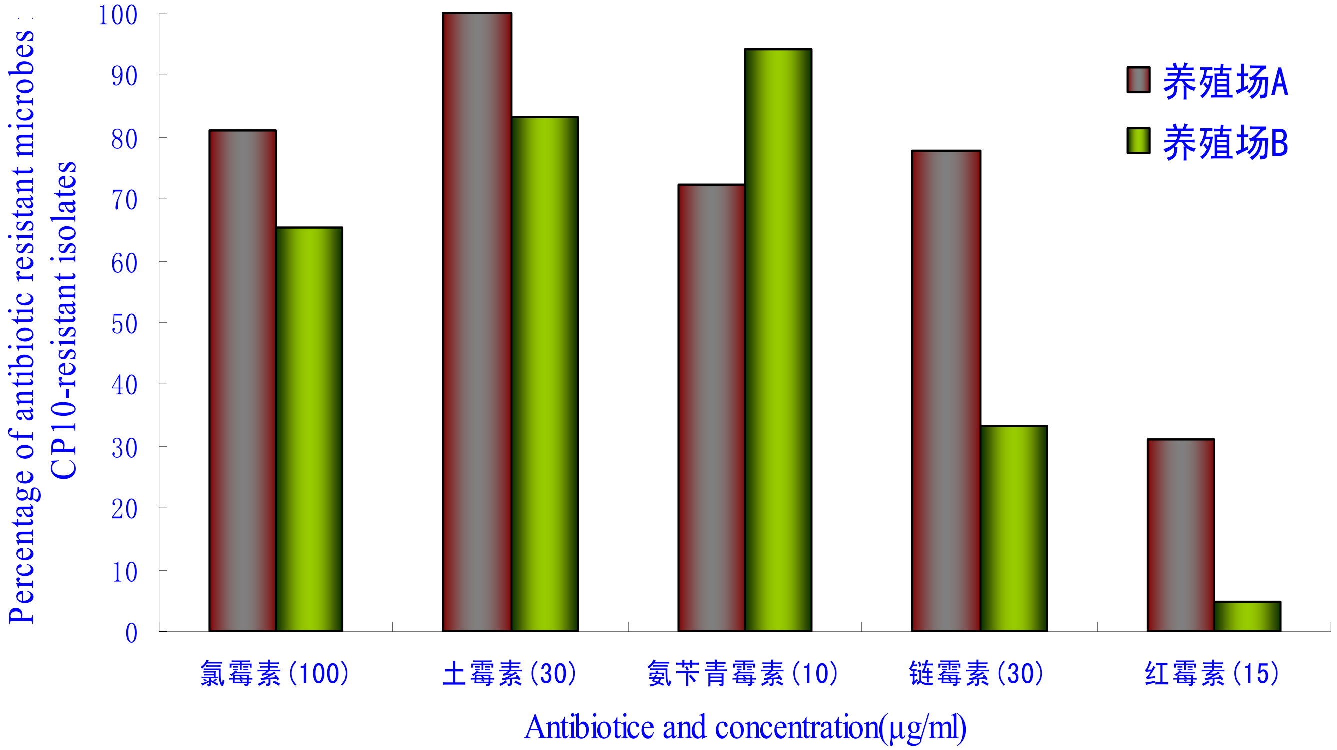
The frequently outbreak of disease threaten the sustainable development of aquaculture

Fish

Shrimp

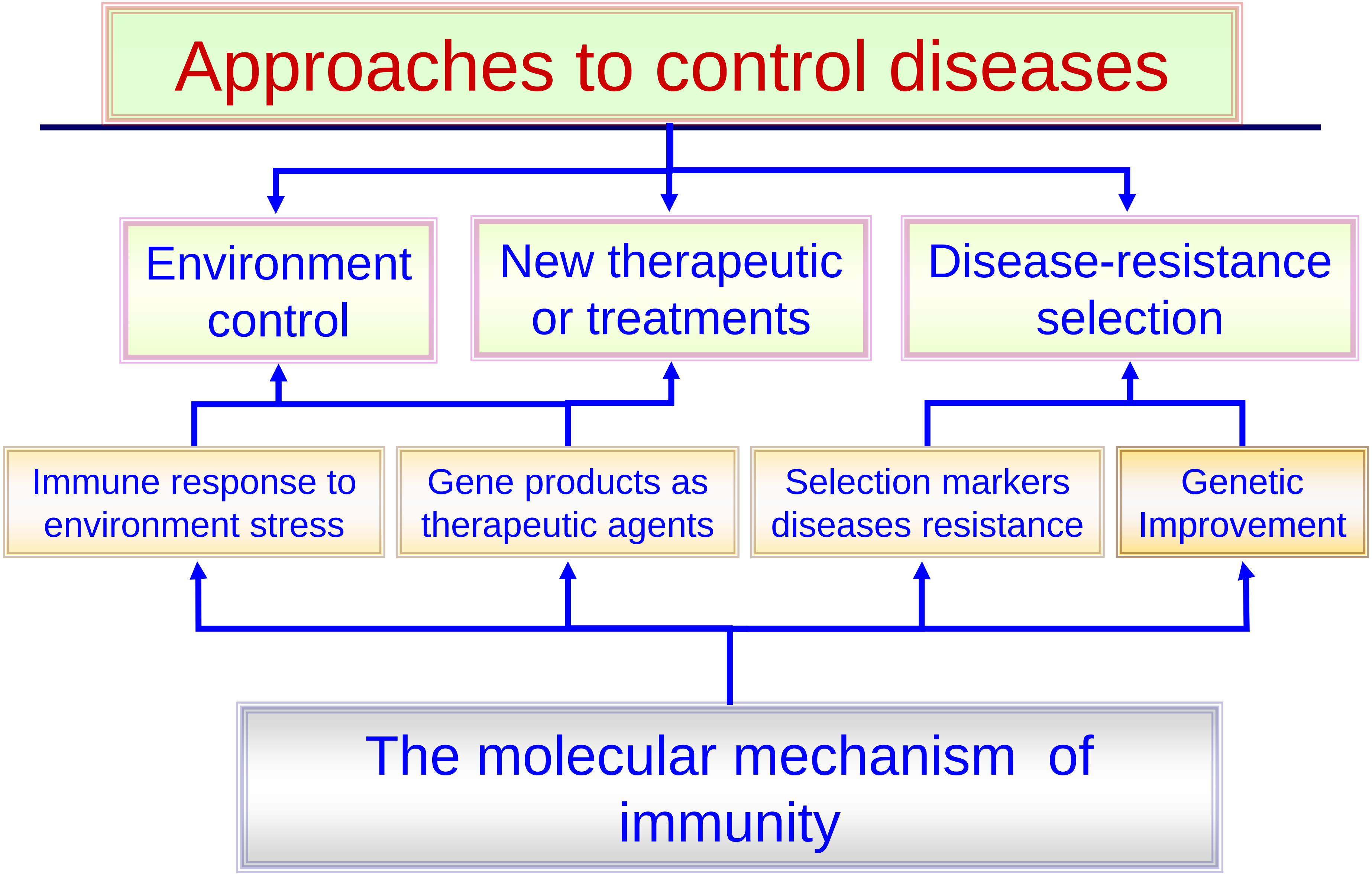
Scallop

The incontrovertible harmful consequence for environment from abuse of antibiotics and chemicals.



The abundance of antibiotic-resistant microbes increased.

Dang et al., Microbiol Ecology, 2006



Approaches to control diseases
Environment control
New therapeutic or treatments
Disease-resistance selection
Immune response to environment stress
Gene products as therapeutic agents
Selection markers diseases resistance
Genetic Improvement
The molecular mechanism of immunity

2. Immune system

Most multi-cellular organisms survive in the world depend on a network of host defence mechanisms

The world is laden so many kinds of microorganisms

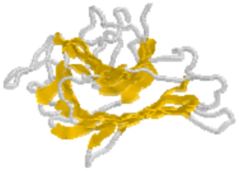
Innate immunity

Acquired immunity

Innate immunity

Invertebrate immunity system is comprised of two branches:

Humoral response:



Antimicrobial peptides;

Macrokines;

Clotting system;

proPO activating system;

Lectins.

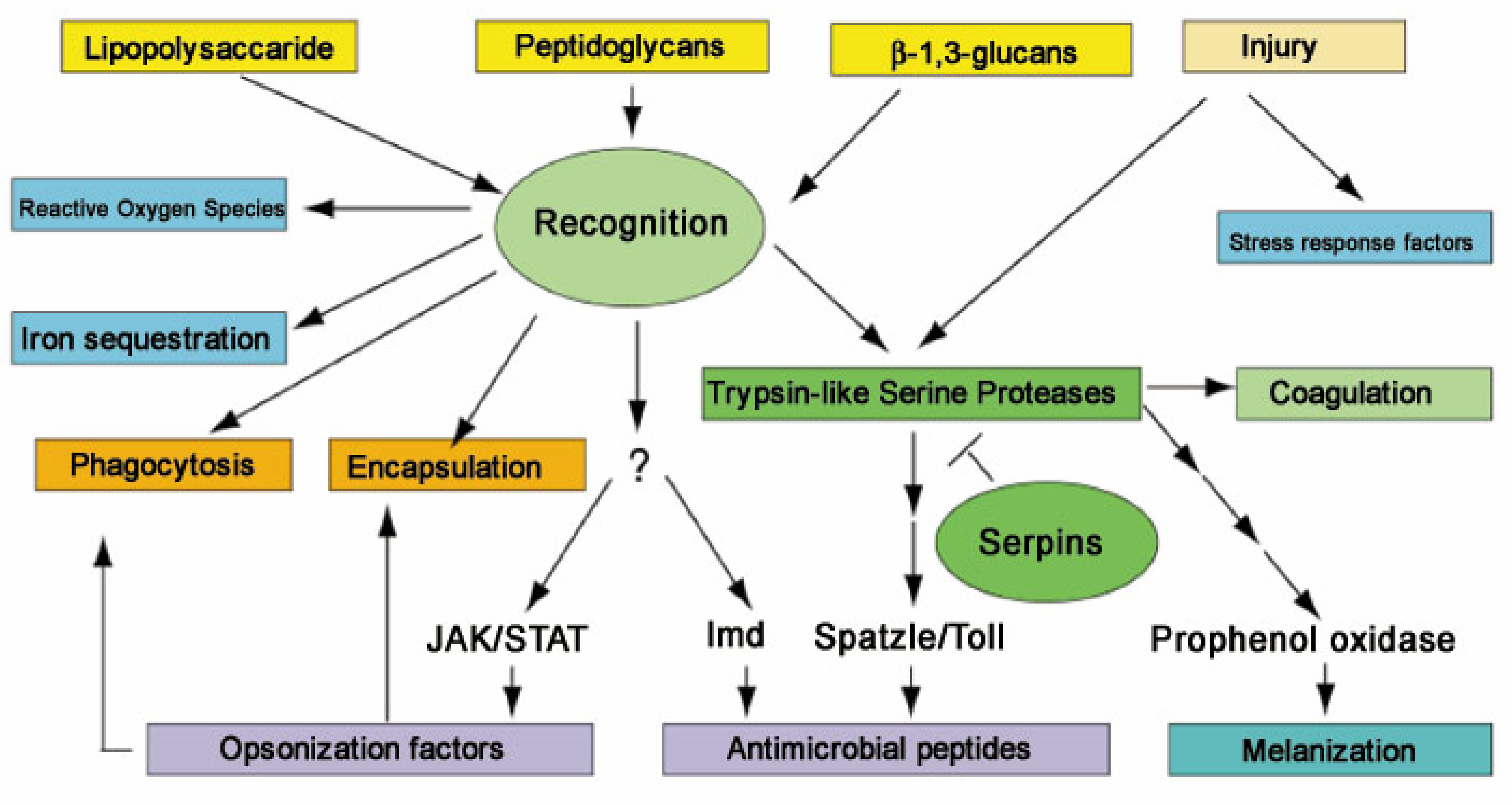
Cellular response:

Phagocytosis

Encapsulation

Nodulation

Drosophila host defence



From Current Opinion in Microbiology

Immunity and Biological Methods of Disease Prevention and Control

i. Molecular diagnostic kit

ii. Vaccine

iii. Immune stimulators

iv. Anti-microbial peptides

Molecular diagnostic techniques in aquaculture

- Serological methods:

  Multifarious ELISA

- Molecular methods:

  DNA

  RNA

  Protein

Vaccination

- Injection
- Immersion methods

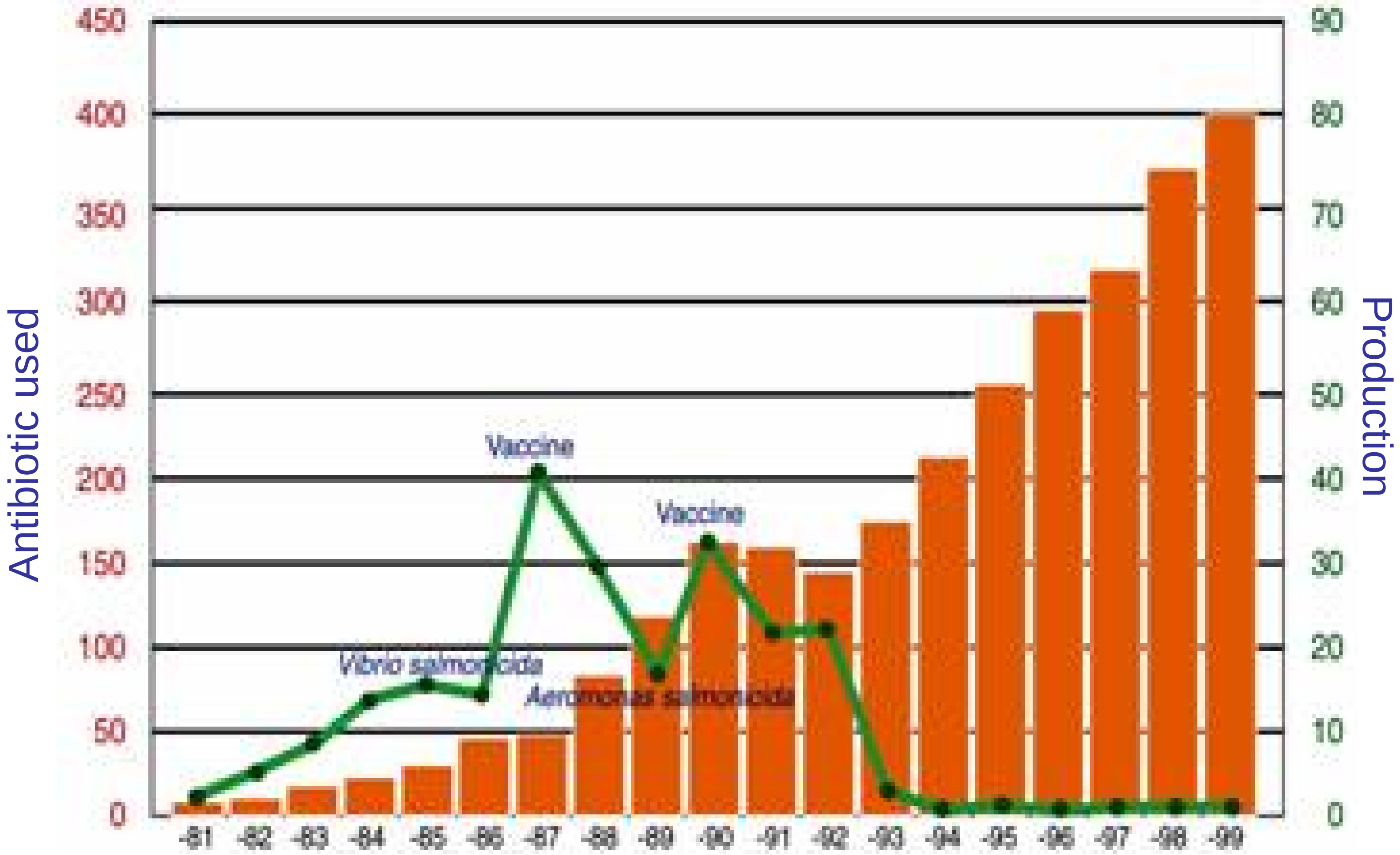
  Dip vaccination

  Bath vaccination
- Oral vaccination

Future development -

Use recombinant DNA techniques produce proteins of pathogens, DNA vaccine

Use of antibiotic relative to salmon and trout production in Norway from 1981 to 1999



(reproduced from Vinitrianthar, 2001)

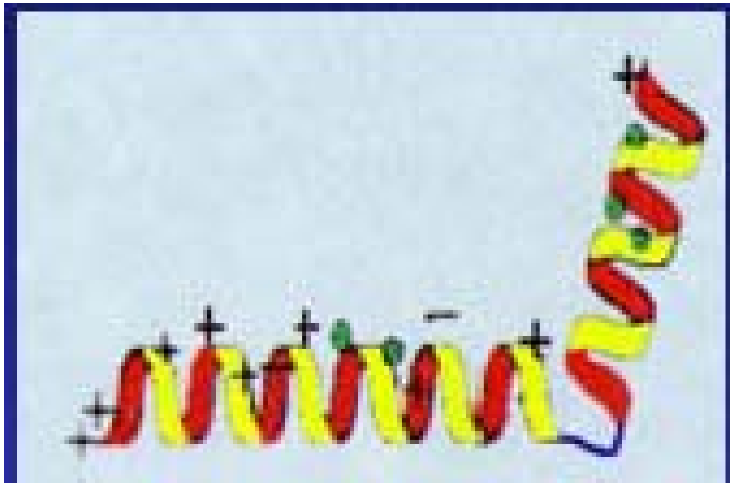
IMMUNOSTIMULANTS

Immunostimulants are compounds that stimulate the non-specific defense mechanisms in organisms.

- Lipopolysaccharide: The cell wall component of Gram-negative bacteria and consists of lipids and carbohydrates.
- Peptidoglycan: The cell wall component of Grampositive bacteria.
- Glucans: Found on the cell walls of fungi.
- Mannan oligosaccharides: Component of yeast cell wall.
- Fucoidan: Sulfated polysaccharide

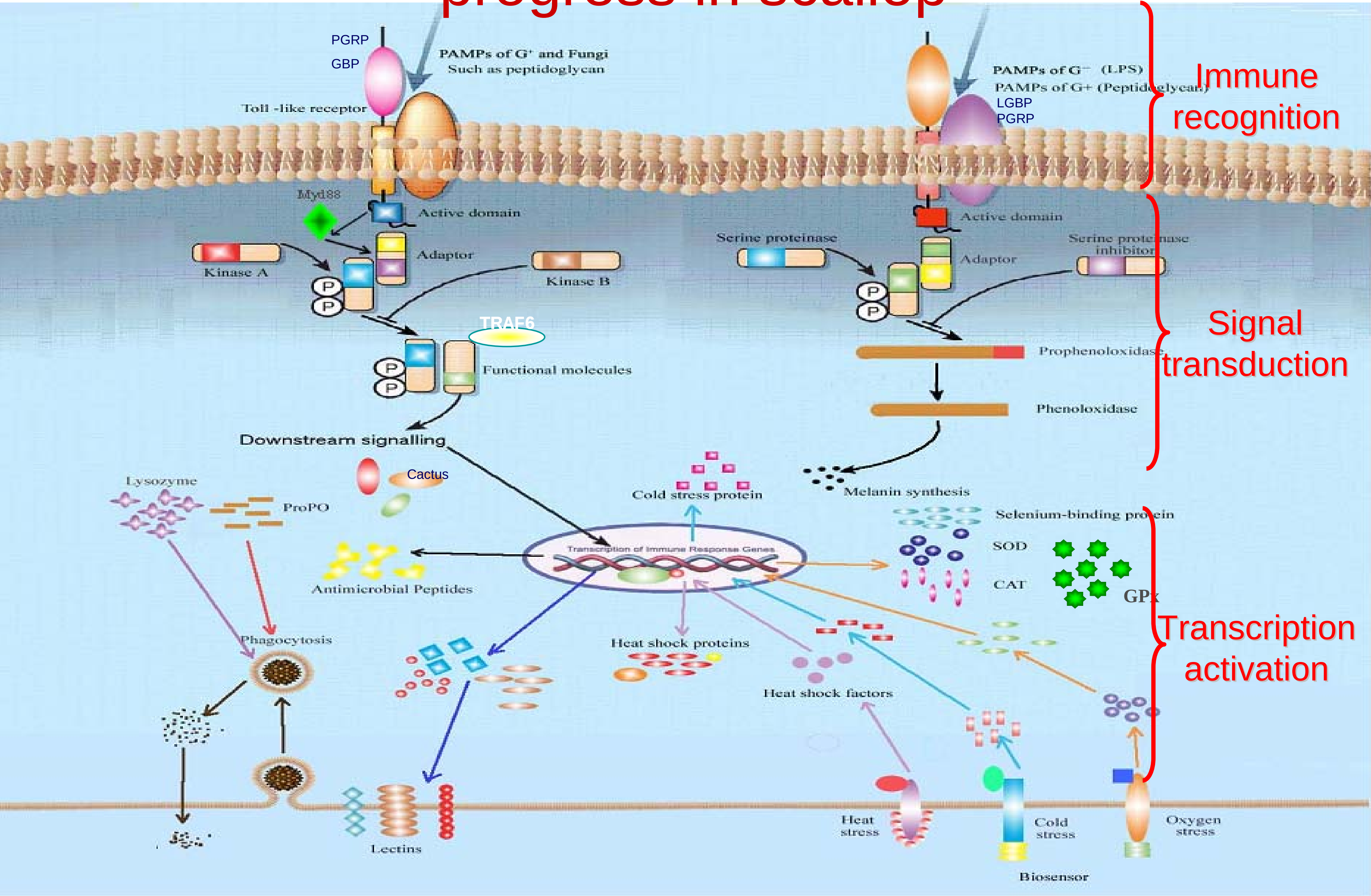
Antimicrobial peptides

- AMP is an Innate immune response of plants, invertebrates and vertebrates
- Ubiquitous lytic peptides in nature
- First line of defense against pathogens
- Amphipathic α helix
- Cationic peptide
- A channel-forming peptide
- 15-75 amino acids in length



3. The preliminary progress in the study of scallop immunity and the potential immunity-based approaches to control disease

The overview of molecular immunological progress in scallop



(1) Genes involved in immune recognition

(LGBP, PGRP, Lectins, TLRs)

Huan Zhang et al., 2007, Mol. Immunol

Duojiao Ni et al, 2007, Dev. Com. Immuonol

Jianguo Su et al., 2007, Fish & shellfish Immunol

Hao Wang et al, 2007, Mol. Immunol

Limei Qiu et al., 2007, Fish & shellfish Immunol

Jianguo Su et al., 2004, Aquaculture

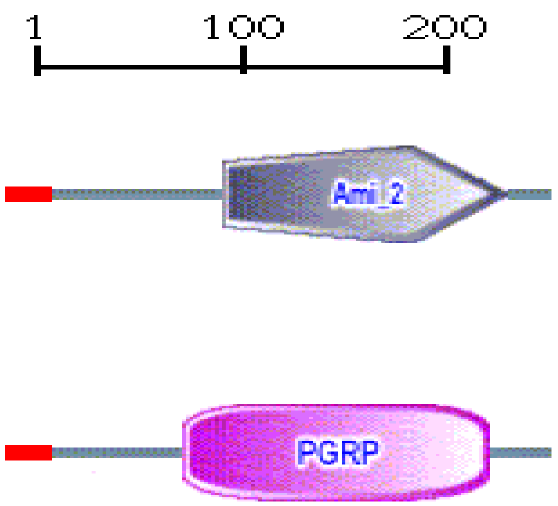
The genes involved in immune recognition (14)

Gene name	Function	Full-length/ ORF (bp)	Accession No.	species
AiPGRP	recognition/G^{+}	1018/615	AY437875	*A. irradians*
CfPGRP		1073/759	AY987008	*C. farreri*
CfLGBP	recognition/G^{-}or Fungi	1876/1320	AY259542	*C. farreri*
CfCTL-A	Recognition the virus carbohydrate/glyco protein	1038/684	AY676311	*C. farreri*
CfCTL-1		708/171	DQ209289	*C. farreri*
CfCTL-2		1772/221	DQ209290	*C. farreri*
CfCTL-3		2257/524	DQ209291	*C. farreri*
CfCTL-4a		2086/633	DQ209292	*C. farreri*
CfCTL-4b		1897/633	DQ2092893	*C. farreri*
CfToll-1	recognition/G^{-}	1695 /650	DQ350772	*C. farreri*
CfTEP	recognition/G^{-}	4616/4446	EF210036	*C. farreri*
CfC1qDC	recognition/G^{-}	777/537	EF536358	*C. farreri*
TLRs(2,5,8)	recognition	1038-3085	--	*C. farreri*
SR	recognition	partial/1439	--	*C. farreri*

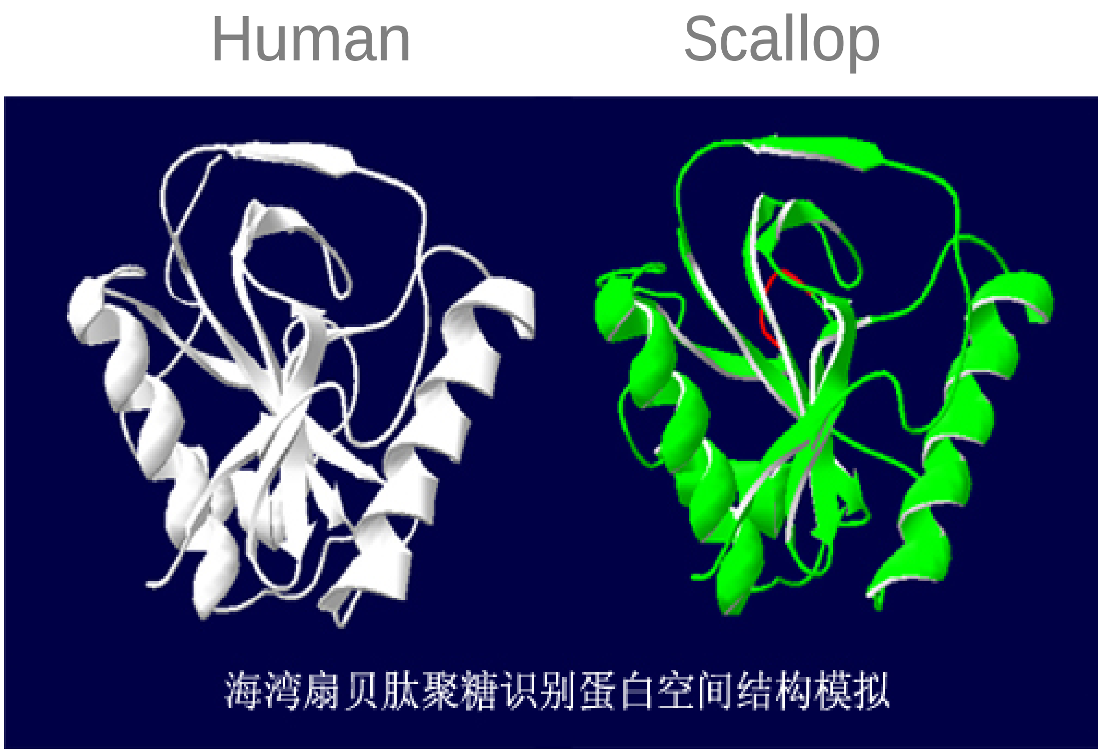
✦ Cloning and mRNA expression of LGBP and PGRP gene from scallops

- LGBP (Lipopolysaccharide-and beta-1,3-glucan-binding protein) and PGRP (Peptidoglycan recognition protein) play a crucial role in the innate immune response as a pattern recognition protein.
- They can recognize and bind lipopolysaccharide in the G^- bacteria and glucan in fungi, or peptidoglycan in the G^+ bacteria to trigger the responses such as phagocytosis, nodule formation, encapsulation, activation of proteinase cascades, and synthesis of antimicrobial peptides.

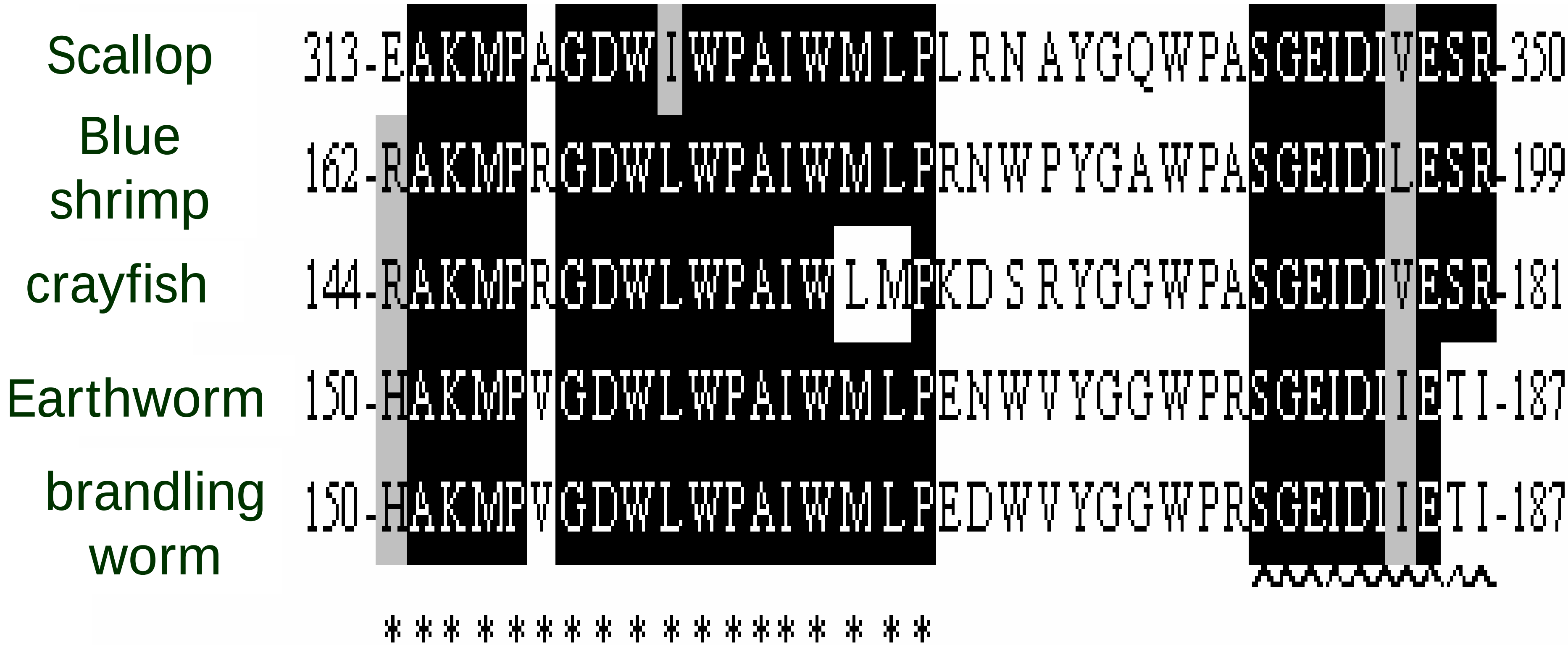
The predicated structure of PGRP



Signal peptide: 1-22 Aa;
PGRP domain: 83-225;
Ami_2 domain: 100-231

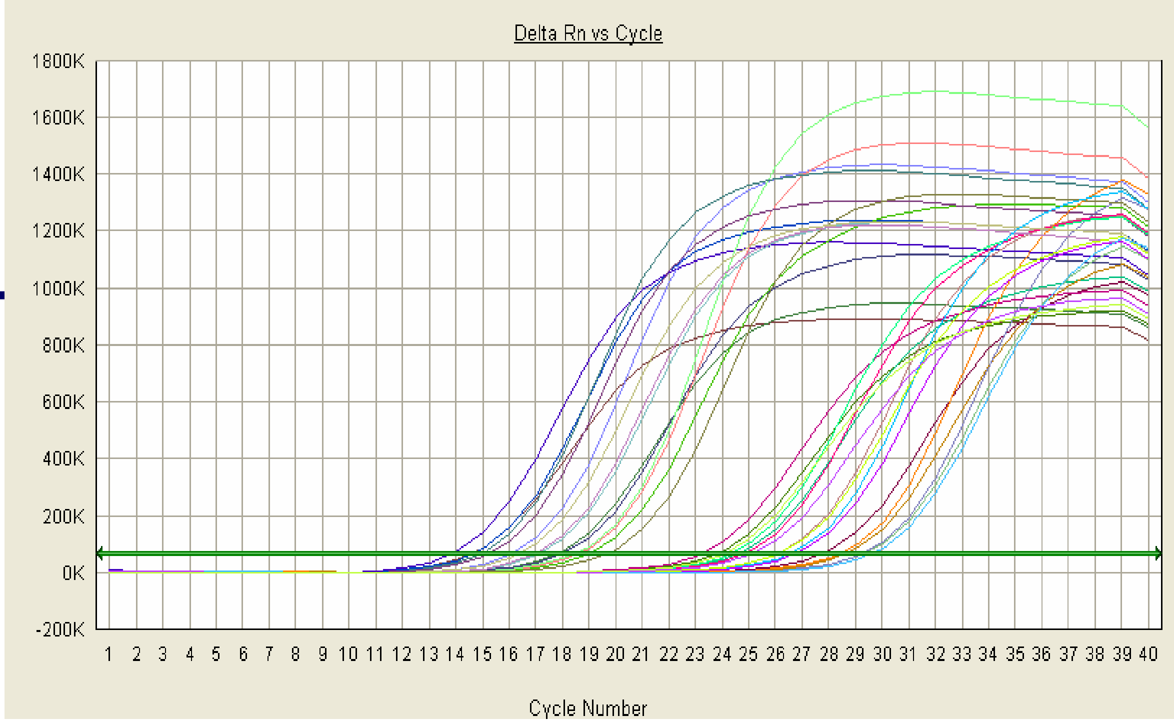


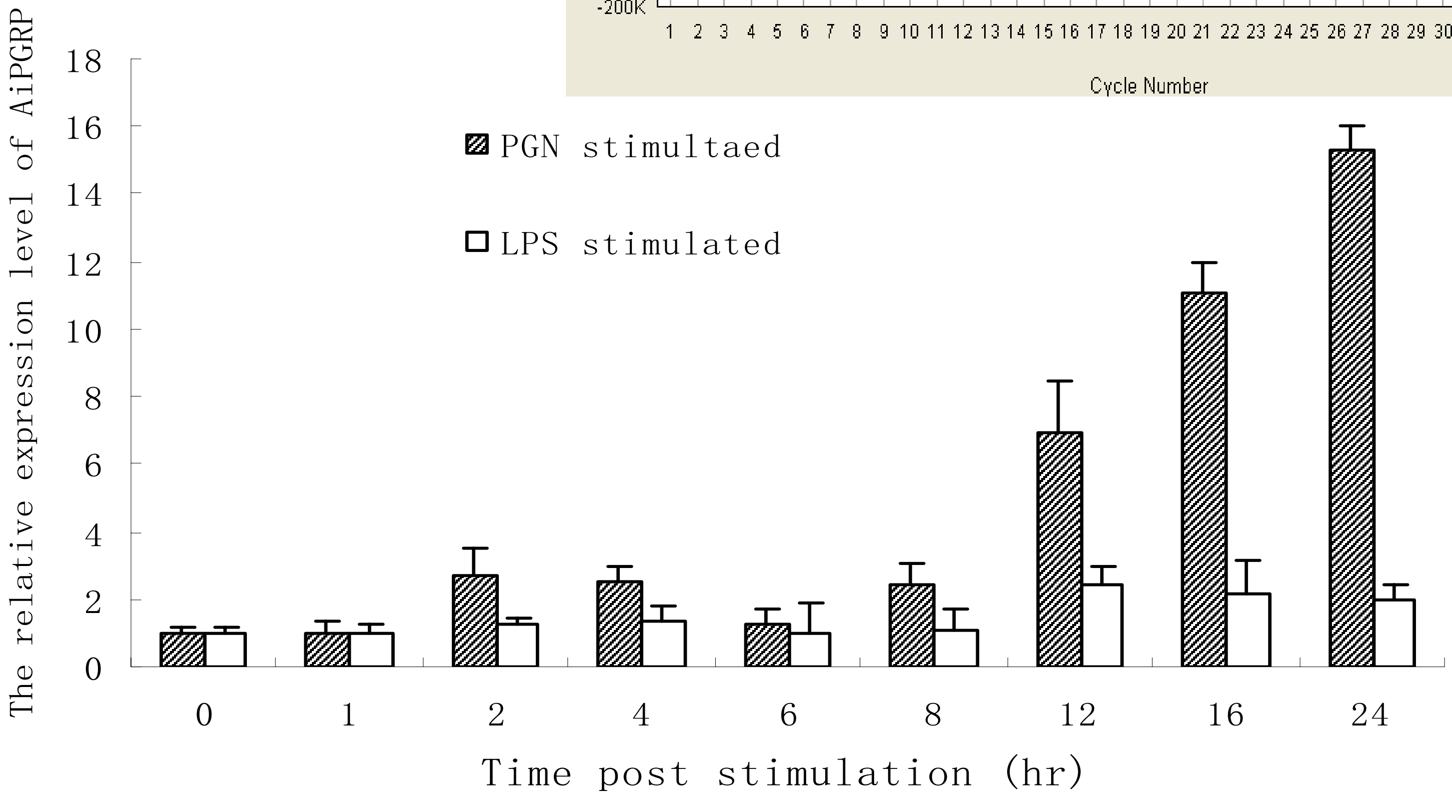
The LGBP binding sites



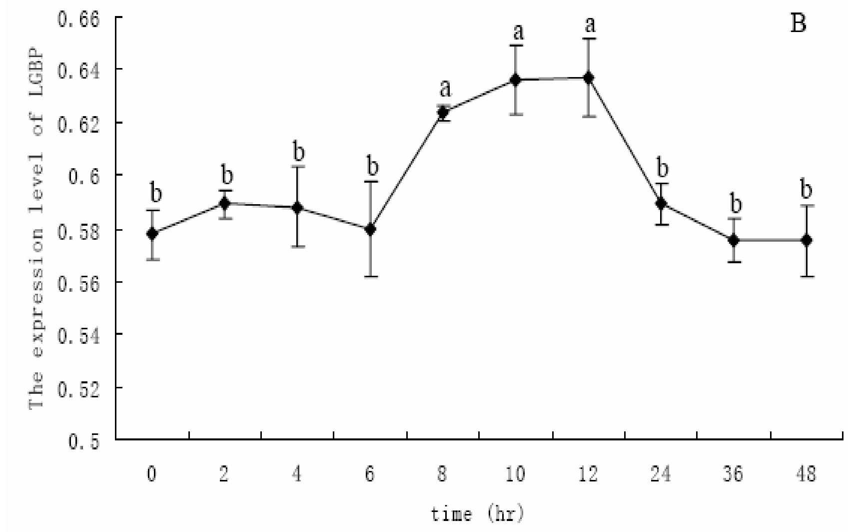
- *:LPS binding site; vvvv: Glucan binding site.

The expression of PGRP in the mixed primary cultured haemocytes stimulated by LPS and PGN.

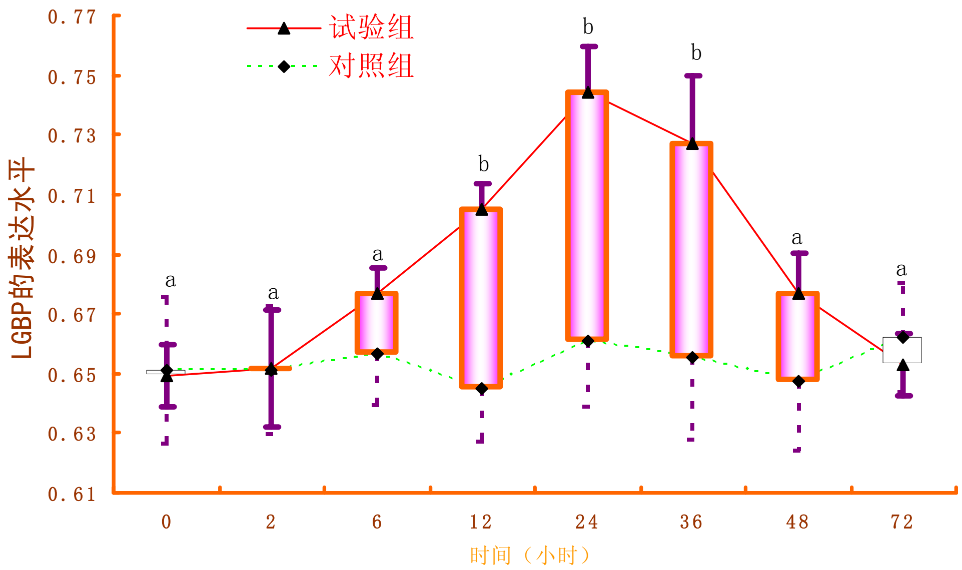




The expression of LGBP in scallop challenged by bacteria or yeast



Vibro



yeast

Data plotted are mean + SD of three replicates. Data that are significantly different are indicated by different letters above the bars(1-way ANOVA, p<0.05)

✧ C type lectin genes cloned from scallop *Chlamys farreri* (5)

Name	Full length	A.a	CLECT domains
CfCTL-A	708	171	1
CfCTL-B	1772	221	1
CfCTL-C	2257	524	3
CfCTL-D1	2086	633	4
CfCTL-D2	1897	633	4

Lectin is a family of sugar-binding proteins of non-immune origin that agglutinates cells or precipitates glycoconjugates.

The predicated structure of scallop C-lectins

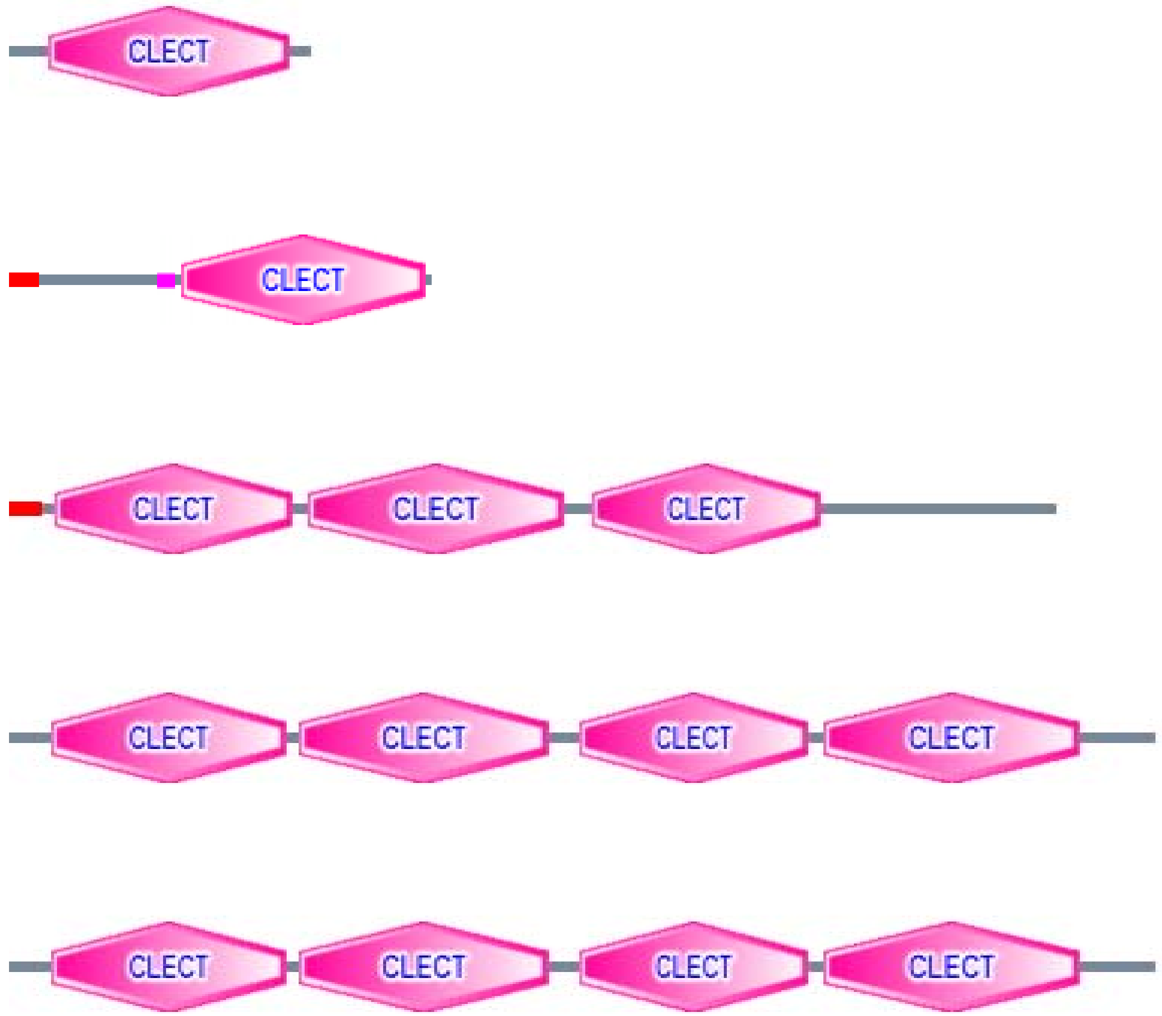
CfCTL-A

CfCTL-B

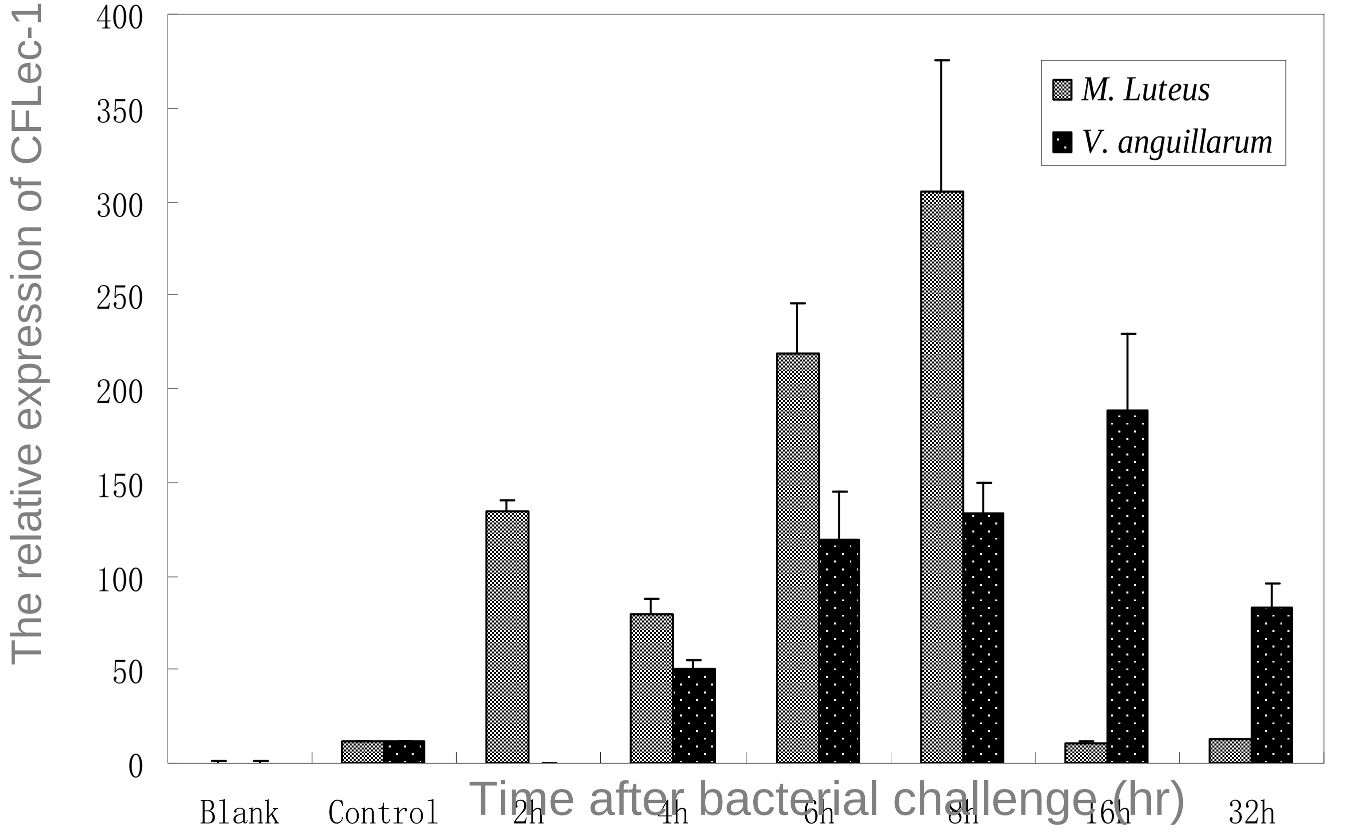
CfCTL-C

CfCTL-D1

CfCTL-D2

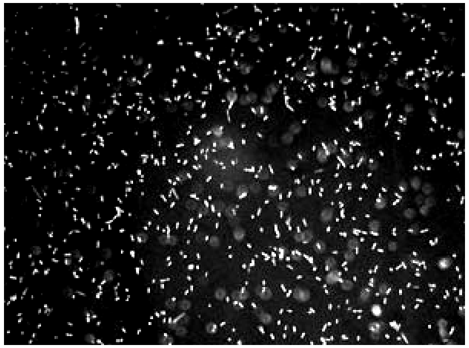


The temporal expression of CFLec-A after bacteria challenges

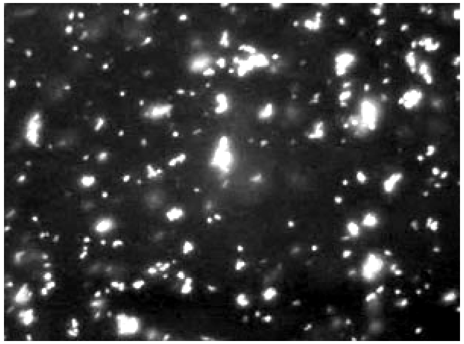


Bacteria (G^+, G^-) challenge up-regulate the expression of CfLec-A.

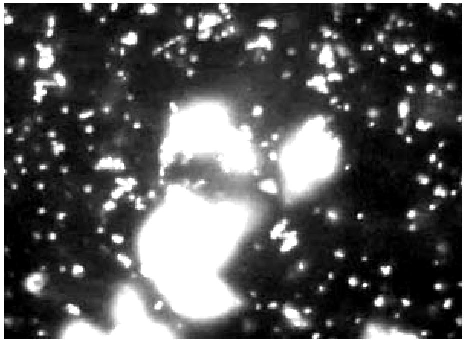
The bioassay of recombinant Lectin



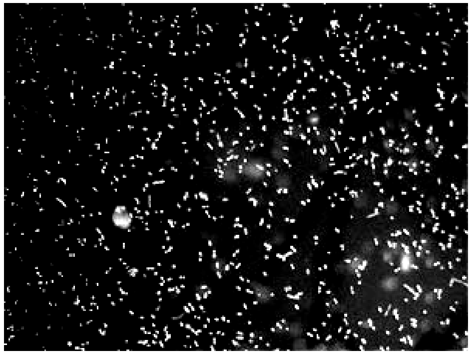
BSA (1mg mL^{-1})



rCFLec-A (~ 20 ug mL^{-1})



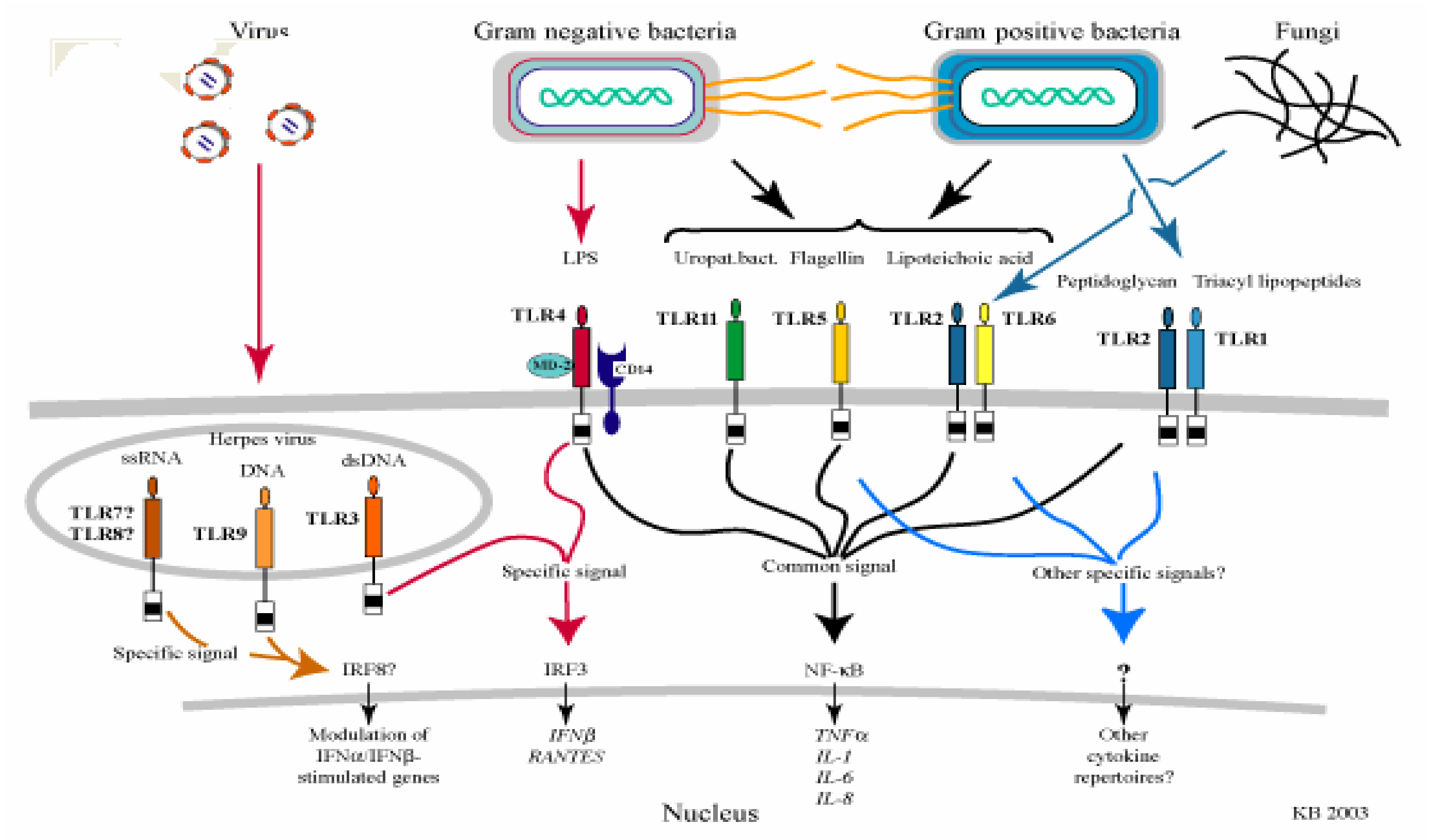
rCFLec-A (~ 80 ug mL^{-1})



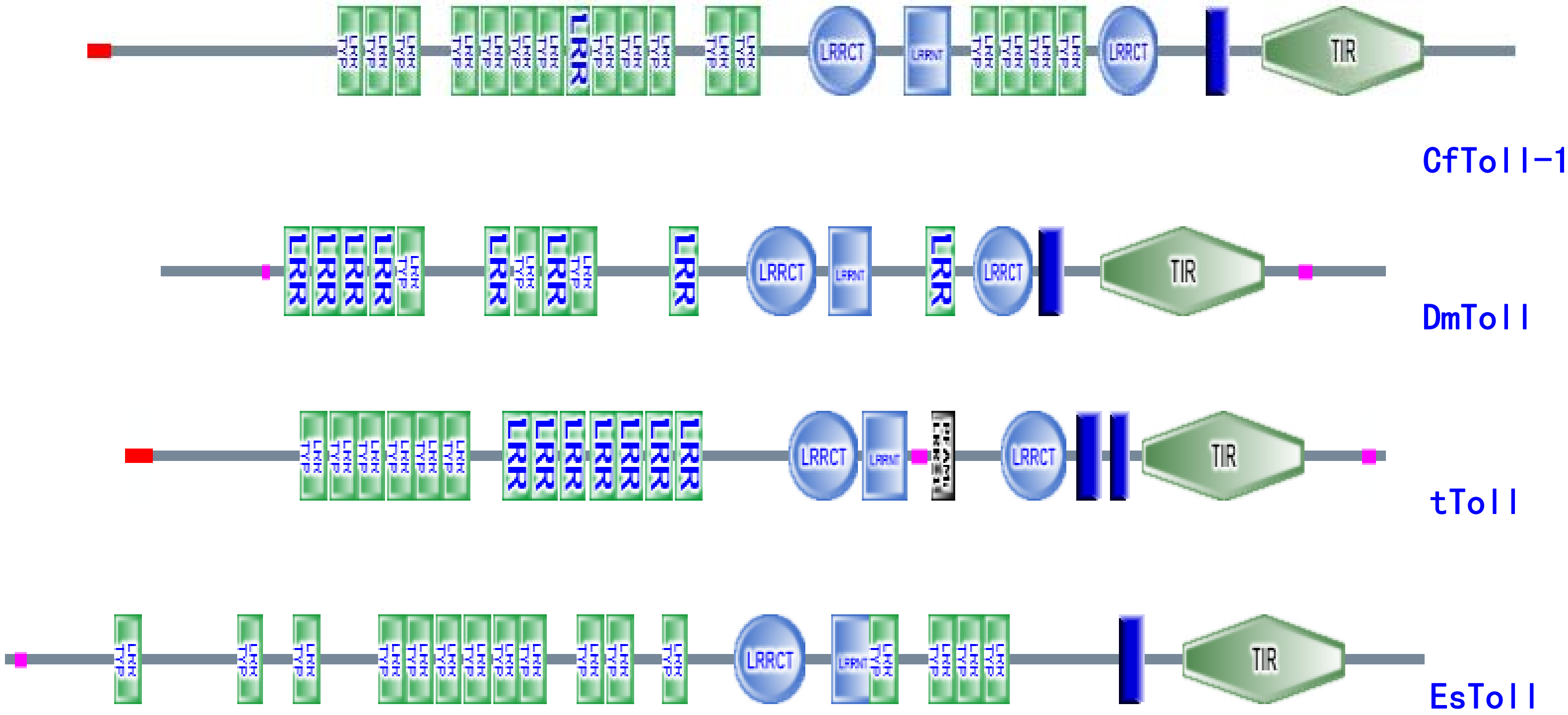
rCFLec-A (~80 umg mL^{-1})
in TBS-EDTA

The recombinant Lectin displayed strong activity to agglutinate bacteria.

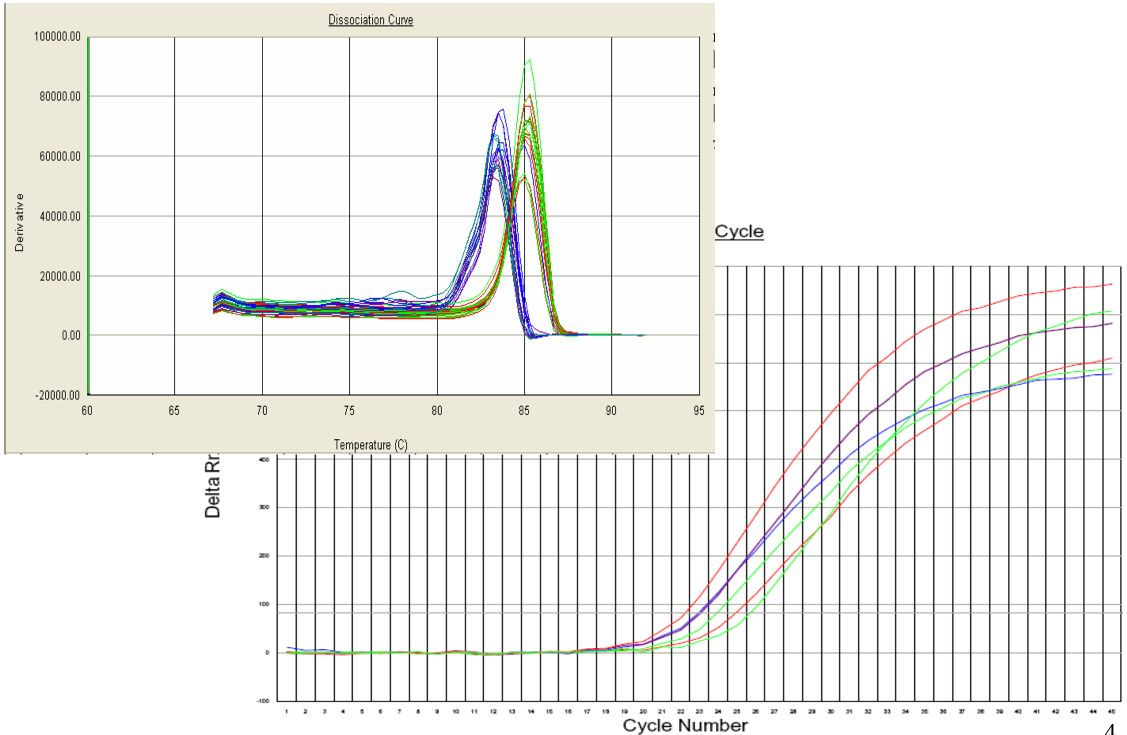
✦ TLR is a family of PRRs which can recognize and bind different PAMPs and plays a crucial role in the innate immune response.

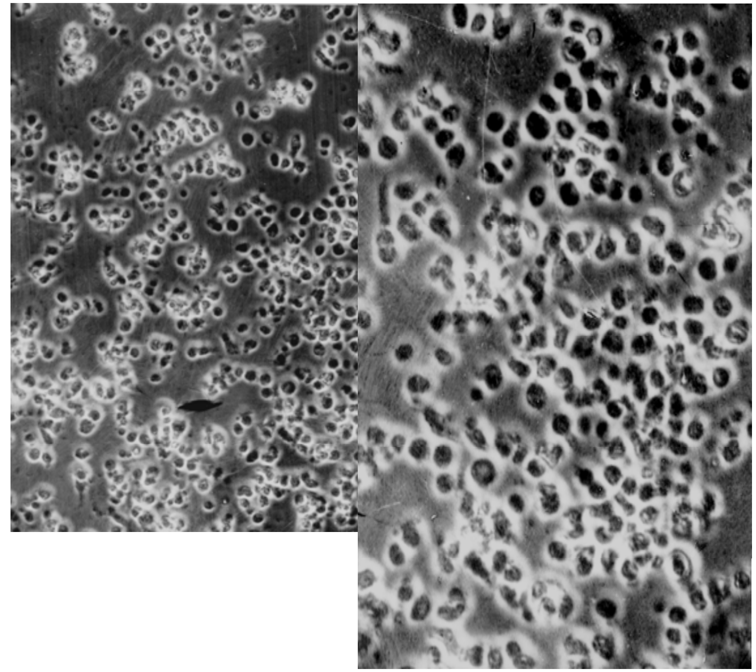


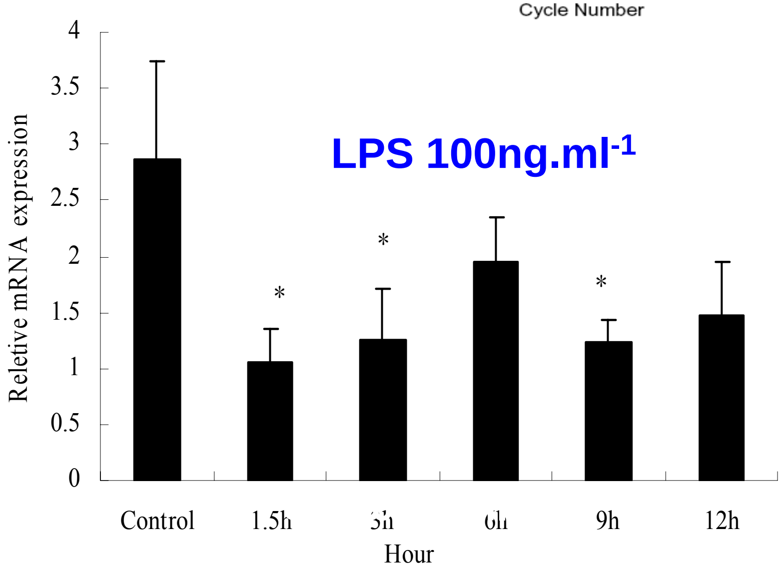
The predicated structure of scallop CfToll-1

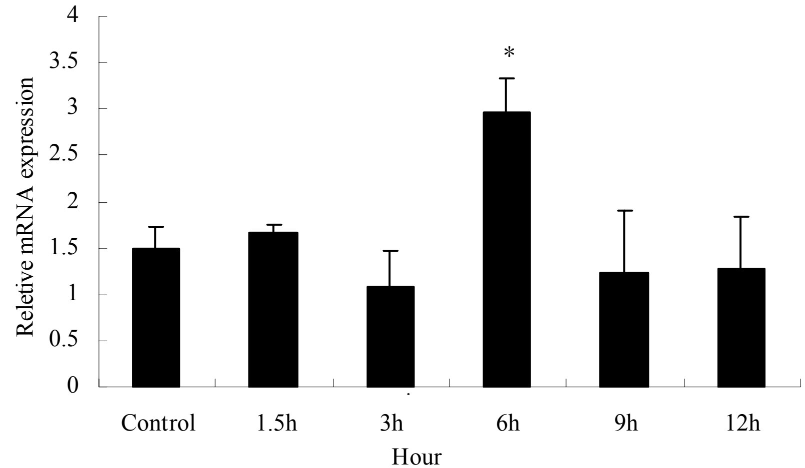


Limei Qiu et al., 2007, Fish & shellfish Immunol





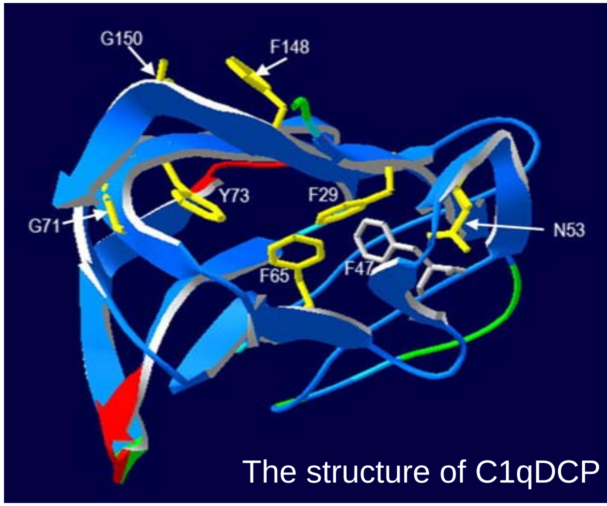


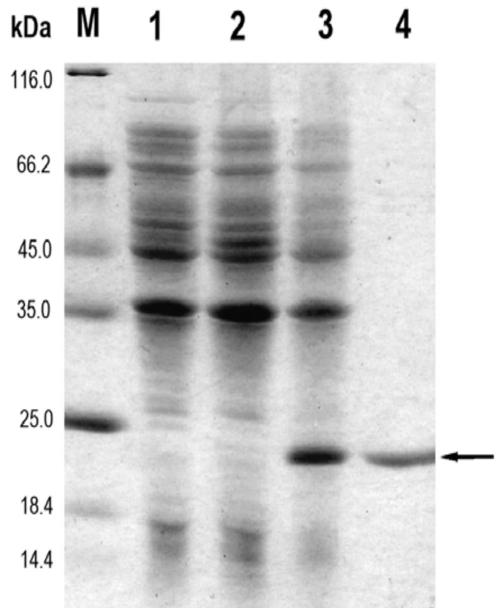


LPS 1μg.ml-1

The expression of CfToll-1 in the heamocytes after LPS stimulation

✧ Thioester-containing protein (CfTEP) and C1q-domain-containing protein (CfC1qDCP) indicating the existence of complement system in scallop.



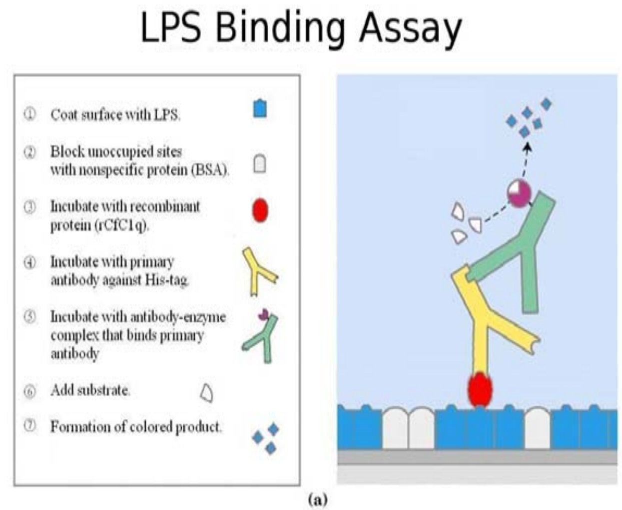


Recombinant expression of CfC1qDCP

(a) Bla

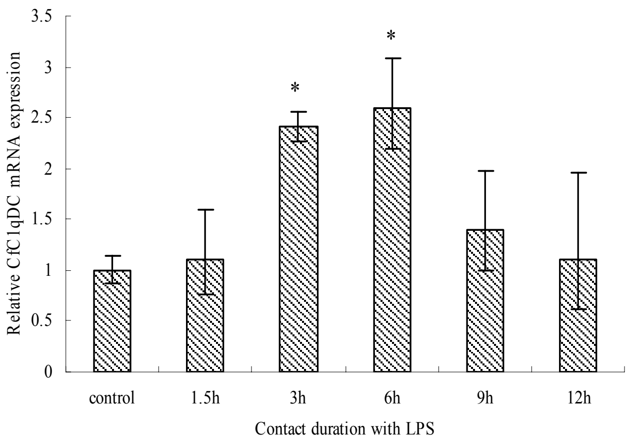
(b) *V. a*

HE: her



(a)

(b)



Huan Zh

rCfC1qDCP displayed LPS binding activity.

LPS up-regulated the expression of CfC1qDC in mixed primary culture of hemocytes

Phylogenetic tree of various members of TEP family

(2) Genes involved in Signal modulation, amplification and transduction

(Myd 88, TRAF6, Cactus, SERPINs)

Limei Qiu et al., 2007, Fish & shellfish Immunol

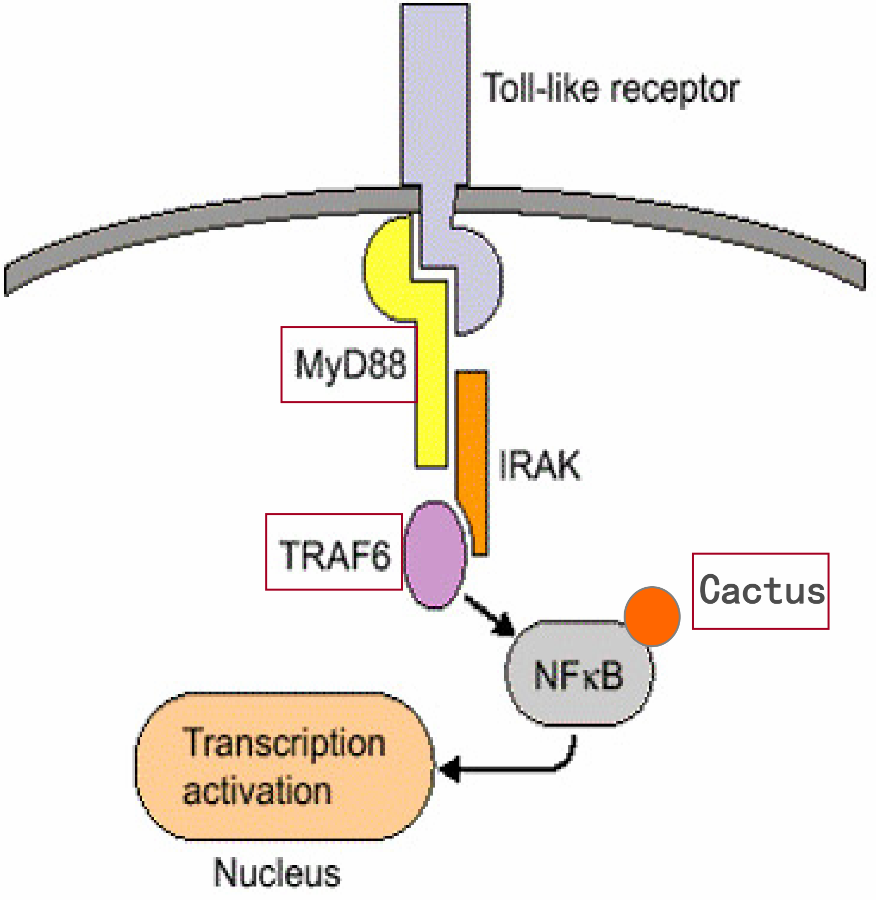
Ling zhu et al., 2006, Fish & shellfish Immunol

Ling zhu et al., 2007, Fish & shellfish Immunol

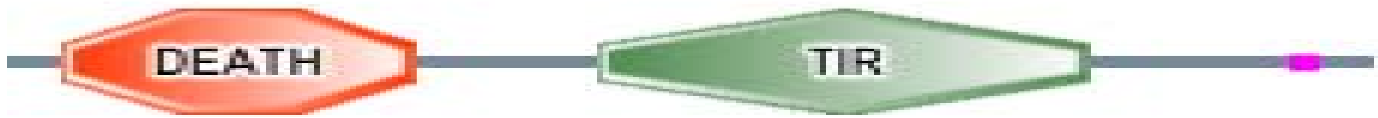
The genes involved in immune signal modulation, amplification and transduction (14)

Gene name	Function	Full-length/ ORF (bp)	Accession No.	species
CfMyd88	Adaptor and transduction	1564 /1101	DQ249918	*C. farreri*
TRAF6		2510 /1965	--	*C. farreri*
Cactus	transduction	2488/827		*C. farreri*
CfSP-1	PPO and PO cascade	1121/1062	DQ186670	*C. farreri*
CfSP-2		1119/1008	--	*C. farreri*
CfSP-3		922/798	--	*C. farreri*
CfSERPIN -1		1841 /1524	QD236243	*C. farreri*
CfSERPIN -2		1358 /1041	QD236244	*C. farreri*
CfSERPIN -3		1187 /1092		*C. farreri*
CfSERPIN -4		1064 /969		*C. farreri*
CfSERPIN -5		632 /279	--	*C. farreri*
AiSERPIN -1		1020 /834	AY830445	*A. irradians*
AiSERPIN -2		714 /897	QD236241	*A. irradians*
AiSERPIN -3		642 /459	QD236242	*A. irradians*

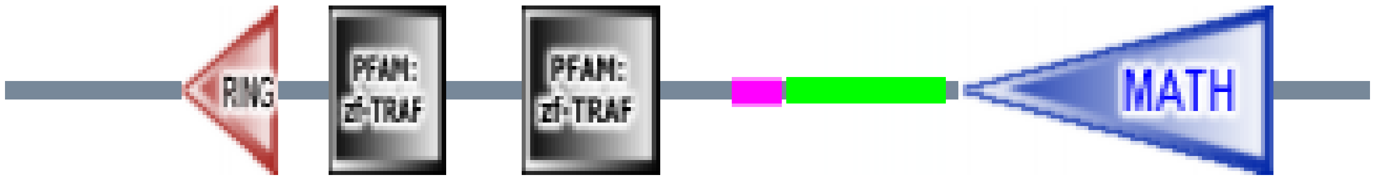
Toll signaling pathway



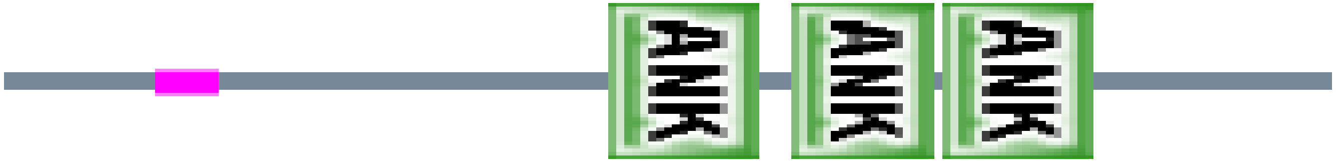
The structure of Myd 88, TRAF6 and Cactus from scallop *Chlamys farreri*



CfMyd88

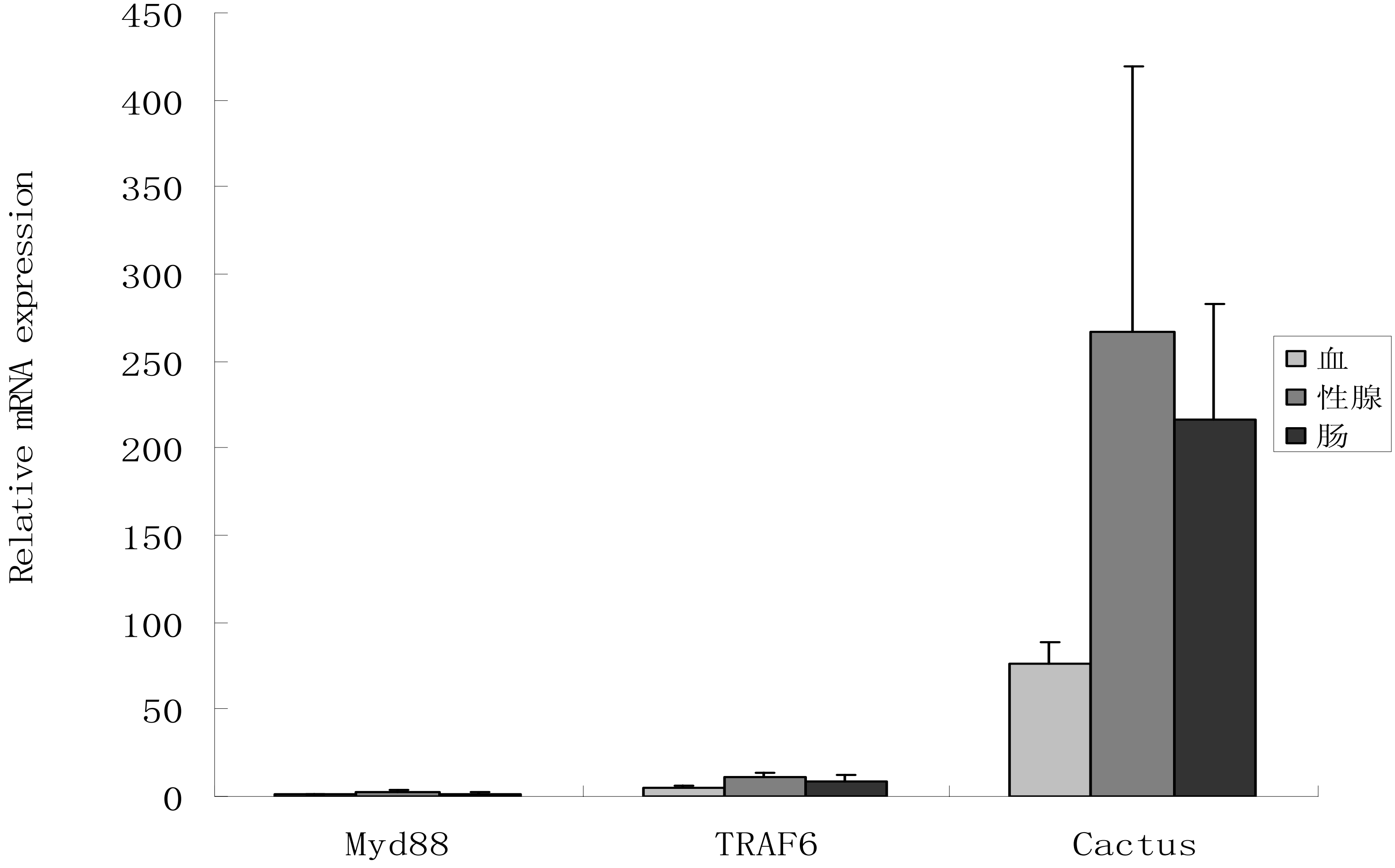


CfTRAF6



CfCactus

The cascade of Myd88-TRAF6-Cactus in different tissue



(3) Immune effecter genes

(Lysozyme, defensin)

Jianmin Zhao et al., 2007a, Mol. Immunol

Jianmin Zhao et al., 2007b, Mol. Immunol

Lysozyme categories

- Lysozyme is of widespread distribution in animals and plants.
- Several types of lysozymes have been described:

  c (chicken);

  g (goose);

  i (invertebrate) ;

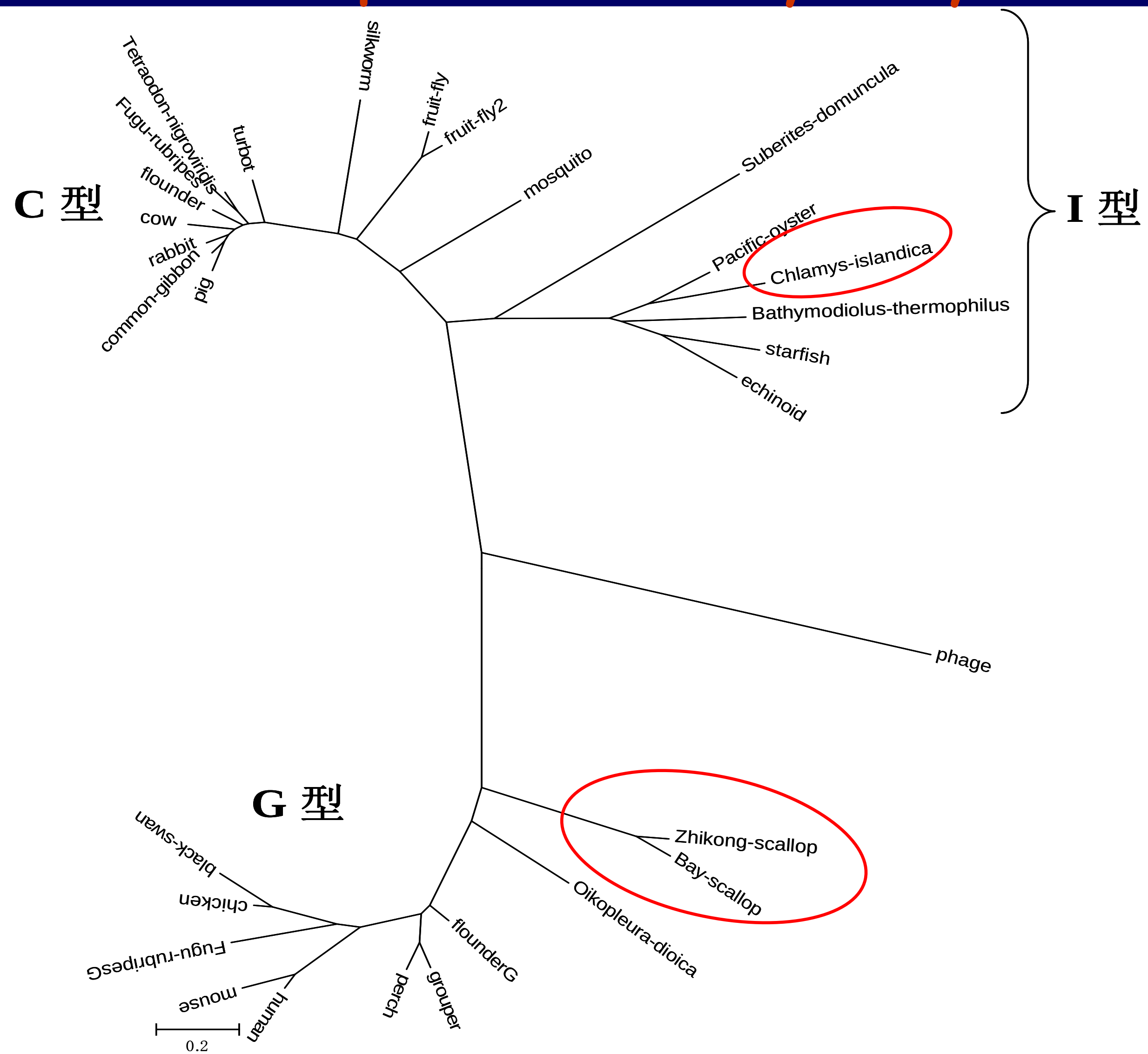
  phage, bacteria;

  plant.

G-Lysozyme from scallops

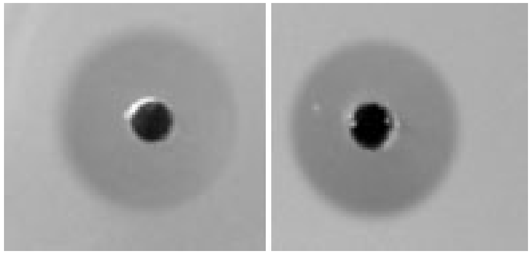
Gene Name	CfLyz-g	AiLyz-g
Full Length (bp)	829	659
5’-UTR (bp)	21	18
3’-UTR (bp)	218	41
ORF (bp)	588	600
Aa coded	196	200
MW (Kd)	22.39	21.99

The phylogenic tree constructed based on amino acid sequences of lysozyme

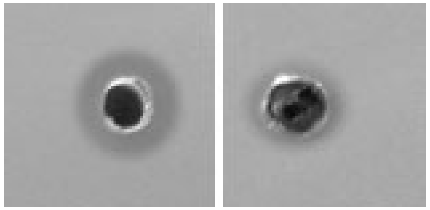


Recombinant expression and bioassay of AiLyz-g

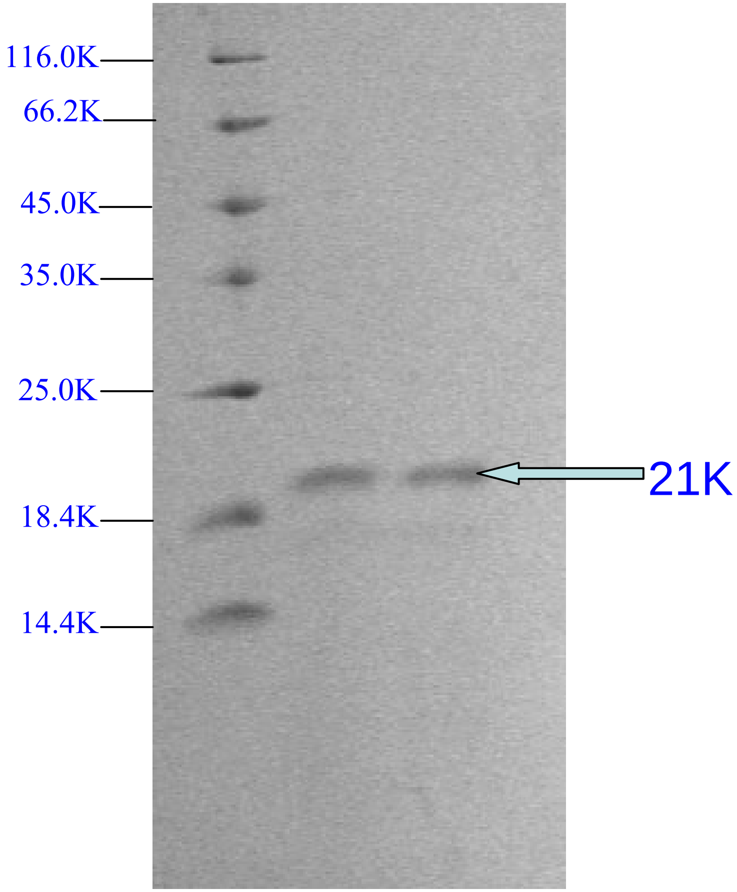
Bioassay of re-lysozyme



rAiLyz-g against G^{+} bacteria

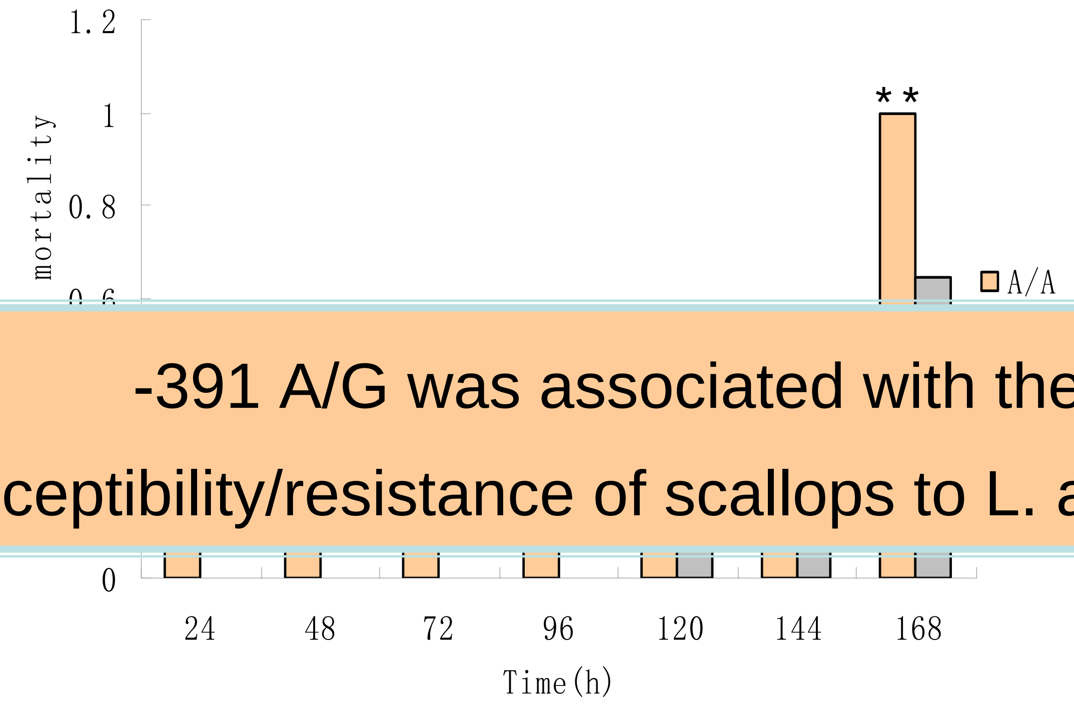


rAiLyz-g against G^{-} bacteria



Polymorphisms of Lysozyme in *Chlamys farreri* and Its Association with Resistance/ Susceptibility to *Listonella anguillarum*

Relation of Susceptibility/Resistance Phenotypes With Genotypes at Locus -391



-391 A/G was associated with the susceptibility/resistance of scallops to L. anguillarum

Cumulative mortality of scallops with -391A/A genotype and -391 A/G genotype during *Listonella anguillarum* challenge

Association Between Exon Polymorphism and Disease Susceptibility/Resistance

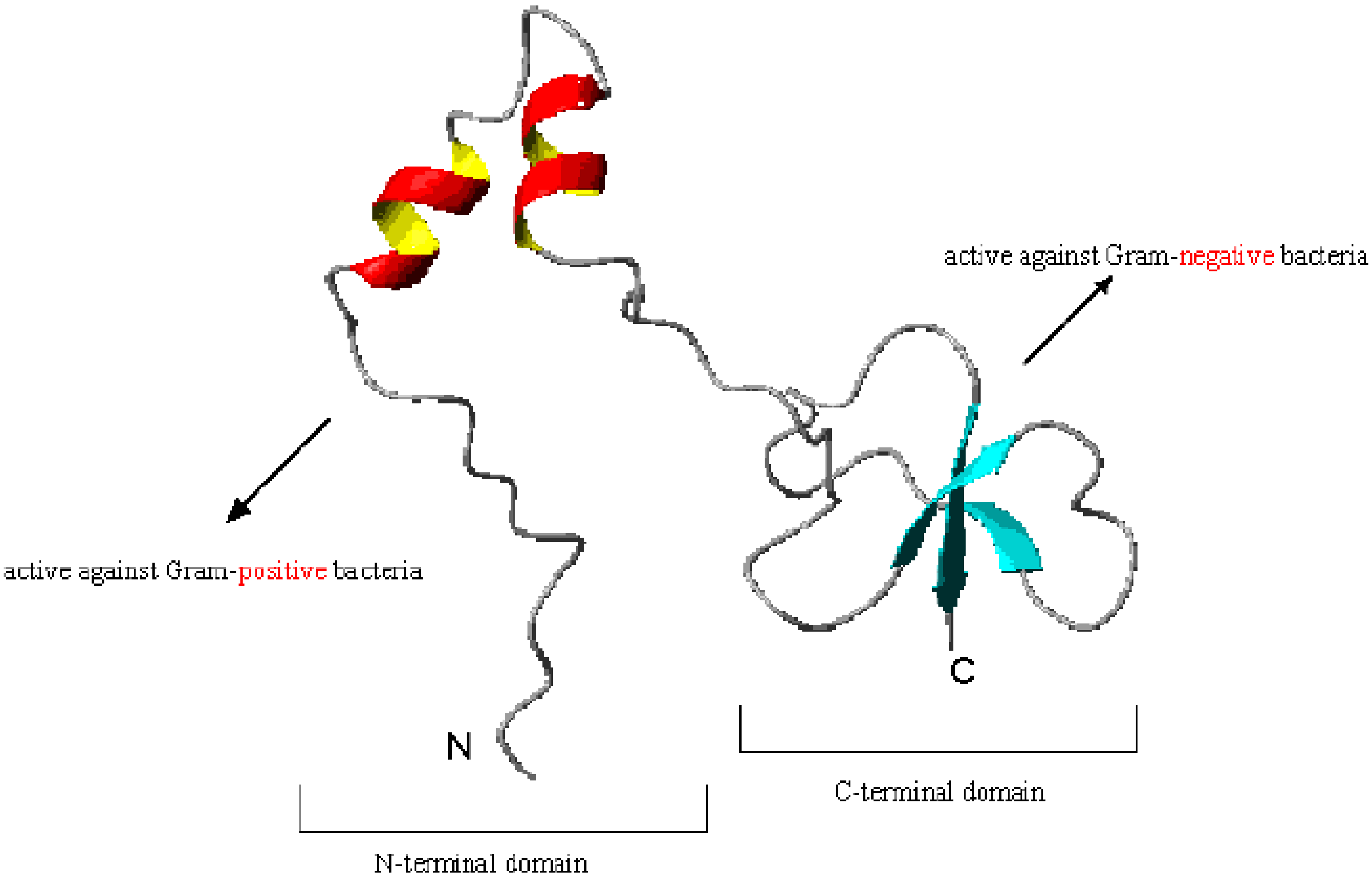
Goose type lysozyme promoter polymorphism	Susceptible stock No. (%)	Resistant stock No. (%)	χ^2(P)
+3473			
Allele			
Genotype			
C/C	10(0.238)	7(0.175)	
C/A	17(0.405)	7(0.175)	7.60(0.022)
A/A	15(0.357)	26(0.650)	

+ 3473 A/C was associated with the susceptibility/resistance of scallops to L. anguillarum

Defensin

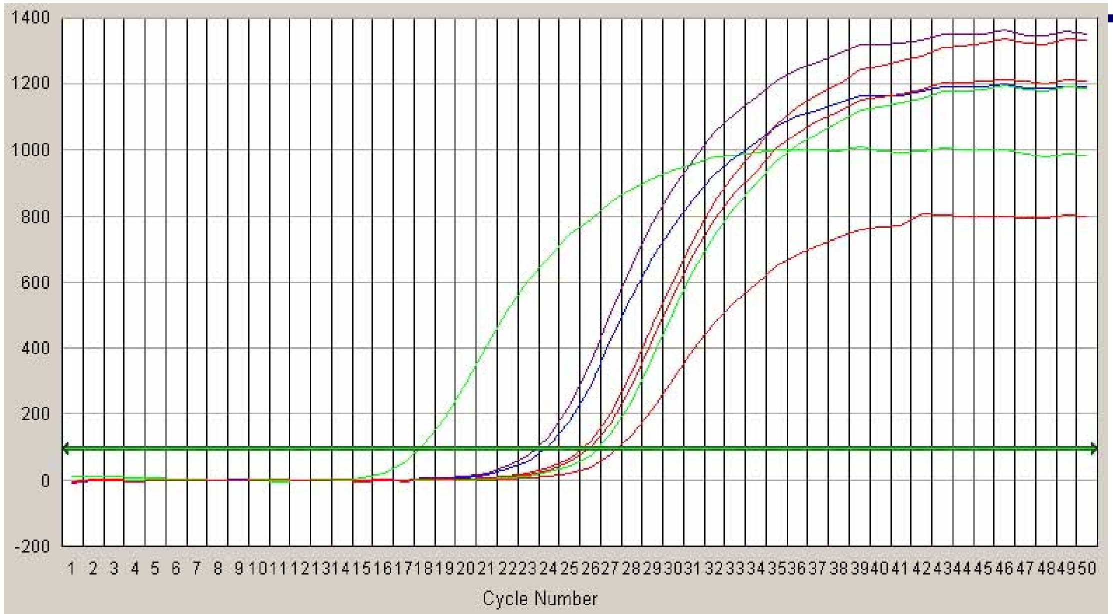
A family of potent antibiotics made within the body by neutrophils and macrophages. They are small peptides unusually rich in the amino acid cysteine (Cys).

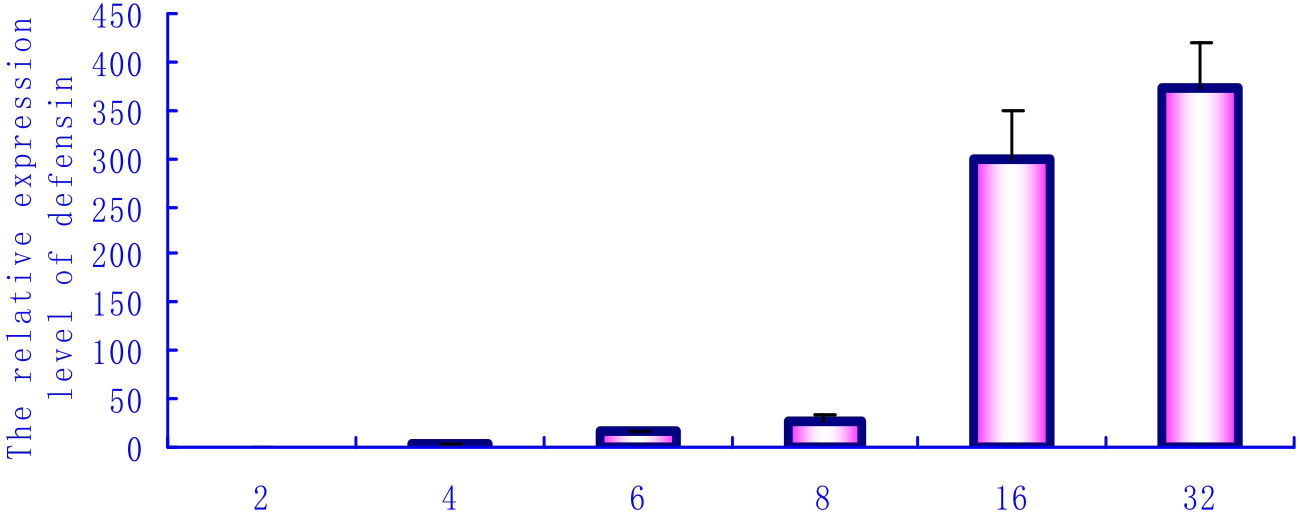
The defensins play important roles against invading microbes. They act against bacteria, fungi and viruses by binding to their membranes and increasing membrane permeability.



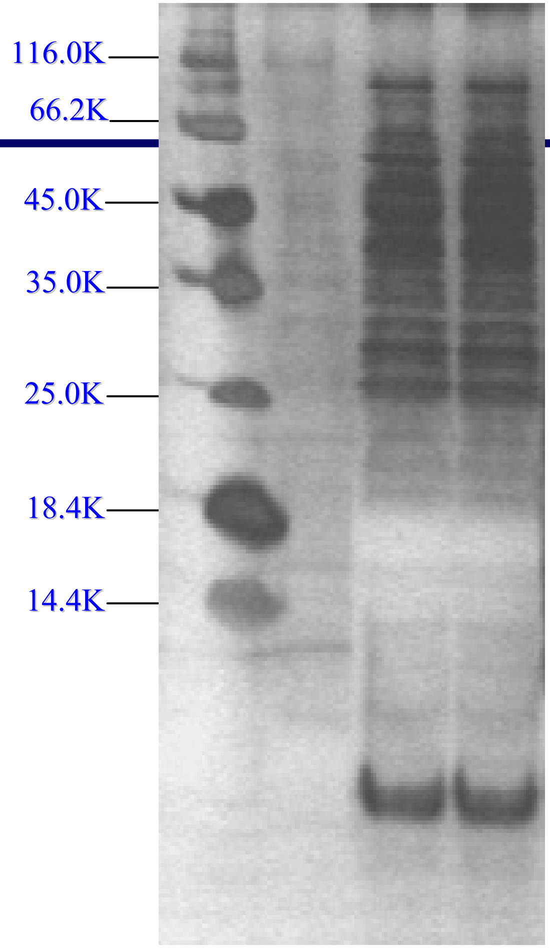
The predicated structure of bay scallop defensin

mRNA expression of scallop defensin after bacteria challenge

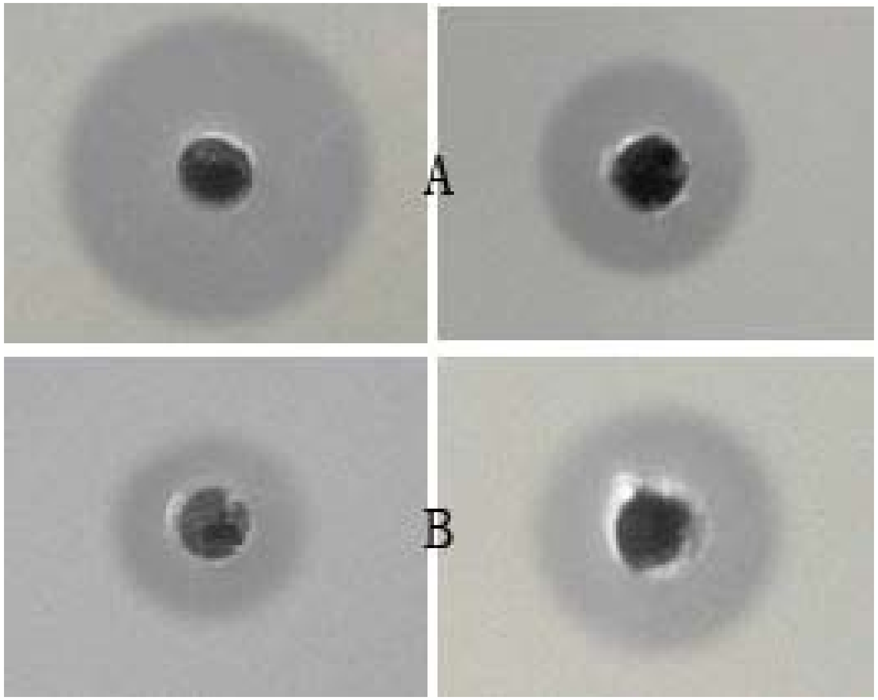




Bioassay of re-defensin



The recombinant expression of scallop defensin in yeast



A: G^{+} bacteria
B: G^{-} bacteria

(4) The genes involved in the oxidation/reduction

(CAT, Prxs, SOD, GPx, GST , Trx， SeBP)

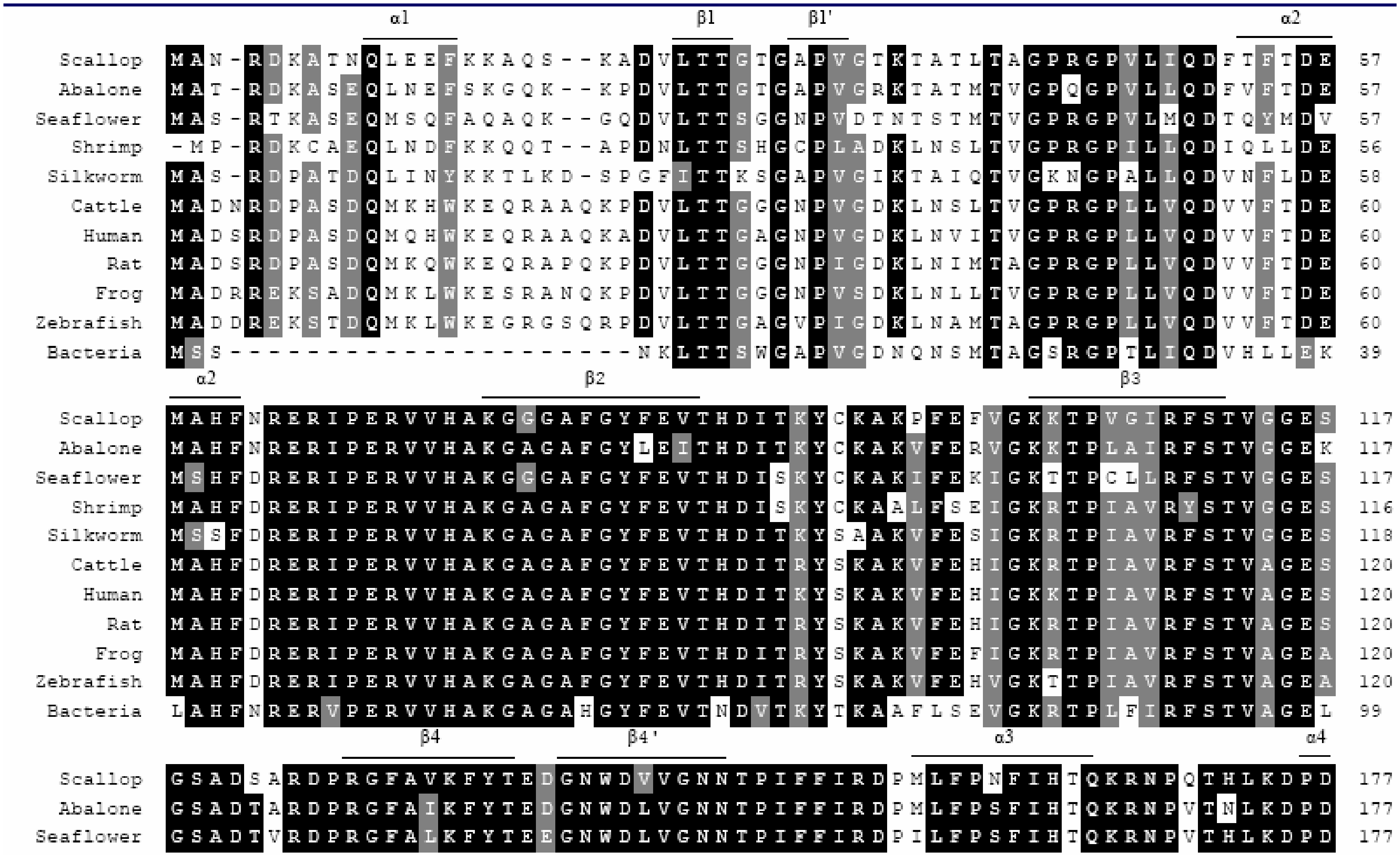
Huibin Zou et al., 2006, Dev. Com. Immunol

Duojiao Ni et al., 2007, Fish & shellfish Immunol

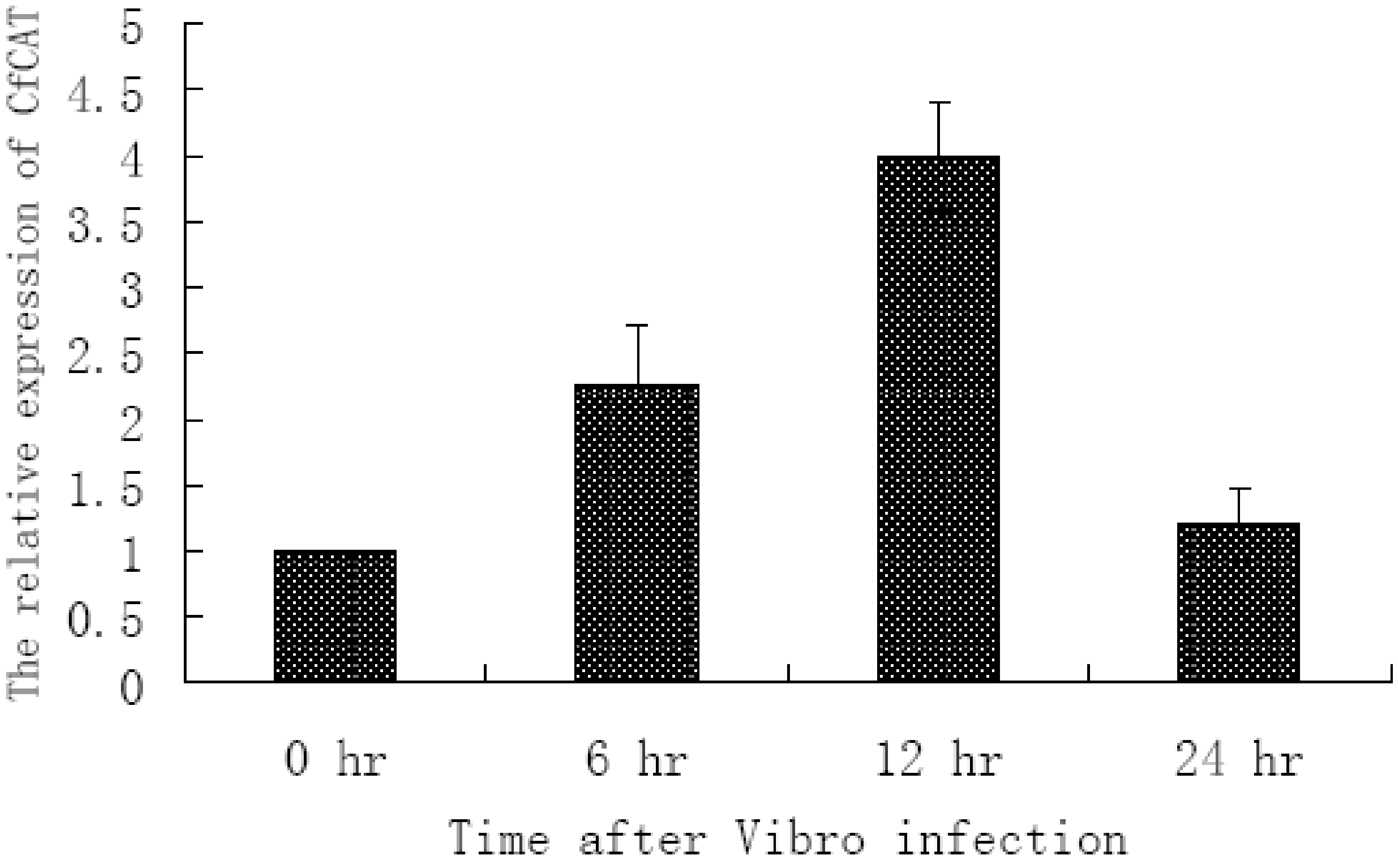
The genes involved in oxidation/reduction

Name	Function	Full-length/ ORF (bp)	Accession No.	species
CfSeBP	Anti-oxidation	1664 /1440	AY835660	*C. farreri*
CfGPX-1	Anti-oxidation	1194 /651	--	*C. farreri*
CfGPX-2		1290/705	--	*C. farreri*
CfSOD	Anti-oxidation	1022/459	DQ400349	*C. farreri*
CfGST-1	Glutathione-necessary	1483 /717	--	*C. farreri*
CfGST-2		954 /672	--	*C. farreri*
CfGST-3		1135/609	--	*C. farreri*
CfGST-4		1387/615	--	*C. farreri*
CAT	H_2O_2 reduce	3144 /975	DQ862859	*C. farreri*
Thioredoxin, Trx	Oxidation /reduction	1494 /324	--	*C. farreri*
tyrosinase, TYR	PO-like	1711 /1458	--	*C. farreri*

The alignment of CfCAT with selected CATs from other species



Temporal expression of the CfCAT transcript in haemocytes after *Vibrio anguilarum* infection



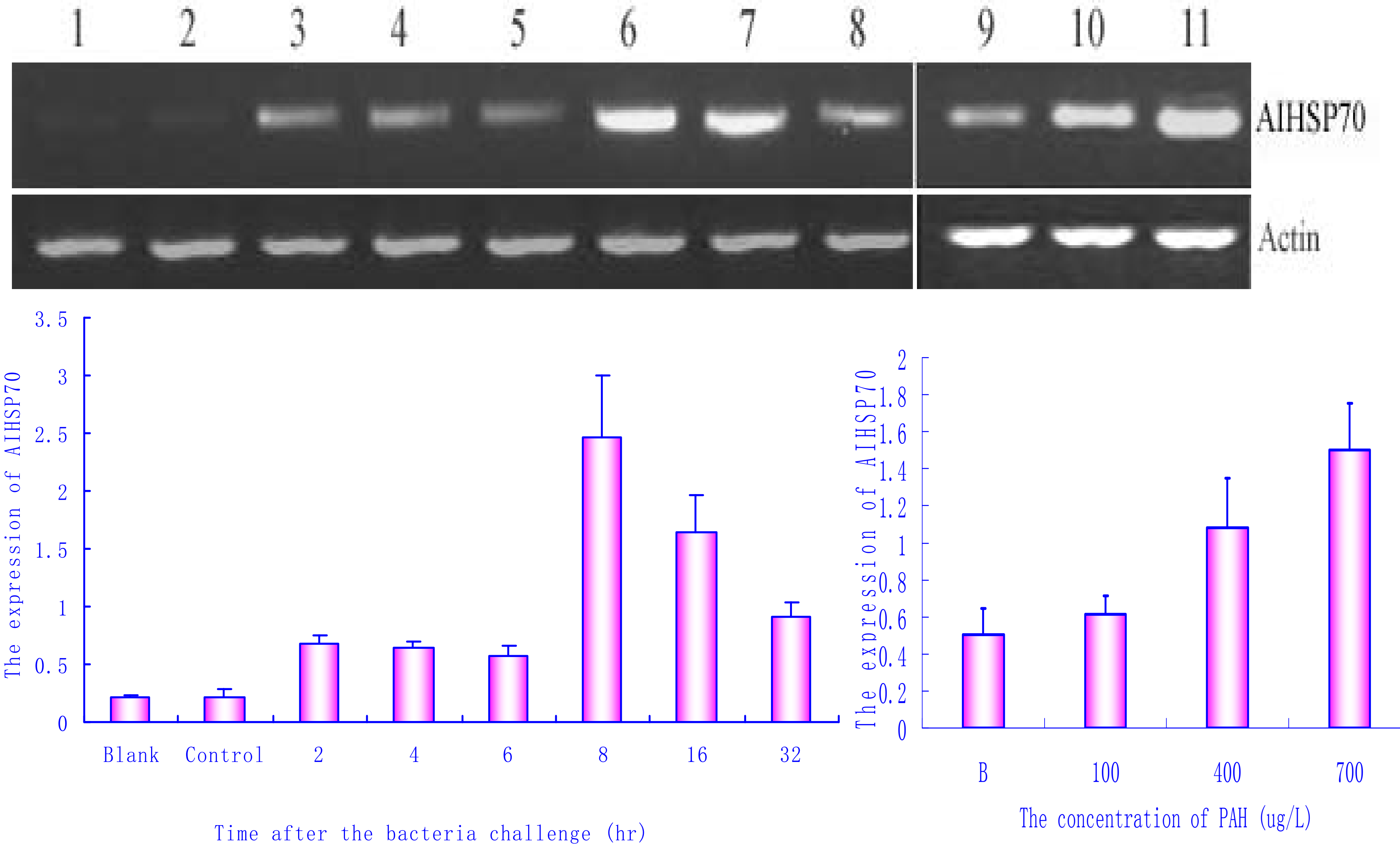
(5) The acute phase protein genes

name	functions	Full-length/ ORF (bp)	Accession No.	species
CfHSP90	Molecular chaperon; Stress response; Heavy metal, ROS binding and clearance; Infection.	2710 /2181	AY362761	*C. farreri*
CfHSP70		2573 /1968	AY206871	*C. farreri*
CfHSP22		849 /576	AY362760	*C. farreri*
AiHSP70		2651 /1980	AY485261	*A. irradians*
MyHSP70		2641 /1974	AY485262	*M. yessoensis*
AiMT-1	Heavy metal binding, anti-oxidation	787 /438	--	*A. irradians*
AiMT-2		664/ 333	--	*A. irradians*
AiMT-3		582 /273	--	*A. irradians*

Qiang Gao et al., 2007, CBP
Lingling Wang et al., 2008, CBP
Linsheng Song et al., 2006, Fish & shellfish Immunol

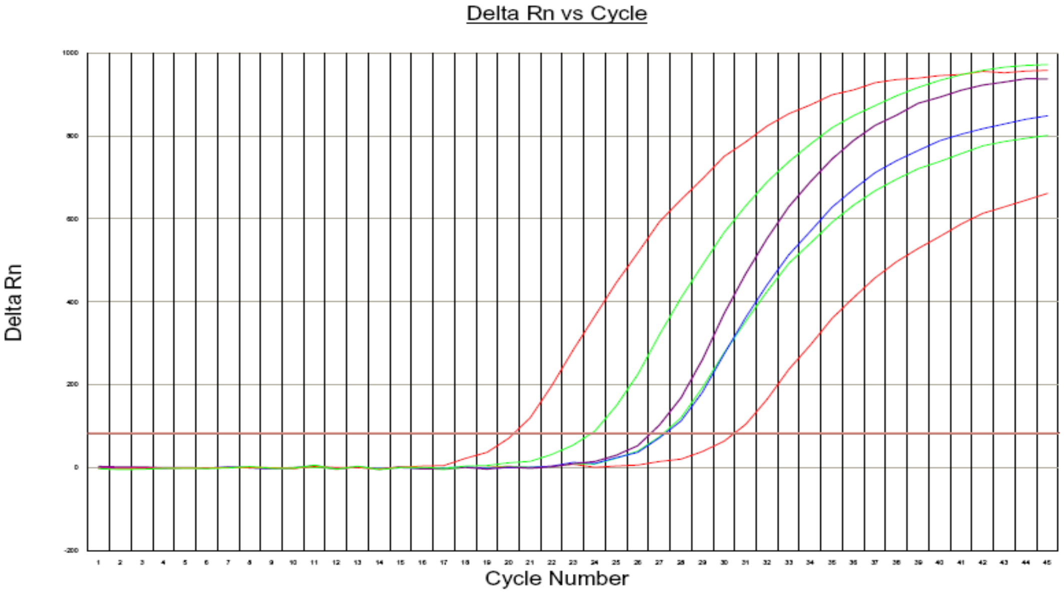
吴龙涛等，2003，高技术通讯
刘维青等，2006， 海洋与湖沼

The expression of AiHSP70 after bacteria challenge and PAH stimulation

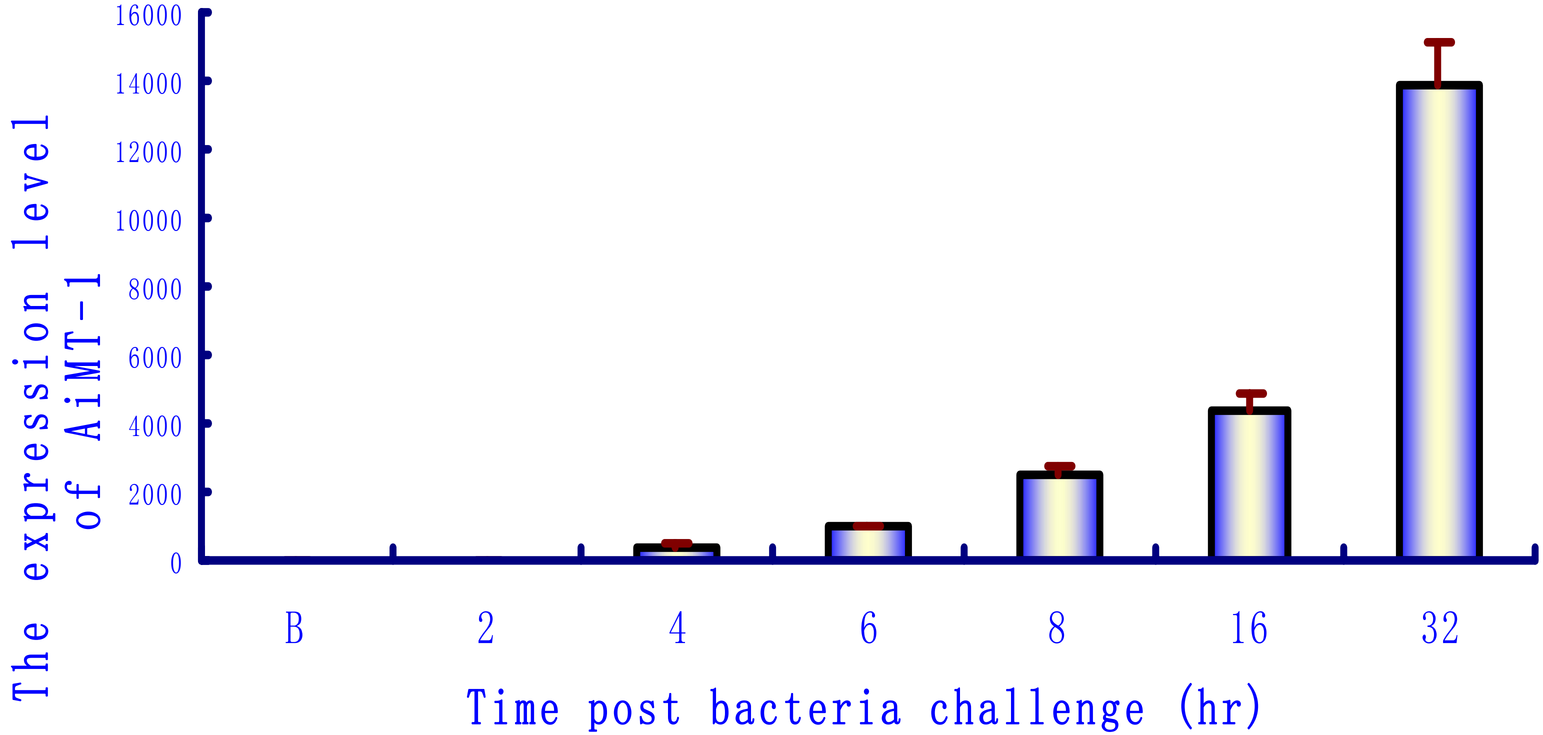


The Metallothionein genes cloned from bay scallop

Gene name	AiMT-1	AiMT-2	AiMT-3
cDNA full length	787	664	582
Aa	145	110	90
Cys	40	28	28
C-C	2	2	2
C-X-C	8	8	10
C-X-X-C	6	6	2
C-X-X-X-C	5	5	6
CKCXXXCXCX	1	1	1

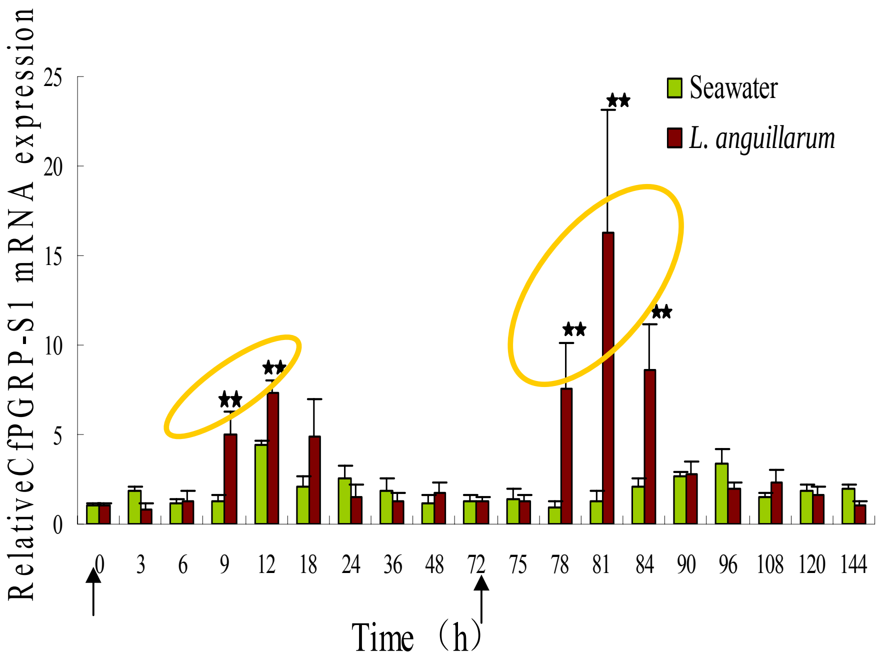


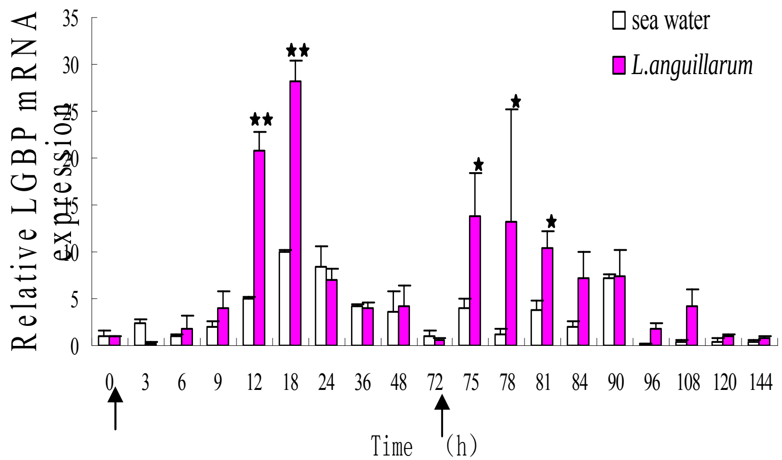
The expression of AiMT-1 after bacteria challenge



(6) The immune priming in scallops

mRNA expression of CfPGRP-S1 and CfLGBP

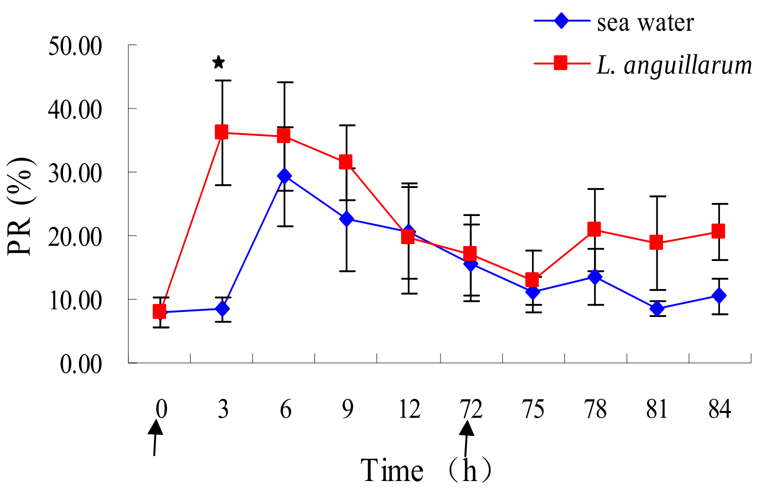




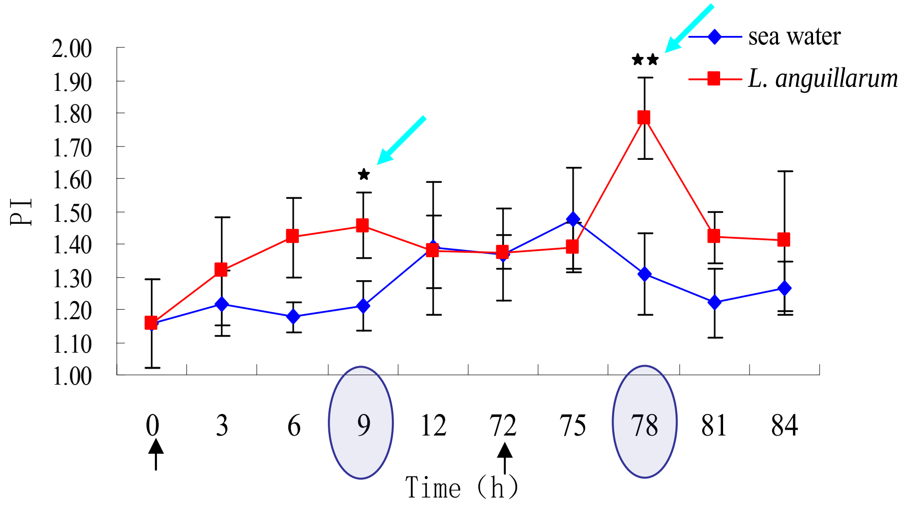
- After scallops were challenged by *L.anguillarum*, the mRNA levels of CfPGRP-S1 and CfLGBP were up-regulated.
- After the second stimulation，the mRNA levels of the two genes were increased significantly stronger and sooner.

The change in phagocytic activity

Phagocytic rate (PR)

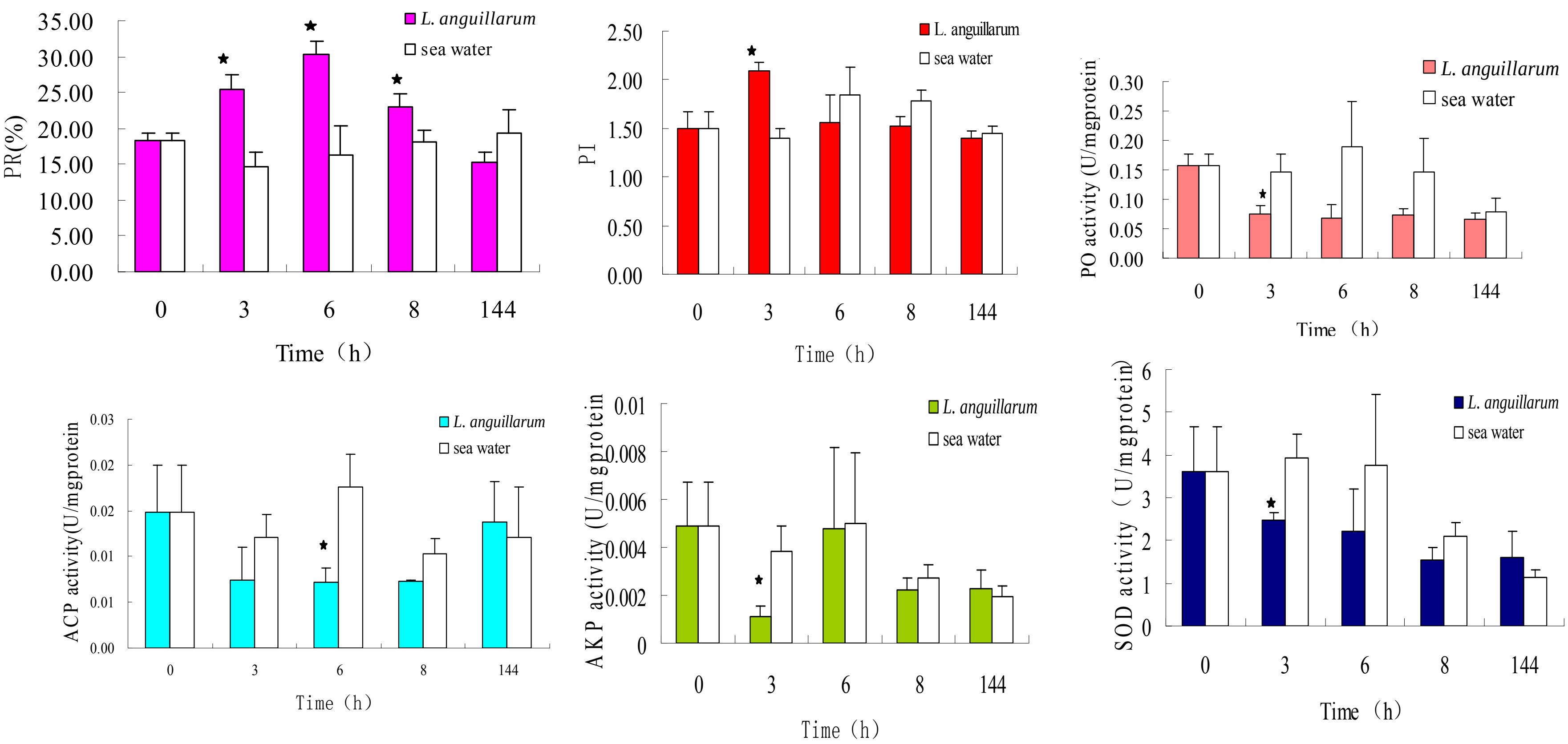


Phagocytic index (PI)



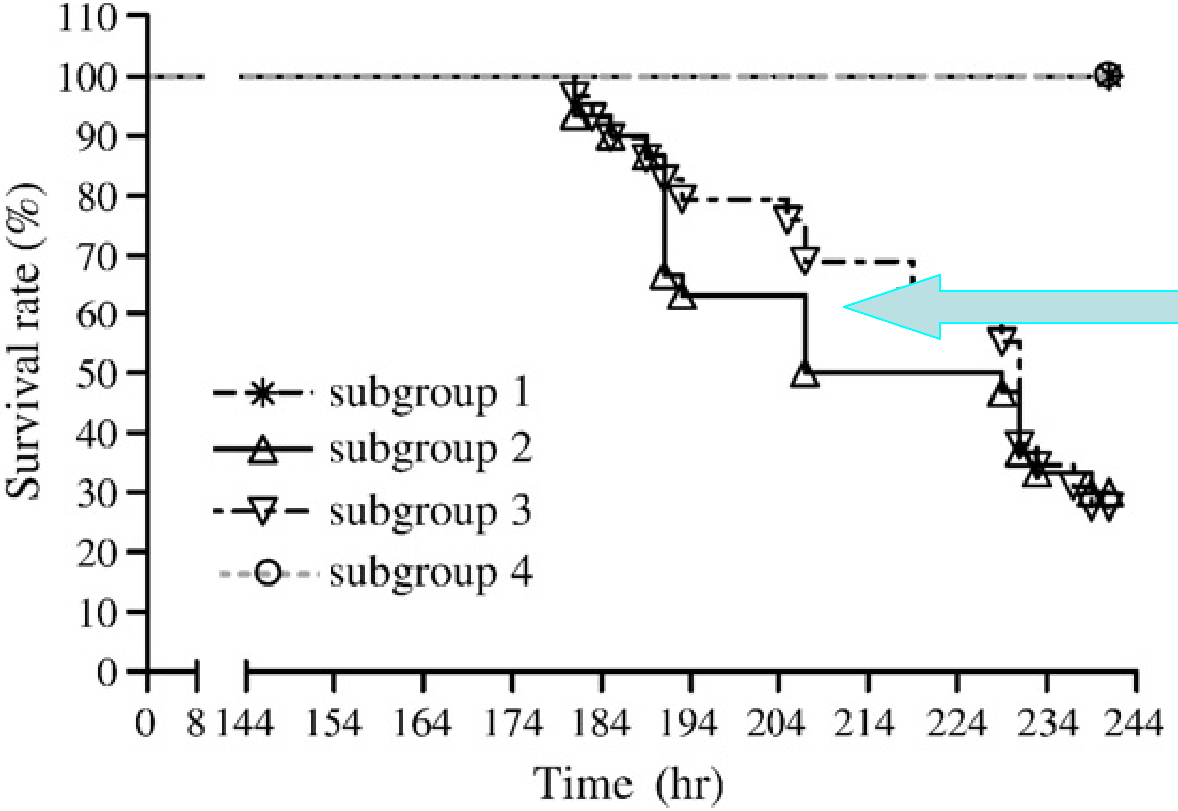
- No significant difference between PR after scallops were stimulated twice.
- After the second stimulation, PI was increased significantly stronger and sooner than that after the first stimulation (P<0.05).

Immune response to 1st challenge



- The significant difference indicated that the scallops were enduring an immune response against short-time challenge by *L. anguillarum*.

Survival rate-time relationships of four subgroups after challenged for twice



Significant difference between subgroup 2 and 3

Short-time challenge made resistance ability of scallops higher against long-time challenge by L. anguillarum.

subgroup 1: only received the first stimulation;
subgroup 2: only received the secondary stimulation ;
subgroup 3: received twice stimulations;
subgroup 4: received no stimulation.

Acknowledgement

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Thanks for your attention!