2021년도
한국어병학회 춘계학술발표회
요 지 집

ABSTRACT
2021 Spring Meeting of the Korean Society of Fish Pathology

▌일시 : 2021년 6월 24일(목)~6월 25일(금)
▌장소 : 제주대학교 해양과학연구소

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2021년도
한국어병학회 춘계학술발표회 일정

전체 행사 일시 및 장소

- 일 시 : 6월 24일(목)
  오후 5시 30분~: 이사회

  6월 25일(금)
  오전 10시 10분~: 구두 발표 및 포스터 게시

- 장 소 : 제주대학교 해양과학연구소

‘21 춘계 한국어병학회 세부일정

일 시	내 용	발 표 (진행)
2021년 6월 24일		
17:30~18:30	이사회	-
2021년 6월 25일		
09:30~10:00	Zoom 연결	-
10:00~10:10	개회사	한국어병학회장 (권준영)
구두 발표 Session-1		**좌장 : 정준범(제주대)**
10:10~10:30	Immune stimulation and protection effect against VHS by delivery of interferon-related gene plasmids	김혜지 (선문대학교)
10:30~10:50	Altered temperature induces a differential immune response in seven-band grouper following nervous necrosis virus infection	Rahul Krishnan (전남대학교)
10:50~11:10	Biological characteristics of *Microcotyle caudata* (*Microcotylidae*: *Monogenea*) from dark-banded rockfish (*Sebastes inermis*) in Korea	남우화 (강릉원주대학교)
11:10~11:30	휴식	
구두 발표 Session-2		**좌장 : 김정호(강릉원주대)**
11:30~11:50	측방 유동 면역 크로마토그래피법 기반 marine birnavirus (MABV) 현장검사용 키트 개발	공경희 (전남대학교)
11:50~12:10	A characterization and functional analysis of tumor necrosis factor new (TNFN) in rock bream (*Oplegnathus fasciatus*)	고성재 (강릉원주대학교)
12:10~12:30	바지락의 염증 정량 기술 개발과 이를 이용한 건강도 측정	김승현 (군산대학교)
12:30~13:30	점심식사	
특별 강연		**좌장 : 권준영(선문대)**
13:30~14:10	*nlrx1*-knockout increases expression of innate immune response genes associated with antiviral pathway in zebrafish	김명진 (경북대학교)
14:10~14:50	Phylogenomic characterization and improvement of molecular diagnostic assays for genus *Megalocytivirus* from cultured fish	김광일 (부경대학교)
14:50~15:30	바이오플락 넙치 내성한계 및 사육지침 마련연구	김준환 (선문대학교)
15:30~15:40	휴식	
구두 발표 Session-3		**좌장 : 이제희(제주대)**
15:40~16:00	A virulent plasmid of *Aeromonas salmonicida* subsp. *masoucida* could be spread to other aquatic pathogens through horizontal gene transfer	강유라 (부경대학교)
16:00~16:20	MIC panel을 이용하기 위한 어병세균의 배양조건 확립	김예지 (제주대학교)
16:20~17:00	총회 및 경품추첨	한국어병학회 총무간사
17:00~17:10	폐회사	한국어병학회장 (권준영)

특 별 강 연

구 두 발 표

° 김예지* · 우수지** · 김명석** · 전려진* · 정준범*

*제주대학교 해양생명과학과

**국립수산과학원 병리연구과

포 스 터 발 표

D. C. Rajapaksha[*], S. L. Edirisinghe[*], Chamilani Nikapitiya[*], Mahanama De Zoysa[*] and Ilsong Whang[**]

[*]*College of Veterinary Medicine, Chungnam National University, Daejeon 34134, Republic of Korea*

[**]*National Marine Biodiversity Institute of Korea (MABIK), 75, Jangsan-ro 101 beon-gil, Janghang-eup, Seochun-gun, Chungchungnam-do 33662, Republic of Korea*

특 별 강 연

SL 1~3

SL-1

nlrx1-knockout increases expression of innate immune response genes associated with antiviral pathway in zebrafish

° Myoung Jin Kim[*], Su mi Jung[**,***], Sarithaa Sellaththurai[**,***], Sung Eun Lee[*] and Je hee Lee[**,***]

[*]Department of Applied Biosciences, Kyungpook National University, Daegu 41566, Republic of Korea
[**]Department of Marine Life Sciences & Fish Vaccine Research Center, Jeju National University, Jeju Self-Governing Province 63243, Republic of Korea
[***]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Nlrx1 is one of the members of the Nod-like receptor (NLR) family that acts as a pattern recognition receptor (PRR) and is known to regulate the innate immune and inflammatory responses during infection with various pathogens, including viruses. Known as the negative regulator of MAVS, Nlrx1 has been studied by several groups, but there is still debate as to whether it increases the expression of innate immune response genes during viral infection due to conflicting results. In this study, we characterized the *nlrx1* gene and generated an *in vivo nlrx1*-knockout model to study its role during viral infection using zebrafish, which has many advantages as an experimental animal.

◎ **Methods** : The sequence of zebrafish Nlrx1 and known orthologs from other species was retrieved using Ensembl and the NCBI GenBank database. *In silico* analyses, such as multiple sequence alignment and pairwise sequence comparison, were performed to compare the zebrafish Nlrx1 with other orthologs using Clustal Omega, NCBI, Motif Scan, the EMBOSS Needle Pairwise Sequence Alignment Tool and PSORT II. Expression patterns were analyzed using whole mount *in situ* hybridization and qRT-PCR method in various developmental stages and adult tissues. Mutation was generated by microinjection of single guide RNA for *nlrx1* with Cas9 protein. *nlrx1*-knockout was confirmed by T7E1 enzyme assay and sequencing analysis of target site. Finally, the expression of innate immune response genes was analyzed using qRT-PCR in *nlrx1*-knockout mutant.

◎ **Results & Discussion** : Zebrafish Nlrx1 contains 1005 amino acids and has a carboxy-terminal leucine-rich repeat (LRR) domain and a central NACHT domain, similar to other species. However, the reported mitochondrial target sequence at the amino-terminal in human NLRX1 has not been observed in zebrafish Nlrx1. In addition, among the species subjected to sequence comparison, the amino acid sequence similarity of zebrafish Nlrx1 was the highest with *Cyprinus carpio* Nlrx1 at 92.2% and was relatively low with the human protein at 57.6%. As a maternal gene, *nlrx1* was evenly distributed throughout the embryo during the early developmental stage and restricted in brain, primary head sinus, and somite boundary after 3 dpf. The expression was exhibited relatively high in the brain, ovary, and blood in adults. An *nlrx1*-knockout *in vivo* model lacking the functional NACHT

and LRR domains was produced using CRISPR/Cas9 system. The *nlrx1*-deficient mutant increased the expression of innate immune response genes that encode Nlrx1-interacting proteins (*mavs, pcbp2, sting, and tbk1*), transcription factors (*irf3, irf7, and nf-κb*), and inflammation-related proteins (*isg15, tnfα, il1β, il6, mxa, mxb, and ifnphi1*) at 7 dpf. These results indicate that *nlrx1* plays an important role in innate immunity, and this mutant will be used as an important model to elucidate the function of *nlrx1* during viral infection.

SL-2

Phylogenomic characterization and improvement of molecular diagnostic assays for genus *Megalocytivirus* from cultured fish

° Kwang Il Kim

Department of Aquatic Life Medicine, Pukyong National University

◎ **Background of This Study** : *Megalocytivirus* (the family *Iridoviridae*), a causative agent of red sea bream iridoviral disease (RSIVD), is one of the most virulent viruses in cultured fish. Since the first outbreak in the 1990s, numerous megalocytivirus infections in a wide range of fish species have been identified worldwide, resulting in endemic disease in several regions. Herein, the genetic relatedness of megalocytiviruses based on their phylogeny associated with various fish species was analyzed. Besides, real-time PCR assays for detecting and genotyping of *Megalocytivirus* were developed and evaluated the methods.

◎ **Methods** : Predominant genotype was analyzed based on the phylogeny of the major capsid protein (MCP) genes from megalocytiviruses in Korea between 2012 and 2018. A genetic variant of RSIV-type *Megalocytivirus* was isolated from a primary cell-derived from rock bream fin and was investigated its genome sequences. From the consensus sequence of MCP genes retrieved from NCBI GenBank, specific primers and peptide nucleic acid (PNA) probes were designed and evaluated their analyte sensitivity and specificity for detection and genotyping. Besides, the TaqMan probe-based real-time PCR assay was developed and validated through the limit of detection ($LOD_{95\%}$) and its diagnostic sensitivity (DSe) and specificity (DSp).

◎ **Results & Discussion** : Two genotypes (RSIV- and TRBIV-types) have been identified from cultured fish in Korea. Of note, RSIV subtype II has been the predominant type in marine fish. Natural infection of RSIV-type *Megalocytivirus* as a major causative agent for RSIVD in marine fish was identified from golden mandarin fish (*Siniperca scherzeri*) as one of the freshwater fish, suggesting that RSIV-type *Megalocytivirus* might be crossed species barriers. Interestingly, an RSIV-type strain (112,360 bp; 115 putative ORFs) showed genetic variation (InDel, SNP and MNP) compared with Ehime-1 strain as an ancestor of RSIV subtype I, indicating that a natural genetic variant has emerged like other countries. PNA probe-based real-time PCR assay allowed the quantification of *Megalocytivirus* (detection limit of 10^2 – 10^3 copies) and simultaneously differentiating genotypes based on melting temperature (T_m) and fluorescent signal in a single-tube reaction. Furthermore, TaqMan probe-based real-time PCR assay also showed to be specific for megalocytiviruses with 5.3 viral gene copies of $LOD_{95\%}$. The DSe and DSp were 100% and 95.2% compared with OIE reference PCR assays, respectively. Developed real-time PCR assays could contribute to the diagnostic tool for qualitative and quantitative analysis.

SL-3

바이오플락 넙치 내성한계 및 사육지침 마련연구

° 김준환, 조아현, 홍수민, 정지호, 주창훈

선문대학교 수산생명의학과

◎ 연구의 배경 및 목적 : 우리나라 어류양식산업은 1970년대 이후로 꾸준히 발전해오고 있으며, 양질의 단백질원을 안정적으로 공급해주는 국가 기반 산업이다. 하지만, 한정된 공간에서 양식장의 무분별한 건립으로 인한 연안 오염은 빈번한 질병 발생을 유발하고 있으며, 사육환경 악화에 따른 어류의 면역력 감소로 질병에 대한 저항성은 낮아져 대량 폐사를 겪고 있다.

바이오플락 기술은 유용미생물을 이용하여, 암모니아 및 아질산과 같은 환경독성물질을 친환경적으로 제거하는 기법으로, 양식장 사육수의 교환이 필요하지 않기 때문에, 유입수를 통한 질병 유입의 우려가 없어 생물방역에도 효과적이고, 배출수에 의한 연안환경 오염도 발생하지 않아 지속가능한 미래양식기법이다. 따라서, 우리나라에서 가장 많이 양식되고 있는 넙치를 바이오플락 양식기법에 적용하여 양식기술개발을 적립한다면, 산업적으로 높은 파급력 뿐만아니라, 연안환경 개선에도 기여할 수 있을 것이다.

본 연구는 넙치를 이용하여 바이오플락 양식기술 개발을 위한 연구의 일환으로 바이오플락 및 유수식 넙치의 건강도 비교평가, 아질산 노출에 따른 내성한계 및 감수성 비교평가, 질병감염실험에 의한 질병 감수성 비교평가 등을 통해, 바이오플락 넙치의 사육을 위한 기본 지침을 마련하고, 바이오플락 넙치와 유수식 넙치의 내성한계 비교평가를 통해 바이오플락 넙치의 특성을 확인하는데 있다.

◎ 실험 방법 (또는 연구방법) : [1]바이오플락 및 유수식으로 양성한 초기 넙치에서 혈액성상 및 면역지표의 비교평가를 통해 건강도의 평가를 수행

[2]바이오플락 및 유수식으로 양성한 넙치에서 아질산 노출에 따른 내성한계를 비교평가하기 위해, 혈액성상, 혈장성분, 항산화효소, 신경독성, 스트레스 반응 및 면역지표 분석 수행

[3]세균성질병 감염에 대한 바이오플락 및 유수식 넙치의 질병저항성을 평가하기 위해, *Edwardsiella tarda* 인위감염 실험을 통해 생존율, 혈액성상, 혈장성분, 항산화효소, 신경독성, 스트레스 반응 및 면역지표 분석 수행

[4]최종적으로 상품사이즈의 바이오플락 넙치를 관능평가를 실시하여, 상품성으로서의 가능성을 확인

◎ 결과 및 고찰 : 실험의 결과 [1]바이오플락과 유수식 넙치의 큰 유의적 차이는 나타나지 않았지만, 바이오플락 넙치의 스트레스 지표가 유수식 넙치에 비해 유의적으로 낮으며, 면역지표가 높음을 확인, [2]바이오플락 넙치가 유수식 넙치에 비해 아질산 노출에 따른 높은 내성한계를 가지며, 아질산 노출에 의한 생리적 영향이 유수식에 비해 덜함을 확인, [3]세균성 감염에서도 바이오플락 넙치가 높은 생존율을 나타내며, 질병저항성이 높고 생리적 지표에서도 우수함을 확인, [4]관능평가 결과 식품으로서 일반 횟집 넙치에 비해 더 높은 선호도를 나타냄을 확인하였다. 바이오플락 넙치의 우수성에도 불구하고 아직 안정적인 양식에서는 어려움이 있으며, 다양한 기반연구 및 시스템 개발이 되어야 할 것이다.

구 두 발 표

OP 1~8

OP-1

Immune stimulation and protection effect against VHS by delivery of interferon-related gene plasmids in zebrafish

° Hye Ji Kim, Jeong Su Park, Jong bin Park, Ji Hyun Lee, Hyun Woo Kim and Se Ryun Kwon

Department of Aquatic Life Medical Sciences, Sunmoon University, Asan, Korea

◎ **Background (or Objective) of This Study** : Viral hemorrhagic septicemia (VHS) is one of the most serious viral diseases of freshwater and seawater fish, causing severe damage to the aquaculture industry in worldwide. In this study, immune genes related to innate and acquired immunity were selected and tried to be expressed in zebrafish. Expression pattern of immune genes and survival rates of zebrafish against VHSV challenge were analyzed.

◎ **Methods** : To construct expression vectors, CRTAM, IFIT14 and the interferon phi (IFN Φ) 1 gene was inserted into pcDNA3.1, the eukaryotic expression vector. In addition, an expression vector was established for IRF10 gene was also selected to confirm a negative regulation on IFNΦ1. Challenge with VHSV at 14 or 28 days post inoculation of expression vectors was performed to investigate the retention duration for activating the immunity. And the concentration (1㎍ or 10㎍) of expression vectors was also considered.

◎ **Results & Discussion** : As the results, in the group inoculated with CRTAM expression vectors, the expression of Mx and IL-1β increased on the 7 and 14 days, the expression of all immune genes (TNF-α, IL-1β, IFNΦ1, IFNγ and Mx) was significantly up-regulated. In the group inoculated with IFIT14 expression vector, expression of IFNΦ1 gene was not observed while Mx gene was up-regulated. In the group inoculated with IFNΦ1 expression vector, all immune genes were highly up-regulated in the early stages of inoculation, but were down-regulated on the 14th. The group inoculated with IRF10 expression vectors, there was no expression of IFNΦ1, suggesting that IRF10 may function as a negative regulator of IRF3, which binds to the IFNΦ1 promoter. As the results of challenge test performed at 14 days after inoculation of the expression vectors, the maximum survival rate for each group was 75% for the CRTAM group, 65% for IFIT14 group, 57.5% for IFNΦ1 group, and 50% for IRF10 group were recorded. Meanwhile, the survival rates of pcDNA3.1 and PBS as the control groups were 10% and 15%, respectively.

This study suggest that expressing genes such as CRTAM and IFIT14 capable of activating various immune responses, rather than inducing interferon alone, which is known to have strong antiviral action, has a higher defense effect against VHS. We propose the possibility of the CRTAM and IFIT14 genes being used as control measures for VHS.

OP-2

Altered temperature induces a differential immune response in seven-band grouper following nervous necrosis virus infection

° Rahul Krishnan[*], Yo Seb Jang[*], Jong Oh Kim[**], Su Young Yoon[*], Myung Joo Oh[*]

[*]Department of Aqualife Medicine, Chonnam National University, Yeosu, Republic of Korea
[**]Institute of Marine Biotechnology, Pukyong National University, Busan, Republic of Korea

◎ **Background of this Study:** The NNV infection in aquafarms is generally observed during July to October when the seawater temperature is higher than 24^{O}C and the fishes seem to be refractory to disease at suboptimal temperatures suggesting a role of thermoregulation in NNV pathogenesis. Previous studies demonstrated a down-regulated NNV multiplication and 100% protection upon rechallenge when the groupers were initially challenged with NNV at suboptimal temperature. However, the studies were inconclusive about the mechanism and effect of suboptimal temperature on antiviral immunity in seven-band grouper. Thus, the current study evaluated the regulation of cytokines, immune markers, and transcriptional control factors during temperature-dependent NNV challenge in seven-band grouper.

◎ **Methods:** Horizontal transmission of NNV was achieved through co-habitation challenge at optimal and suboptimal temperatures. Periodic sampling of brain and blood was undertaken following post-primary challenge and rechallenge. mRNA transcript analysis and Granzyme B assay were performed with brain tissue and blood was used for serological analysis including anti-NNV IgM ELISA, virus microneutralization, myeloperoxidase assay, and NBT assay.

◎ **Results & Discussion:** The data suggest that the challenge at 17° C showed a sustained expression of proinflammatory cytokines and following rechallenge, the survivors also exhibited a stable expression. The 100% survival following the challenge and rechallenge at 17° C might be due to the stable and sustained activation of the immune response. However, at the 25° C, the rechallenge displayed a priming effect showing a hyperactivation of the immune system. Thus, the mortality observed might co-relate with the cytokine storm observed from the expression profile. neither of the adaptive immune markers was suggestive of humoral immune response in the 17° C group. Moreover, the data suggested a possible role of NK cell and microglia in mediating antiviral immune response in the brain at differential temperature with the former being beneficial in restricting viral infection with higher host tolerance.

◎ **Reference:**

Gye, H.J., Oh, M.J. and Nishizawa, T., Lack of nervous necrosis virus (NNV) neutralizing antibodies in convalescent sevenband grouper *Hyporthodus septemfasciatus* after NNV infection. Vaccine, 36(14):1863-1870, 2018.

Oh, M.J., Gye, H.J. and Nishizawa, T., Assessment of the sevenband grouper *Epinephelus septemfasciatus* with a live nervous necrosis virus (NNV) vaccine at natural seawater temperature. Vaccine, 31(16):2025-2027, 2013.

Nishizawa, T., Gye, H.J., Takami, I. and Oh, M.J., Potentiality of a live vaccine with nervous

necrosis virus (NNV) for sevenband grouper *Epinephelus septemfasciatus* at a low rearing temperature. Vaccine, 30(6):1056-1063, 2012.

OP-3

Biological characteristics of *Microcotyle caudata* (*Microcotylidae*: *Monogenea*) from dark-banded rockfish (*Sebastes inermis*) in Korea

U Hwa Nam, Jeong Ho Kim

Department of Marine Bioscience, Gangneung-Wonju National University, Gangneung, Korea

◎ **Background of This study**: *Microcotyle caudata* is known as a gill parasite of Dark-banded rockfish (*Sebastes inermis*). A congeneric species, *Microcotyle sebastis* is a well-known gill parasite of Korean rockfish (*Sebastes schlegelii*), causing economic loss to the aquaculture industry in Korea. Although *M. caudata* can be a potential threat to dark-banded rockfish aquaculture industry, there is only scarce information on the biology of *M. caudata*. In this study, we investigated the reproductive biology of *M. caudata* and compared with those of *M. sebastis*. In addition, we investigated the prevalence of infection of these monogeneans in the aquaculture facilities with the PCR-RFLP method established in our previous study.

◎ **Methods**: We investigated *in vitro* egg-laying rate and egg-hatching rate of *M. caudata* and *M. sebastis* with various temperature conditions (10, 15, 20, 25℃). 240 adults and 240 eggs (120 for *M. caudata* and 120 for *M. sebastis*) were used and all the experiments were triplicated. In addition, the monogeneans were collected from the hosts (34 *S. inermis* and 47 *S. schlegeli*), and 309 monogeneans were randomly selected (135 from *S. inermis* and 174 from *S. schlegeli*) and identified by PCR-RFLP with subsequent sequencing.

◎ **Results & Discussion**: The temperature strongly influenced on fertility and viability of *M. caudata* and *M. sebastis*. The average amount of oviposition of *M. caudata* after 48 hours' incubation were 36.5±0.9 per individual at 10℃, 45.3±8.2 at 15℃, 30.3±2.9 at 20℃, 30.3±2.9 at 25℃. And those of *M. sebastis* were 31.1±0.4 at 10℃, 44.8±8.9 at 15℃, 58.2±5.8 at 20℃, and 26.2±1.8 at 25℃. *M. caudata* showed the highest fertility at 15℃, whereas *M. sebastis* showed the highest fertility at 20℃. The average time required to hatch eggs decreased as the temperature increased. The highest egg hatching rate was observed at 15℃ for *M. caudata* and 20℃ for *M. sebastis*. Of 135 individual monogeneans from dark-banded rockfish, 130 worms were identified as *M. caudata* (96.3%) and 5 worms as *M. sebastis* (3.7%). Of 174 individual worms from Korean rockfish, 168 were identified as *M. sebastis* (96.5%) and 5 were identified as *M. caudata* (2.9%). Interestingly, we found *M. caudata* from Korean rockfish and *M. sebastis* from dark-banded rockfish. We confirmed that they are not hybrid (*M. caudata*×*M. sebastis*) by PCR-RFLP of mtDNA and nuclear DNA (ITS region) with subsequent sequencing. This is probably because the net-pen cages of the two different host species are closely located, causing the accidental cross-infection. But more evidence are necessary to confirm our speculation.

OP-4

측방 유동 면역 크로마토그래피법 기반 marine birnavirus (MABV) 현장검사용 키트 개발

° 공경희[*] · 오명주[*] · 김춘섭[**] · 김위식[*]

[*]전남대학교 수산생명의학과

[**](주)엔바이로젠

◎ 연구의 배경 및 목적 : Marine birnavirus (MABV)는 넙치 치어에 복수와 두부 발적을 유발하며 대량 폐사를 일으키는 원인 병원체로 알려져 있다. 넙치 양식장 (종묘장 및 양성장)에서는 치어뿐만 아니라 성어에서도 MABV의 감염이 확인되고 있다. MABV를 검사하는 방법으로는 어류 주화세포를 사용한 분리배양법과 reverse transcriptase polymerase chain reaction (RT-PCR)이 주로 사용되고 있다. 이들 방법들은 민감도와 특이도가 우수하다는 장점이 있으나, 전문 인력 및 장비가 필요하며 검사에 최소 1일 이상의 시간이 소요된다. 이에 반해 측방 유동 면역크로마토그래피법을 기반으로 하는 현장검사용 키트는 특수한 장비와 복잡한 사용 방법 없이 분석 시료를 신속하게 검사할 수 있는 방법이다. 본 연구에서는 양식현장에서 사용 가능한 MABV 현장검사용 키트를 개발하고자 하였다.

◎ 실험 방법 (또는 연구방법) : MABV에 대한 단클론 항체 5종을 사용하여 현장검사용 키트를 제작하였다. 5종의 단클론 항체 중 최적의 조합 (금 나노 입자 축합체용 항체/검사선용 항체)을 선별하기 위하여 pair test를 실시하였다. 최종적으로 선별된 항체를 사용하여 키트를 제작한 후, 특이도와 민감도를 조사하였다. 특이도는 5종의 어류 바이러스 (MABV, HIRRV, IHNV, NNV, VHSV) 배양액 100 μl를 사용하였고, 민감도는 MABV 배양액을 buffer로 10배씩 희석 ($10^{4.3}$ - $^{8.3}$ $TCID_{50}$/100 μl)하여 strip 투입구에 떨어뜨리고 반응을 확인하였다. 임상적 성능 평가를 위해 164 마리의 넙치 (외관상 건강한 넙치 94 마리와 감염 실험을 통해 얻은 넙치 70 마리)를 대상으로 개발된 키트와 MABV 검사 방법 (어류 주화세포를 사용한 분리배양법 + RT-PCR)을 비교하였다.

◎ 결과 및 고찰 : 5종의 MABV에 대한 단클론 항체를 사용하여 pair test를 실시한 결과, 38B5/47E3 (금 나노 입자 축합체용 항체/검사선용 항체)에서 가장 강한 반응을 보였다. 민감도와 특이도를 조사한 결과, 키트의 검출 한계는 $10^{5.3}$ $TCID_{50}$/100 μl로 나타났으며, MABV를 제외한 4종의 어류 바이러스들에는 음성 반응을 보였다. 임상적 성능을 평가한 결과, 94 마리 (외관상 건강한 넙치)에서는 키트와 MABV 검사 방법 (어류 주화세포를 사용한 분리배양법 + RT-PCR) 모두에서 음성으로 나타났다. MABV 감염 실험을 통해 얻은 70 마리의 넙치에서는 84.3% (59/70 마리)에서 키트와 MABV 검사 방법 모두 양성으로 나타났으며, 15.7% (11/70 마리)에서는 키트에서 음성, MABV 검사 방법에서 양성으로 나타났다. 본 연구에서 개발된 현장검사용 키트는 MABV 검사 방법 (어류 주화세포를 사용한 분리배양법 + RT-PCR)보다 검출 감도가 낮았으나, 100%의 특이도를 보였으며, $10^{5.3}$ $TCID_{50}$/100 μl 이상의 MABV를 검출 가능하므로, 양식현장에서 넙치의 MABV를 검출하는데 유용하게 사용될 수 있을 것으로 사료된다.

OP-5

A Characterization and functional analysis of Tumor necrosis factor NEW (TNFN) in rock bream (*Oplegnathus fasciatus*)

° Sung jae Ko, Jong won Lim, Su hee Hong

Department of Marine Biotechnology, Gangneung Wonju National University, Gangneung 210-702, South Korea

◎ **Background (or Objective) of This Study** : Tumor necrosis factor New (TNFN) gene has been found only in teleost, sharing a high homology with mammalian lymphotoxin (LT)-β. In teleost, there is no report about bioactivity of TNFN. In mammals, LT-α1β2 activates via LT-βR, leading to chemokine, cytokine, adhesion molecule expression, cell proliferation and cell survival. Although it is not clear whether teleost TNFN is bioactive via LT-βR signaling, there is a possibility TNFN that TNFN can bind to other TNF receptor superfamily members. In mammals, LT-α2β1 as diminutive LT form was reported to bind TNFR1 (TNFRSF1A) and TNFR2 (TNFRSF1B) as well as LT-βR. Thus, there is a possibility TNFN may be bioactive via LT-βR or TNFR1 and TNFR2 in fish. The purpose of this study is to help understand the function of TNFN in fish by identifying the TNFN gene and ascertaining its bioactivity in rock bream.

◎ **Methods** : The sequence of Rrock bream TNFN gene was obtained from Next Generation Sequencing (NGS) of the RNA library and confirmed by PCR and blast search. TNFN gene expression was analyzed by real-time quantitative PCR (qPCR) *in vitro* and *in vivo* for tissue distribution and various stimulants. In addition, recombinant TNFN was produced in *E. coli* system, and its bioactivity upon stimulation with recombinant TNFN was analyzed *in vitro* and *in vivo* by qPCR.

◎ **Results & Discussion** : Bioinformatics analysis revealed that RB-TNFN slightly differs from mammalian LT-β in genomic structure, phylogenetic relation, and predicted protein tertiary structure, while the synteny analysis implicated that genomic location of TNFN is the same as LT-β at just behind TNFα. In healthy rock bream, RB-TNFN gene expression levels were the highest in liver and midgut while it was the lowest in head kidney. *In vitro*, RB-TNFN gene expression was significantly upregulated by stimulation with pathogen-associated molecular patterns (PAMPs) and RBIV. *In vivo,* RB-TNFN gene expression was significantly upregulated in blood, head kidney by RBIV vaccine and after RBIV infection. In functional analysis, recombinant rock bream TNFN (RB-rTNFN) significantly upregulated interleukin-1β (IL-1β), interleukin-8 (IL-8) gene expression in immune organs. Conclusively, although mammalian LT-β is known to be active as heterotrimer with LT-α and there is no evidence of LT-α in teleost, RB-rTNFN was functionally active, playing a role in innate and adaptive immunity like mammalian LT-β.

OP-6

바지락 염증 정량 기술개발과 이를 이용한 건강도 측정

° 김승현* · 남기웅** · 박경일*

*군산대학교 해양생명응용과학부 수산생명의학과
**충청남도 수산자원 연구소 연구개발과

◎ 연구의 배경 및 목적 : 염증은 척추동물에서 생물학적 또는 비생학적 자극에 대한 선천 면역반응의 한 형태로 생물의 생리적인 상태를 판단하는데 중요한 척도로 이용되고 있다. 후천면역이 부재한 바지락과 같은 연체동물 역시 염증반응은 생체 방어에 중요한 역할을 담당하는 것으로 알려져 척추동물과 같이 생물의 건강상태를 판단하는 유용한 지표로 추정되고 있다. 그러나 현재까지 해산이매패의 염증은 조직절편을 이용한 혈구의 침윤상태를 육안으로 판단하는데 그치고 있어 이를 정량하는 것은 매우 힘든 실정이다. 따라서, 본 연구는 척추동물의 염증 정량 기술로 널리 이용되고 있는 nitric oxide (NO), cyclooxygenase 2 activity (COX-2 activity) 및 allograft inflammatory factor-1 (AIF-1) 등을 바지락의 혈구에서 측정함으로써 바지락 염증 유발 기작의 척추동물과의 유사성을 확인하고, 염증 정량을 통한 바지락의 건강도 판정 가능성을 조사하기 위하여 수행되었다.

◎ 실험 방법 (또는 연구방법) : 척추동물의 염증 기작이 바지락에서도 유효한지를 확인하고자 척추동물에서 이용되고 있는 염증 유도제 (lipopolysaccharide)와 항염증제 (ibuprofen, diclofenac)를 바지락의 혈림프에 24시간 노출 한 후, nitric oxide (NO) 농도, cyclooxygenase 2 activity (COX-2 activity) 및 allograft inflammatory factor-1 (AIF-1) 유전자의 변화를 확인하였다. 또한 바지락의 건강도 판정에 이러한 기술의 적용가능성을 판단하기 위하여 단세포 기생충인 *P. olsnei* 영양체를 바지락에 노출 시킨 후 염증량의 변화를 측정하였으며, 사육수질의 변화에 의한 바지락에 발생한 염증량을 확인하기 위하여 여과장치의 보유 유무에 따른 염증량을 정량하였다. 또한 흔들림 스트레스의 바지락내 염증 발생 유무를 확인하기 위하여 바지락을 100 rpm에서 6시간 노출 시킨 후 염증량을 측정하였다.

◎ 결과 및 고찰 : 본 조사 결과 염증유도제나 염증저해제에 의해 바지락 혈구의 NO 농도, COX-2 농도 및 AIF-1 유전자의 발현이 유의하게 증가하거나 또는 감소하는 것으로 밝혀졌다. 또한 *P. olseni*의 농도가 증가할수록 염증량이 유의적으로 증가하였으며, 여과장치의 유무에 따라 발생한 수질차이가 바지락의 염증량 변화에 영향을 주는 것을 확인하였다. 또한 흔들림 스트레스에 노출된 바지락에서 높은 염증량이 확인되었다. 이상의 결과를 종합해 볼 때, 바지락은 척추동물과 유사한 염증 발생 기작을 보유하고 있으며, 다양한 병리적, 물리적, 화학적인 스트레스에 대한 반응으로 염증 반응을 나타남이 확인되었다. 따라서 이러한 염증반응은 바지락의 광범위 스트레스를 측정하는데 유용한 지표로서 이용될 수 있을 것으로 기대된다.

OP-7

A virulent plasmid of *Aeromonas salmonicida* subsp. *masoucida* could be spread to other aquatic pathogens through horizontal gene transfer

° Yu Ra Kang and Do Hyung Kim

Department of Aquatic Life Medicine, Pukyong National University, Korea

◎ **Background (or Objective) of This Study** : *Aeromonas salmonicida* causes furunculosis with clinical symptoms such as skin ulcers and exophthalmos in various fish species worldwide. In this study, a plasmid pBY3 of *A. salmonicida* subsp. *masoucida* BR19001YR, which is a high virulent strain originating from Korean rockfish (*Sebastes schlegelii*), was sequenced and characterized, and the strain was used to determine whether the plasmid could confer invasive properties to low- and non-pathogenic *Edwardsiella tarda*.

◎ **Methods** : For comparison of pathogenicity of *A. salmonicida* subsp. *masoucida* with (strain B19001YR) and without (strain RFAS1) pBY3, black rockfish (average weight = 1.77±0.21 g) were injected with RFAS1 at the concentration of 1.67 * 10^9 CFU $fish^{-1}$ for the determination of LD_{50}. Also, comparative genome analysis between two strains was conducted using EDGAR. Both strains were used for the determination of antibiotic susceptibility and invasive ability in red seabream (*Pagrus major*) fin (PMF) cells. Antibiotic resistance genes (ARGs) were searched using CARD (Comprehensive antibiotic resistance database). In this study, for pathogenicity test, the plasmid pBY3 was transferred into a low pathogenic strain of *Edwardsiella piscicida* KE2 and a non-pathogenic strain of *Edwardsiella tarda* SU100 using conjugation. Olive flounder (average weight = 5.66±0.33 g) were challenged with the two strains at 10^6 CFU $fish^{-1}$, and mortality and clinical signs were observed.

◎ **Results & Discussion** : Comparative analysis of whole genome sequence of the two strains shows that the plasmid pBY3 was present only in BR19001YR. Genome analysis revealed that the plasmid harbored *sul2*, *tetA, tetR*, responsible for the categorization as non-wild type for sulfonamide and tetracycline, and *virB/D4* type IV secretion system. This plasmid is unique, and it has not been found in the genus *Aeromonas*. Partial sequence of pBY3 was almost identical to approximately 55% of each sequence of the plasmid pEIB202 of *E. piscicida* EIB202, and P3PS10 of *Piscirickettsia salmonis* AY3800B. Rockfish challenged with RFAS1 did not show any mortality, but the LD_{50} of BR19001YR was 7.14*10^2 CFU $fish^{-1}$. Also, strain BR19001YR showed 14 times higher invasion ability in PMF cells than that of RFAS1. BR19001YR was categorized as non-wild type for tetracycline and sulfonamide, while RFAS1 was wild type for those antibiotics. It was confirmed that pBY3 was transferred to *E. piscicida* and *E. tarda* through bacterial conjugation. KE2, which acquired pBY3, seemed to have higher pathogenicity than the wild type strain. The results show that ARGs and virulence-related genes on this novel plasmid may have possible contribution to virulence and can spread into other species and aquatic environment. Further study will be needed to better clarify the role of the plasmid pBY3 in its virulence, and also evaluate it as an epidemiological marker.

OP-8

MIC panel을 이용하기 위한 어병세균의 배양조건 확립

° 김예지* · 우수지** · 김명석** · 전려진* · 정준범*

*제주대학교 해양생명과학과

**국립수산과학원 병리연구과

◎ 연구의 배경 및 목적 : 수산업에서는 양식현장에서 연중 발생하는 연쇄구균병, 에드워드병, 비브리오병 등과 같은 세균성 질병에 의한 피해를 막고 생산성 향상을 위한 수단으로서 항생제를 사용한다. 의약품의 사용량이 증가함에 따라 오남용으로 인한 위험성이 고조되고 있으나 즉시 항생제의 사용을 줄이는 것은 불가능하기 때문에 현재로서는 항생제 감수성 검사를 실시하여 유효 항생제를 선택한 다음 용법과 용량에 맞도록 사용하는 것이 최선의 방법이다. 다양한 항생제가 농도별로 coating되어 있어서 간편하고 신속하게 항생제 감수성 검사를 실행할 수 있는 MIC (minimal inhibitory concentration) panel은 인체 및 가축에서 유래된 세균을 대상으로 제작되어 사용되어졌기 때문에 어병세균에 적용하기에는 항생제의 종류와 배양조건이 적합하지 않다. 따라서 본 연구에서는 18종의 수산용 항생제들이 96 well plate에 coating된 Sensititre™ KRAQ1, CAMPY2 panel (TREK Diagnostic system, East Grinstead, UK)을 이용하여, 어류에서 분리한 세균 종에 대한 MIC 분석을 실시하고 효율적인 항생제 감수성 검사를 위한 최적화 조건을 확립하고자 하였다.

◎ 실험 방법 : 실험균주는 어류에서 분리되는 대표적인 병원성 세균인 *Streptococcus* spp., *Edwardsiella piscicida, Vibrio* spp., *Aeromonas* spp. 및 *Pseudomonas* spp.를 각각 5균주씩 본 연구에 사용하였다.

MIC panel을 이용한 항생제 감수성 검사 : *Streptococcus* spp.의 경우, 5%의 lysed horse blood (LHB)가 첨가된 cation-adjusted Mueller-Hinton broth with TES buffer (CAMHBT) 배지와 1% NaCl의 첨가 유무를 다르게 하여 실험을 진행하였고, Mueller-Hinton broth (MHB) 배지에서 배양했을 때 MIC 값의 차이점을 확인하고자 하였다. *E. piscicida*, *Vibrio* spp. 그리고 *Aeromonas* spp.는 CAMHBT 배지, 1% NaCl이 첨가된 CAMHBT 배지 그리고 1% NaCl이 첨가된 MHB에서 배양하였다. *Pseudomonas* spp.의 경우는 CAMHBT 배지에서 온도의 조건을 22℃, 28℃와 35℃로 나누어 실험을 진행하였다.

◎ 결과 및 고찰 : *Streptococcus* spp.를 배양하기 위한 최적의 조건은 5% LHB가 첨가된 CAMHBT를 28℃의 온도에서 배양하는 것으로 설정하였다. 염분에 민감한 *Vibrio* spp.의 경우, 1% NaCl이 첨가된 CAMHBT 배지를 이용하여 28℃에서 panel을 배양하는 것으로 최적화 조건을 수립하였으며, *E. piscicida*, *Aeromonas* spp, *Pseudomonas* spp.의 MIC 값을 확인하기 위한 배양조건은 CAMHBT 배지를 이용하여 28℃의 온도가 적합한 것으로 확인하였다.

◎ 참고문헌

CLSI (Clinical and Laboratory Standards Institute). Methods for broth dilution susceptibility testing of bacteria isolated from aquatic animals; proposed guideline. CLSI document M49-P. CLSI, Wayne, PA, U.S.A., 2005.

포스터 발표

PP-1 ~ 42

PP-1

Complete genome sequence and phylogenetic analysis of RSIV-type *Megalocytivirus*

Min a Jeong and Kwang Il Kim

Department of Aquatic Life Medicine, Pukyong National University

◎ **Background of This Study** : *Megalocytivirus* is classified into three genotypes, red sea bream virus (RSIV), infection spleen and kidney necrosis virus (ISKNV) and turbot reddish body iridovirus (TRBIV), based on the major capsid protein (MCP) and adenosine triphosphatase (ATPase) genes. Although megalocytiviruses have emerged in several countries since the 1990s, only a few complete genome sequences have been reported. This study reported phylogenetic analysis and the complete genome sequence of two RSIV-type megalocyviruses: 17SbTy originating from sea bass (*Lateolabrax japonicus*) and 17RbGs originating from rock bream (*Oplegnathus fasciatus*).

◎ **Methods** : Two megalocytiviruses (17SbTy and 17RbGs strains) isolated from a primary cell derived from rock bream fin (RBF) were subjected to complete genome sequencing and analysis. The whole-genome sequencing was carried out by pair-end sequencing using the Illumina Hiseq platform. Complete genome sequence alignment was performed using the Clustalw (Ver. 2.1). Gene prediction was performed using Prokka (Ver. 2.1). The predicted ORFs were subjected to a homology search using the NCBI BLASTp program. Based on MCP and ATPase genes, phylogenetic trees were constructed by the Maximum-likelihood algorithm with 1,000 bootstrap replicates using MEGA program (Ver. 5.05). The genomic DNA composition, structure, and homologous regions were analyzed using CCT software.

◎ **Results & Discussion** : The 17RbGs strain was identified as a typical RSIV subtype II being rampant in the marine fish species in East and Southeast Asian countries. The 17SbTy strain belonged to RSIV subtype I and subtype II sequences based on the MCP and ATPase genes, respectively. Furthermore, the 17SbTy comprises 112,360 bp of genome sequences coded 115 putative open reading frames (ORFs) and the 17RbGs consisted of 112,235 bp genome sequences coded 114 ORFs. From the comparison with reference strain (Ehime-1 strain as an ancestor of RSIV subtype I), genetic variations (InDel, SNP and MNP) were significantly identified in the 17SbTy strain, suggesting that this strain is a natural genetic variant type.

◎ **Reference** : Kim K. I., Lee E. S., Do J. W., Hwang S. D., Cho M., Jung S. H., Jee B. Y., Kwon W. J. and Jeong H. D.: Genetic diversity of *Megalocytivirus* from cultured fish in Korea. Aquaculture., 509:16-22, 2019.

Puneeth T. G., Baliga P., Girisha S. K., Shekar M., Nithin M. S., Suresh T. and Kumar B. N.: Complete genome analysis of a red seabream iridovirus (RSIV) isolated from Asian seabass (*Lates calcarifer*) in India. Virus research., 291:198199, 2021.

PP-2

Characterization of a primary cell from rock bream (*Oplegnathus faciatus*) and its susceptibility of *Megalocytivirus*

Ye jin Jeong and Kwang Il Kim

Department of Aquatic life Medicine, Pukyong National University

◎ **Back ground (or Objective) of This study**: A cell line that has high susceptibility to a virus is one of the important tools for viral research. Genus *Megalocytivirus* has been identified as the causative agent of a red sea bream iridoviral disease (RSIVD) that cause mass mortality in fish. Although several cell lines were developed, these cell lines are difficult to obtain as well as some of them showed decreasing in viral concentration post serial passages of the *Megalocytivirus*. In this study, we established RBF (rock bream fin) cell-derived fin and confirmed its susceptibility to *Megalocytivirus*.

◎ **Methods** : Fin culture from rock bream (*Oplegnathus faciatus*) as primary cell was optimized. Cell growth conditions dependent on the temperature and fetal bovine serum (FBS) were analyzed. Based on the phylogeny of cytochrome c oxidase subunit 1 (COI) in the cell, the origin of the fish species was identified. Besides, the chromosome numbers were analyzed. For the susceptibility of *Megalocytivirus*, three genotypes (RSIV, ISKNV and TRBIV) were inoculated to cells as well as quantified its titer by TaqMan probe-based real-time PCR assay.

◎ **Results & Discussion** : Fibroblast-like cells obtained from the caudal fin of rock bream and their origin was confirmed based on the phylogeny of COI gene. Primary cells showed successful serial passages at 25℃ in L-15 supplemented with 10-15% FBS. The chromosome numbers of developed cells ranged from 39 to 57 with a modal chromosome number of $2n$ = 48. From the inoculation of *Megalocytivirus* (RSIV subtype II, ISKNV and TRBIV-type), all genotypes were successfully isolated and appeared obvious CPE within 3-7 days. Furthermore, the viral gene copies of infected cells with RSIV subtype II, ISKNV and TRBIV reached 7.00×10^8, 2.72×10^7 and 1.78×10^7 copies/mL, reaching more than 10 - 100 times of the initial number (1×10^6 viral gene copies/mL), respectively.

◎ **Reference** : Kawato, Y., Yamashita, H., Yuasa, K., Miwa, S., & Nakajima, K.: Development of a highly permissive cell line from spotted knifejaw (*Oplegnathus punctatus*) for red sea bream iridovirus. Aquaculture, 473:291-298, 2017.

Le, Y., Li, Y., Jin, Y., Jia, P., Jia, K., & Yi, M.: Establishment and characterization of a brain cell line from sea perch, *Lateolabrax japonicus*.. In Vitro Cellular & Developmental Biology-Animal, 53(9):834-840, 2017.

PP-3

Production of *Penaeus vannamei* nodavirus (*Pv*NV) virus-like particles (VLPs) for delivery of double-stranded RNA

Soon Joo Hong and Ki Hong Kim

Department of Aquatic Life Medicine, Pukyong National University, Busan, Republic of Korea

◎ **Background (or Objective) of This Study** : Various viral diseases in cultured shrimp have brought a huge economic loss in shrimp aquaculture. Unfortunately, no effective control measure against viral diseases has been available. Although many studies have demonstrated that double-stranded RNA (dsRNA) targeting viral mRNA had a protective effect against viruses through RNA interference (RNAi) pathway, technologies for the practical delivery of dsRNA to shrimp have not yet been established. Therefore, the development of efficient delivery tools is urgently required. In this study, we aimed to use *Penaeus vannamei* nodavirus (*Pv*NV) virus-like particles (VLPs) containing dsRNA binding domain (DRBD) as a long dsRNA delivery tool. *Pv*NV genome consists of 2 segments of positive-sense, single-stranded RNA, and RNA2 contains a single ORF encoding viral capsid protein. The N-terminal end of the capsid protein has a motif that interacts with RNA molecules, and is located the inside of viral particles. Therefore, we modified the N -terminal domain of *Pv*NV capsid protein to efficiently bind to dsRNAs.

◎ **Methods** : To produce *Pv*NV VLP containing DRBD, we constructed two vectors which had T7 promoter, dsRNA binding domain (DRBD), linker protein, *Pv*NV RNA2 ORF and 6x his tag. One had *Pv*NV RNA2 full ORF (pET28-DRBD-RNA2), and the other had the 32 amino acids-deleted N-terminal end of capsid protein (pET28-DRBD-RNA2-△32). Each vector was transformed into *Escherichia coli* HT115(DE3), and DRBD fused *Pv*NV capsid protein was expressed at 20℃, 20 hours with IPTG (0.4 mM) induction.

◎ **Results & Discussion** : The expression of *Pv*NV capsid protein and DRBD fused *Pv*NV capsid protein was verified by SDS-PAGE and western-blot analysis. The production of each VLP was analyzed by Bio-Transmission Electron Microscope analysis, and the EM photos showed the successful production of *Pv*NV VLPs. The binding of dsRNA to the modified capsid protein and the delivery of the VLPs to shrimp are being conducted.

◎ **Reference** : Goh ZH, Mohd NAS, Tan SG, Bhassu S, Tan WS.: RNA-binding region of *Macrobrachium rosenbergii* nodavirus capsid protein. J Gen Virol, Sep;95(Pt 9):1919-1928, 2014.

PP-4

Generation and characterization of the primary koi carp (*Cyprinus rubrofuscus*) caudal fin (KCF) cell

Seong Mok Kim, Ki Hong Kim

Department of Aquatic Life Medicine, Pukyong National University, Busan, Republic of Korea

◎ **Background (or Objective) of This Study** : Cyprinid herpesvirus-3 (Koi herpesvirus, KHV) is a virus belonging to the family Alloherpesviridae and can induce high mortality (80~90%) in all ages of carp. The KHV was first reported in Germany and Israel in 1988. But now, the virus is widespread in the world due to trading koi carp (*Cyprinus rubrofuscus*) and has been listed in the World Organization for Animal Health (OIE) since 2006 as a notifiable pathogen. Among several KHV detection methods, a cell culture-based detection method has a limitation in that KHV can be grown in a few susceptible cell lines (e. g. KF-1, CCB) and shows low PCR detectability from the cell lines. Therefore, in this study, we newly established a koi carp caudal fin cell line from caudal fin tissue (KCF cell). Through the cell proliferation assay, KHV inoculation, and PCR detection, we analyzed the suitability of the KCF cell line for research on KHV.

◎ **Methods** : The koi carp caudal fin (KCF) cell line was established from minced caudal fin tissue. Cells were cultured in a T-25 cell culture flask with appropriate culture media and a 25℃ incubator with 5% CO_2. The cell proliferation assay was performed using CytoX solution. All procedure of cell proliferation assay was done according to the manufacturer's protocol. The KHV inoculation and isolation were conducted with KCF cells and cytopathic effect (CPE) was observed daily. The virus detection was done 10 days after infection by PCR.

◎ **Results &Discussion** : In this study, we established the koi caudal fin cell line and conducted a cell proliferation assay. Through the proliferation assay result, we could determine the optimum cell culture conditions. The CPE formation and the PCR amplification from KHV inoculated KCF cells suggest that the present established cell line has a high susceptibility to KHV, and can be used as another cell line for KHV culture.

◎ **Reference** : Wang, Y., Zeng, W., Wang, Q., Li, Y., Bergmann, S. M., Zheng, S., & Lepp, P.: Establish and charaterization of a new cell lone from koi brain (*cyprinus carpio L.*). Journal of Fish Diseases, 41(2):357-364, 2017.

Zhou, J., Wang, H., Zhu, X., Li, X., Lv, W., & Zhang, D.: The primary culutre of miiror carp snout and caudal fin tissues and the isolation of Koi herpesvirus. In Vitro Cellular & Developmental Biology - Animal, 49(9):734-742, 2013

PP-5

Efficacy of live vaccine against viral hemorrhagic septicemia virus (VHSV) infection in juvenile olive flounder, *Paralichthys olivaceus*

Yo seb Jang, Rahul Krishnan, Su Young Yoon, Myung Joo Oh

Department of Aqualife Medicine, Chonnam National University, Yeosu, Republic of Korea

◎ **Background of this Study** : The aquaculture industry of olive flounder (Paralichthys olivaceus) is suffering massive economic losses from VHS in the seasons of March to May. VHS infection occurs when juvenile olive flounders were shifted from hatchery system where the water temperature is around 18° C to the grow out system where the water temperature is below 15° C. To date, several studies on the VHSV vaccines have been reported. However, so far there is no feasible vaccine for juvenile olive flounder against VHSV infection. Many studies developed inactivated vaccines that render them safe to use. Previously we have formulated a method for live vaccinating flounders using temperature optimized challenge with live VHSV. Thus, in the present study, we studied the efficacy of live viral vaccine compared to that of an inactivated vaccine.

◎ **Methods** : Juvenile olive flounder were separated into groups A, B, and C. For immunization, fishes were acclimated at 17° C and challenged with VHSV at a dose of 105.5 TCID50/ml by immersion for 1 hour into group A as a live viral vaccine. In group B, fish were challenged with ultraviolet (UV) irradiated VHSV as inactivated vaccine keeping challenge conditions constant. Fishes of group C were immersed with DMEM0. Following the challenge, fishes were moved to 10° C and acclimated for 3 days. To confirm the efficacy of vaccination, fishes from all groups were challenged with VHSV at a dose of 104.5 TCID50/100ul/fish by injection. Fishes were monitored for 20 days and heart, spleen, and kidney tissues were collected at 5-, 10-, 15-, and 20-days post-infection to analyze viral titer and the pattern of VHSV mRNA replication in targeted tissue of vaccinated flounder using an in-situ hybridization assay.

◎ **Results & Discussion** : Efficacy of live VHSV vaccination at 17° C was demonstrated with no mortality while non-vaccinated flounder and UV treated inactivated VHSV vaccinated flounder resulted in 100% cumulative mortality within 20 days. Although the immunization period at 17° C was 10 days to allow fish to acquire the immune response against VHSV infection, it seemed like enough to immunize the fish. Viral titer was observed in live VHSV vaccinated flounder group at 5, 10, and 15 dpi, titer even less than 105.5 TCID50/ml. No viral particles were localized in the spleen and kidney of live VHSV vaccinated flounders following the infection suggesting a live vaccine-induced immunity in the olive flounder. From the comparing with results of the viral load titer and the survival curves, no mortality among the live vaccinated flounders.

◎ **Reference** : Kim, Soo-Jin, Syed Shariq Nazir Qadiri, and Myung-Joo Oh.: Juvenile olive flounder immersed in live VHSV at 17° C and 20° C shows resistance against VHSV

infection at 10° C. Virus research, 273:197738, 2019.
Kim, Soo-Jin, and Myung-Joo Oh.: Potentiality to natural immunization inducement against VHS in olive flounder by live VHSV immersion vaccination at temperature-controlled culture condition. Virus Research, 288:198140, 2020.

PP-6

Effects of orally administered betaine on immune responses and protection against viral and bacterial diseases in olive flounder (*Paralichthys olivaceus*)

Hee Jae Choi*, Ji Hoon Lee**, Saran Hori*, Da Yeon Choi*,
Hyuk Chu Kwon* and Yue Jai Kang*

*Department of Aquatic Life and Medical Sciences, Sun Moon University
**Aquatic Disease Control Division, National Fishery Products Quality Management Service

◎ **Background of This Study** : *Edwardsiella tarda* and viral hemorrhagic septicemia virus (VHSV) have been known the important pathogen among each the bacteria and viral species in aquaculture. Also, these major diseases are representative one of disease leading cause of mass mortality among pathogens in cultured olive flounder (*Paralichthys olivaceus*) which is important for industry. Oral administration of immunostimulants can be more efficient than injection of using ineffective vaccine or antibiotics. In this study, we performed research that olive flounder were fed diets containing betaine of various concentration for 30 days, and the effects on growth in fish, innate immune activities, and cumulative mortality rate by pathogens infection.

◎ **Methods** : Each groups were fed diets containing 0 (control), 0.1, 0.5, or 1.0% betaine for 30 days. Experimental groups were randomly sampled at 6 fish/group and those were isolated the serum and extracted the head-kidney, respectively. To measure nonspecific immune activity, lysozyme and respiratory burst activity assay were performed. Also, we checked bactericidal and virucidal activity by obtain serum in each groups to measure complement activity. After the sampling, the fish of remained each groups were checked cumulative mortality against *E. tarda* and VHSV challenge.

◎ **Results & Discussion** : Fish growth was better in the 0.5 and 1.0% betaine groups than in the control groups. The innate immune activity was examined by the lysozyme and complement activities in the serum and the respiratory burst activity in leucocytes these activities were found to be significantly increased in fish fed 1.0% betaine. Moreover, each group was challenged with 2 representative pathogens, namely bacterial pathogen *E. tarda* or viral pathogen VHSV. While survival rates were significantly higher in the 1.0% betaine groups than control groups after *E. tarda* challenge, these rates were significantly higher in the 0.5 and 1.0% betaine groups than the control groups until 11 days after VHSV challenge. Thus we indicate these results that have increased survival rate against pathogens infection and increased innate immunity activities by oral administration of betaine in olive flounder for 30 days.

PP-7

제주 연안에 서식하는 *Vibrio alginolyticus* 분포

최원선 · 문채윤 · 허문수

제주대학교 해양과학대학 수산생명의학과

◎ 연구의 배경 및 목적 : 비브리오 종은 해양 생태계에 상존하는 그람음성 호염균으로 수온이 높아짐에 따라 증가하는 경향이 있다. 그중 *V. alginolyticus*는 대게 오염된 해수에 피부가 노출될 경우 감염이 이루어지며 인간이나 해양생물들에게 감염을 일으키기도 한다. 전 지구적으로 지구 평균 해수면 온도가 상승하였고 한반도 역시 증가하였으며 또한, 제주 지역은 양식장의 밀집도가 높은 곳으로 양식장 배출수에는 용존유기물질과 고형물이 모두 포함되어 연안 해역의 수질과 수중 생물에 많은 영향을 미친다. 따라서 제주 지역의 해양환경 인자와 *V. alginolyticus* 검출률의 상관관계를 알아보고자 한다.

◎ 실험 방법 (또는 연구방법) : 제주도의 4개 지역 (성산, 표선, 한림, 대정)에서 지역별로 해수 및 소라를 채취하여 *Vibrio alginolyticus*를 동정하고 분포를 조사하였다. 그리고 분리된 균의 검출률과 해양환경인자와의 상관관계를 알아보기 위해 샘플링 지점 인근 해안의 수온, 염분, DO, pH의 수치를 함께 조사하였으며 분리된 균의 온도, pH, 염도별로 최적 배양조건과 항생제 감수성 test를 실시하였다.

◎ 결과 및 고찰 : 제주도 연안 4개 지역의 *V. alginolytucus* 분포는 해수의 경우 표선지역에서 13건 (21.7%)으로 가장 많은 분포율을 나타냈으며 한림지역이 8건 (13.3%)으로 가장 낮은 분포율을 나타냈다. 소라의 경우 표선지역이 7건 (23.3%)로 가장 많은 분포율을 나타냈으며 성산지역과 한림지역이 4건(13.3%)으로 가장 낮은 분포율을 보였다. 전체 검출률은 표선지역이 20건(22.2%) 가장 높았으며 한림지역이 12건(13.3%)으로 가장 낮았다. 해양 환경변화에 따른 검출률은 수온이 가장 높은 시기에 가장 높았다. *V. alginolyticus*의 검출률은 인근 해안의 수온 상승률과 비례했으며 수온과 연관성이 높음을 확인할 수 있었다. 항생제 감수성 시험 결과 4개 지역에서 분리된 균들은 공통적으로 Oxytetraxycline, Nalidixic acid에 모두 감수성을 보였으며 이외에도 Oxolinic acid, Doxycycline, Cprofloxacin 에도 대부분이 감수성을 보여 분리 균에 대한 효과적인 항생제임을 확인하였다.

◎ 참고문헌 :

Craig Baker-Austin, James D. Oliver, Munirul Alam, Afsar Ali, Matthew K. Waldor, Firdausi Qadri and Jaime Martinez-Urtaza: Vibrio spp. Infections. Nat Rev Dis Primers, 4:1-19, 2018.

Luigi Vezzulli, Rita R. Colwell and Carla Pruzzo: Ocean Warming and Spread of Pathogenic Vibrios in the Aquatic Environment. Microb Ecol, 65:817-825, 2013.

T. Gregersen: Rapid method for distinction of gram-negative from gram-positive bacteria. European journal of applied microbiology and biotechnology, 5:123-127, 1978.

PP-8

Expression analysis of osmotic regulatory genes in seawater-adapted *Oncorhynchus mykiss* in response salinity challenge

Young Kwang Choi*, Ji Hoon Kim**, Yi Kyung Kim**

*Department of Wellness BioIndustry, Gangneung-Wonju University
**Department of Marine Biotechnology, Gangneung-Wonju University

◎ **Background (or Objective) of This Study** : Salinity is one of the critical factors limiting the distribution patterns of all aquatic organisms. In Korea aquaculture domain, *Oncorhynchus mykiss* has been attention as a potential species due to larger harvest sizes during seawater farming. Recently, research has been actively conducted as a method of gradual seawater adaptation to increase salinity in stages. In comprehensive insight into environmental cues of seawater acclimation in rainbow trout, the objective of this study is to scrutinize their expression patterns of osmoregulation-related genes from rainbow trout in response to salinity challenge.

◎ **Methods**: About 600g of *O. mykiss*, was gradually adapted to seawater at 0, 7, 14, 21, 28, and 32 ppt for 5 days. Total RNA was extracted from the tissue samples using RNAiso Reagent (Takara Bio, Shiga, Japan) with DNase I, RNase-free (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. After blood was collected from the caudal vein using syringes and heparinized tubes, plasma was separated by centrifugation and then stored at -80℃.

◎ **Results & Discussion**: Among the genes related to water and ion regulation in the gills, the osmotic organ of *O. mykiss*, the expression levels of aquaporin3 (AQP3) and cystic fibrosis transmembrane conductance regulator (CFTR) increased significantly at 14, 28, 32 ppt, and solute carrier family12 Member2 (SLC12A2) significantly increased at 7 ppt and then decreased as the salinity increased. In the genes related to osmoregulation hormone, Growth Hormone Receptor (GHR) tended to increase significantly at 7 ppt and then decreased. Glucocorticoid receptor (GR) had a tendency to increase and then decrease with increasing salinity. As a result of blood analysis, the values of GPT and GOT, which are used as a indicator of hepatotoxicity when there is a problem with hepatic function, did not show a significant difference between from the experimental group and the control group. There was no significant trend in triglycerides used as energy sources. Rainbow trout is considered to have low stress in the process of seawater adaptation and this study is expected to be used as a basic data for stable seawater adaptation of Rainbow trout by comparing the expression changes of osmosis-related genes in each salinity challenge stage.

◎ **Reference** : Comparative transcriptome profiling of selected osmotic regulatory proteins in the gill during seawater acclimation of chum salmon *(Oncorhynchus keta)* fry.

PP-9

Optimization of quantitative PCR combined with propidium monoazide (PMA-qPCR) for the detection and quantification of *Edwardsiella piscicida*

A Hyun Kim and Do Hyung Kim

Department of Aquatic Life Medicine, Pukyong National University

◎ **Background (or Objective) of This Study** : *Edwardsiella piscicida* is a widespread waterborne pathogen and is able to enter into a viable but nonculturable (VBNC) state in unfavorable conditions. Propidium monoazide (PMA) can penetrate only membrane-compromised, dead microbial cells and inhibit DNA from being amplified during PCR. In this work, conditions for PMA concentration and photo-activation time were optimized using different concentrations of live and dead cells of *Edwardsiella piscicida* isolated from diseased olive flounder (*Paralichthys olivaceus*).

◎ **Methods** : The optimal concentration of propidium monoazide was determined using live and heat-killed (treated at 90℃ for 10 min) *E. piscicida* cells at a concentration of 10^8 CFU/ml, respectively. The both cells were treated with PMA at a concentration of 0, 5, 10, 20, and 40 μM, respectively, and the cell-PMA mixtures were kept in the dark for 10 min. Then, they were exposed to a 500-W halogen light at a distance of 40 cm for 30 min. Also, in order to determine the time for photo-activation, live and heat-killed cells treated with 20 μM of PMA were exposed to the halogen light for 0, 10, 20, 30, and 40 min. After optimization, PMA-qPCR of live and heat-killed cells were performed separately to draw standard curves. Ten-fold serial dilutions (10^1- 10^8 CFU/ml) of both cells were treated with 20 μM of PMA and exposed to the light for 30 min, and then qPCR and PMA-qPCR standard curves were generated. Additionally, varying ratios of viable to dead *E. piscicida* cells (0:100, 25:75, 50:50, 75:25, and 100:0) were used for PMA-qPCR.

◎ **Results & Discussion** : When bacterial DNA was treated with PMA at various concentrations (0, 10, 20, and 40 uM), the C_t values of PMA-qPCRs increased as PMA increased from 0 to 20 uM, but the value decreased at 40 uM of PMA. As a result, 20 uM of PMA and exposure of the mixture to halogen light for 30 min are optimal for PMA-qPCR. In this study, PMA-qPCR assay shows that it can distinguish and quantify live cells from dead cells when bacterial concentration is high, and its sensitivity is getting lower as bacterial load decreased. This PMA-qPCR assay may provide a sensitive and specific method for detecting VBNC state of *E. piscicida* cells in seawater samples.

PP-10

Quantification of viable but nonculturable (VBNC) state of *Streptococcus parauberis* using quantitative PCR combined with propidium monoazide

Hyo Young Kang and Do Hyung Kim

Department of Aquatic Life Medicine, Pukyong National University

◎ **Background (or Objective) of This Study** : Recent studies have found that *Streptococcus parauberis,* the causal agent of streptococcosis in olive flounder (*Paralichthys olivaceus*), can enter into viable but nonculturable (VBNC) state in aquatic environment. Propidium monoazide combined with quantitative PCR (PMA-qPCR) can not only distinguish viable cells from dead cells, but also quantify the viable cells. Therefore, the aims of this study were to establish the method of PMA-qPCR in order to detect nd quantify the VBNC state of *S. parauberis* in natural seawater.

◎ **Methods** : To determine the optimal PMA conditions, mixture of live and dead *S. parauberis* cells (ratio of 1:1 at $2*10^8$ CFU/mL) were treated with PMA at 10, 20, 30, 40, and 50 μM. After incubation in the dark for 10 min, the cell-PMA mixtures were placed on ice and exposed to 500W of halogen light at distance of 20 cm for 10 min, and then bacterial DNA was extracted. To determine the optimal exposure time for halogen light, three cell mixtures (ratio of live to dead cells=100:0, 50:50, 0:100) treated with 10 μM PMA were exposed to 500 W halogen light at distance of 30 cm for 10, 15, and 20 min, and then bacterial DNA was extracted. To determine the detection limit of PMA-qPCR assay, three cell mixtures treated with and without 10μM of PMA (ratio of live to dead cells=100:0, 10:90, 0:100) were adjusted to 10^3 , 10^4 , 10^5, 10^6 and 10^7 CFU/mL, respectively, and incubated in the dark for 10 min. After exposure to halogen light at 500W for 15 min, and then bacterial DNA was extracted. The extracted DNA in each experiment was used for PMA-qPCR using primer sets targeting the gyrB gene (condition : denaturation at 95° C for 10 min, followed by 45 cycles of 15 s of denaturation at 95° C and 60 s annealing/extension at 60° C).

◎ **Results & Discussion** :In this study, 10μM of PMA and exposure to halogen light for 15 min at a distance of 30 cm were optimal conditions for PMA-qPCR assay for *S. parauberis*. Also, the PMA-qPCR method can detect the VBNC state of *S. parauberis* when its concentration is higher than 10^5 CFU/ml. This optimized PMA-qPCR method can be used for detect and quantify *S. parauberis* in the VBNC state to understand dynamics of various physiological state of the pathogen in association with environmental conditions such as water temperature in reservoirs.

PP-11

Cephalexin의 흰다리새우(*Litopenaeus vannamei*)에 대한 독성 연구

양찬영 · 배준성 · 이채원 · 정은하 · 박영신 · 박관하

전북 군산시 미룡동 산 68번지 군산대학교 해양과학대학 수산생명의학과

◎ 연구의 배경 및 목적 : Cephalexin은 수산동물에서 경구투여가 가능한 1세대 cephalosporin계 광범위 반합성 항생제로서 한국, 미국 등 여러 나라에서 수산용으로 어류에 사용이 허가되어 있다. 어류양식에서 유결절증, 연쇄상구균증, 절창병 등 세균성 질병에서 치료효과를 기대할 수 있으며, 수산동물에서의 사용이 허가되어 있기 때문에 흰다리새우에서의 사용 가능성이 있다고 판단된다. 따라서 본 연구를 통하여 흰다리새우에 활용할 수 있는지를 평가하고자 할 때 필요한 자료 중 독성학적 영향에 대한 평가를 수행하였다.

◎ 실험 방법 (또는 연구방법) : 체중 10-20g의 흰다리새우를 입수하여 각 1톤의 순환여과수조에서 사육하였다. Cephalexin을 체중 1 kg당 10, 20, 40 mg 용량으로 사료에 흡착하여 7일 동안 경구투여 하였고, 대조군의 경우 증류수를 사료에 흡착하여 경구투여 하였다. 투여가 끝난 후 24시간 뒤 혈림프에서의 GPT, GOT, glucose, total protein 및 total cholesterol, 간췌장에서의 ACP, T-AOC, GST, SOD 및 GPx, 간췌장 및 근육에서의 병리조직학적 영향을 평가하였다.

◎ 결과 및 고찰 : Cephalexin의 독성 연구 결과를 종합적으로 평가하면 10, 20 및 40 mg/kg의 농도로 투여하였을 때 모든 지표에서 대조군과 유의적인 차이를 보이지 않았다. 본 연구 결과를 통해 흰다리새우 양식장에서 cephalexin의 사용에 필요한 자료로써 활용될 수 있을 것이다.

PP-12

LC-MS/MS를 이용한 doxycycline의 흰다리새우 조직 내 잔류 연구

이채원 · 배준성 · 양찬영 · 정은하 · 박영신 · 박관하

전북 군산시 미룡동 산 68 번지 군산대학교 해양과학대학 수산생명의학과

◎ 연구의 배경 및 목적 : 흰다리새우의 양식업은 빠르게 증가하고 있지만, 부적절한 사육 환경, 세균 또는 바이러스 감염에 의한 질병 발생, 그로 인한 사망률로 인해 생산량이 영향을 받고 있다. Tetracycline계 항생제는 광범위 항생제로서 그람 양성, 그람 음성, 세균이나 말라리아 같은 원생동물류에도 부분적으로 작용한다. 또한, tetracycline계 항생제인 doxycycline은 다양한 항염증 및 항종양성 역할에도 기여하고 있다. 거의 모든 수산양식에서는 tetracycline계 항생제의 사용이 증가하면서 새우양식에서도 유효한 항생제로 도입되고 있다. 본연구에서는 동물용 의약품인 doxycycline을 흰다리새우에 활용할 수 있는 분석법을 이용하여 잔류 농도를 확인하였다.

◎ 실험 방법 (또는 연구방법) : Doxycycline을 어체중 1kg당 20, 50, 100mg의 용량으로 사료에 흡착하여 체중 13g 정도 크기의 흰다리새우에 투여하였다. 실험은 적수온 28℃와 저수온인 20℃로 잔류 시험을 하였으며, 투여가 종료된 날을 0일로 하여 3, 7, 14, 28, 42일마다 새우를 채취한 뒤 HPLC-MS/MS를 사용하여 분석하였다.

◎ 결과 및 고찰 : 흰다리새우 근육 내 doxycyline의 잔류 농도 20, 50, 100 mg/kg을 투여하였을 때 100mg/kg의 시험 구에서 투여가 종료된 3일째일 때 최댓값을 나타내었다. 또한, 모든 용량에서 투여 종료 후 42일째부터 잔류 농도가 검출되지 않았다.

PP-13

넙치(*Paralichthys olivaceus*)에서 tylosin 경구 투여에 따른 잔류 연구

배준성 · 이채원 · 양찬영 · 정은하 · 박영신 · 박관하

전북 군산시 미룡동 산 68 번지 군산대학교 해양과학대학 수산생명의학과

◎ 연구의 배경 및 목적 : Tylosin은 marcolide계열의 항생제로서 세균의 ribosimal 50S unit과 결합하여 단백질 합성을 저해하는 기전으로 정균작용을 발휘한다. 가축에서 호흡기감염과 피부감염에 대한 치료목적으로 주사제와 경구제가 모두 사용중이며 gram 양성균에 우수한 선택적인 약효를 발휘한다. 연쇄구균증은 넙치에서 대량폐사를 야기하는 가장 중요한 세균성 질병으로 평가된다. 하지만 기존의 수산용의약품은 내성발생으로 인하여 tylosin이 새로운 수산용의약품으로의 활용이 기대되어 본 연구에서는 넙치에서 tylosin을 경구투여한 뒤 수온별 잔류량을 평가하였다.

◎ 실험 방법 (또는 연구방법) : 시험에 사용된 넙치는 평균 50g의 tylosin 투여 경력이 없는 개체를 13±3℃ 및 22±3℃로 나누어 사용하였다. 13±3℃에 순치된 넙치는 tylosin tartrate (Sigma, USA)를 어체중 kg당 10 및 40 mg이 되도록, 22±3℃에 순치된 넙치는 어체중 kg당 10 mg이 되도록 5일간 1일 1회 경구용 feeding needle을 사용하여 경구투여하였다. 투여가 종료된 날을 0일로 하여 3, 7, 14 및 20일 마다 13±3℃는 15마리씩, 22±3℃는 10마리씩 근육을 채취한 뒤 HPLC-MS/MS를 사용하여 잔류량을 측정하였다.

◎ 결과 및 고찰 : 어체중 kg당 tylosin을 13±3℃에서 10 및 40 mg, 22±3℃에서 10 mg을 5일간 경구투여한 뒤 3, 7, 14 및 20일에서의 잔류량은 다음과 같다. 13±3℃에서 10 mg을 투여한 결과 각각 618, 99, 218 및 190 μg/kg이 잔류하였고, 40 mg을 투여한 결과 각각 5359, 2086, 1591 및 813 μg/kg이 잔류하였다. 22±3℃에서 어체중 10 mg을 투여한 결과 각각 444, 280, 95, 39 μg/kg이 잔류하였다.

PP-14

나일틸라피아(*Oreochromis niloticus*)에서 polydeoxyribonucleotide (PDRN) 투여에 따른 상처 수복 효과

정은하 · 배준성 · 이채원 · 양찬영 · 박영신 · 박관하

전북 군산시 미룡동 산 68 번지 군산대학교 해양과학대학 수산생명의학과

◎ 연구의 배경 및 목적 : Polydeoxyribonucleotide (PDRN)은 아데노신 수용체를 통해 작용하여 혈과내피세포 성장인자(VGEF) 생성을 자극할 수 있는 화합물로서 다양한 생물에서 상처의 수복 효과에 대한 많은 연구가 보고되어 있다. 어류에선 이동, 선별 및 포획 등의 상황에서 체표에 많은 창상이 발생하게 된다. 상처를 통한 병원체의 침입으로 2차 감염이 빈번히 발생하기 때문에 상처의 수복 능력은 상품성에 큰 영향을 미칠 뿐만이 아니라 양식과정에서 2차감염의 예방목적으로도 중요하다. 따라서 본 연구는 틸라피아에 PDRN이 함유된 사료를 급이하여 인위적인 상처를 가한 뒤 수복 효과를 측정하였다.

◎ 실험 방법 (또는 연구방법) : 시험물질로 사용된 PDRN은 올인원진텍(부산, 한국)에서 특허 진행중인 김(*Porphyra* sp.)에서 추출한 것을 공급받아 사용하였다. 시험어는 약 100g 전후의 건강한 나일틸라피아(*Oreochromis niloticus*)를 사용하였으며 틸라피아용 배합사료인 피쉬탑파워 1호(우성사료주식회사, 대전)에 PDRN을 어체중 kg당 10 및 20 mg이 투여 되도록 사료에 흡착시켜 체중의 1.5%를 1일 3회 급이하였다. 시험사료를 5일간 투여한 후 인위적인 상처를 가한 뒤 4, 7 및 14일에 상처의 크기를 측정하였다. 시험사료는 인위적으로 상처를 가한 날을 제외하고 계속 투여하였다. 상처는 일회용 biopsy punch를 사용하여 근육에 각각 직경 1, 2 및 4mm의 인위적으로 가하였다. 상처 직경의 순서로 각각 개체를 구별하였다. 상처의 수복 정도는 매 측정마다 동일한 위치에서 실체현미경을 사용하여 촬영한 뒤 imageJ 프로그램을 사용하여 직경을 측정하였다.

◎ 결과 및 고찰 : 19일간 시험물질 투여에 따른 폐사율 및 증체량은 차이가 나타나지 않았다. 직경 1, 2 및 4mm의 biopsy punch를 사용하여 인위적으로 상처를 가한 뒤 4일에는 모든 그룹에서 뚜렷한 차이가 나타나지 않았으나 7일, 14일에는 10 및 20 mg/kg 모두 control과 비교하여 상처 회복 수복이 빠르게 진행되었으며 특히 20 mg/kg 투여군에서 가장 좋은 효과를 보였다.

PP-15

Preparation of oral vaccine against *Miamiensis avidus* using chitosan-based microspheres

Su Mi Shin and Sung Ju Jung

Department of Aqualife Medicine, Chonnam natural University

◎ **Background of This Study** : Scuticociliatosis is a serious disease in marine fishes worldwide, including olive flounder (*Paralichthys olivaceus*) in Korea, and is caused by the ciliate *Miamiensis avidus* (syn. *Philasterides dicentrarchi*). *M. avidus* causes serious economic losses to the Korean flounder industry every year, but there are no effective remedial measures to control the parasite. Therefore, there is a urgent need for preventive or curative measures to counter the disease problem. Previously, we developed an inactivated vaccine viz., formalin-inactivated *M. avidus* mixed with adjuvant, but, the mode of administration was intraperitoneal injection which is not feasible for small sized fingerling fish. To overcome the problem, the present study was conducted to develop preventive strategies via oral delivery methods involving chitosan microsphere encapsulated inactivated vaccine.

◎ **Methods** : The development of oral vaccine as preventive measure against scuticociliate, we encapsulated the inactivated *M. avidus* vaccine with Chitosan Microsphere (Chitosan MS - IV) followed by evaluation of protective efficacy in olive flounder. In this context, first we evaluate the efficacy of microsphere as antigen carrier using chitosan. The immunization (orally fed with MS incorporated feed for 5 days) trials was undertaken to investigate the role of IV antigen in protection against *M. avidus*, wherein efficacy evaluation of Chitosan MS with or without IV were conducted. In addition, our study also displayed that the specific (anti-*M. avidus*) antibody titer in the fish sera using the competitive enzyme-linked immunosorbent assays (c-ELISA) and Agglutination test.

◎ **Results & Discussion** : The results showed that Chitosan MS with IV was more effective (41.7% RPS) against *M. avidus* as compared to Chitosan MS without IV (16.7% RPS). In addition, our study also displayed that the specific (anti-*M. avidus*) antibody titer in the fish sera and mucus of the immunized groups were significantly ($p<0.05$) enhanced following vaccination compared to control group. Thus, it can be inferred that scuticociliate antigen encapsulation can be an effective immunization strategy that can protects the scuticociliate antigen from digestive degradation, facilitates a more targeted vaccine delivery to the host immune organs as well as help in orchestrating protective immune induction in the host.

◎ **Reference** : Kole, S., Qadiri, S. S. N., Shin, S.-M., Kim, W.-S., Lee, J., & Jung, S.-J.: Nanoencapsulation of inactivated-viral vaccine using chitosan nanoparticles: evaluation of its protective efficacy and immune modulatory effects in olive flounder (*Paralichthys olivaceus*) against viral haemorrhagic septicaemia virus (VHSV) infection. *Fish & shellfish immunology, 91*:136-147, 2019.

PP-16

Evaluation of antibacterial effects of graphene oxide on *Aeromonas salmonicida*

Ji Hyun Lee, Jeong Su Park, Hye Ji Kim, Jong Bin Park, Hyun Woo Kim and Se Ryun Kwon

Department of Aquatic Life Medical Sciences, Sunmoon University, Asan, Korea

◎ **Background (or Objective) of This Study** : As the importance of the domestic aquaculture industry increases, continuous development and expansion were being made, and as a result, the production of effluent including pathogenic microorganisms and antibiotic-resistant bacteria is increasing, causing damage to the aquaculture. On the other hand, water purification facilities and methods require the development of cost-efficient technologies because of the high initial cost of establishment and maintaining operations. Graphene oxide is a carbon-based nanomaterial that is easy to manufacture and low cost, and the carbon radicals of graphene oxide promote reduction on the surface of the cell membrane and induce dialysis, causing antibacterial effects. Products that apply these antibacterial effects are already being developed in domestic, but not much research has been done on the antibacterial effects of fish pathogenic diseases contained in effluent. Therefore, this study analyzes the antibacterial effects of graphene oxide on fish pathogenic bacteria *Aeromonas salmonicida* to evaluate the suitability of water treatment system techniques and related products.

◎ **Methods** : To evaluate the ability to inhibit the proliferation of fish pathogenic bacteria by graphene oxide (grapheneol Co., Ltd.), *Aeromonas salmonicida* (1×10^8 CFU/㎖) was inoculated into a TSB medium mixed with 1g of graphene oxide and incubated for 10 minutes, 30 minutes, 1 hour and 6 hours at 27℃. Each culture was diluted 10 times to 10^{-1}~$10^{-6}$with phosphate buffer saline (PBS) and then 100㎕ was added to the TSA medium and incubated for 24 hours at 27℃. In the case of the control group, we proceed in the same method using a TSB medium without graphene oxide. Then, to measure the antibacterial effect of graphene oxide for each period, the colony was counted and calculated as CFU/㎖.

◎ **Results & Discussion** : As a result, the antibacterial effect of graphene oxide on *A. salmonicida* was not significantly different from the control group when incubated for 10, 30 minutes, and 1 hour, while the control group incubated for 6 hours was 2.8×10^7 CFU/㎖, while the group with graphene oxide was 2×10^6 CFU/㎖. These results showed 99% of a high antibacterial effect on fish pathogenic bacteria *A. salmonicida* of graphene oxide, indicating that it may be suitable for water treatment systems.

PP-17

Molecular characteristics, spatial and temporal mRNA expression analysis of the peroxiredoxin-5 from yellowtail clownfish (*Amphiprion clarkii*)

Chaehyeon Lim[*], Jeongeun Kim[*] and Jehee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Peroxiredoxins are a conserved family of thiol-specific antioxidant enzymes that play important role in protecting cellular components from oxidative stress, H2O2 signaling and regulation of inflammatory response. In this study, peroxiredoxin-5 from yellowtail clownfish (AcPrx-5) was characterized by in sillico study and transcriptional analysis.

◎ **Methods** : After challenge experiment, transcriptome library of Amphiprion clarkii(*A. clarkii*) cDNA sequence was constructed. Using cDNA sequence of AcPrx-5, in-silico, tissue distribution and temporal mRNA expression analysis were performed to identify and characterize their role in blood and spleen.

◎ **Results & Discussion** : The full sequence of AcPrx-5 was identified from yellowtail clownfish cDNA database. The identified full sequence of AcPrx-5 was 880 bp including 62 bp of 5’ UTR, 245 bp of 3’ UTR and 573 bp of open reading frame (ORF) encoding a protein of 190 amino acids. Multiple sequence alignment and phylogenetic analysis were performed to determine the conserved protein domains and evolutionary position between other orthologs and suggested that AcPrx-5 has highly conserved domain and closely related with Amphiprion ocellaris. AcPrx-5 was ubiquitously expressed in all tested tissues with the highest expression in the heart. After immune challenge with LPS, polyI:C and Vibrio harveyi, AcPrx-5 expression was upregulated in blood and spleen. Above all, this study provides potential involvement of AcPrx-5 in innate immune responses upon pathogenic stress in yellowtail clownfish (*Amphiprion clarkii*).

PP-18

Molecular characterization and expression profiling of gludaredoxin 1 from *Amphiprion clarkii*

Cheong Uk Park[*,**], W.K.M. Omeka[*], Kishanthini Nadarajapillai[*,**], Hyuk jae Kwon[*,**], Qiang Wan[*,**] and Je hee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Glutaredoxins (GRXs) are antioxidant enzymes known for reducing oxidative stress in all living organisms. GRXs enzymes are affiliated to the thioredoxin superfamily and have identical thioredoxin and catalytic motifs. GRXs participate in many cellular processes, such as anti-oxidation, anti-apoptosis, regulation of cell differentiation, protein folding and regulation of the activity of transcription factors by catalyzing the redox reaction between glutathione and protein disulfide.

◎ **Methods** : In this study, we molecular characterized GRX1 gene from *Amphiprion clarkii* (AcGRX1). Furthermore, the tissue-specific mRNA expression of the AcGRX1 was studied using qPCR. Healthy clownfish were challenged with LPS, poly I:C and Vibrio harveyi to study the effect of the immune stimulants on the mRNA expression of AcGRX1 in the head kidney, and spleen.

◎ **Results & Discussion** : The gene sequence was identified from constructed transcriptomic cDNA library of A.clarkii. The full length of the AcGRX1 was 892bp, containing an open reading frame (ORF) of 321bp encoding 106 amino acids. The molecular weight and the theoretical isoelectric point (pi) of GRX1 are predicted as 11.59 kDa and 6.82, respectively. The highest sequence identity (99.91%) and similarity (99.91%) were shown with Acanthochromis polyacanthus. According to the results of tissue specific mRNA expression qPCR, AcGRX1 has ubiquitous expression in all the tested tissues. Moreover, the brain tissues have shown the highest GRX mRNA expression. The brain is associated with enormous oxidative stress; therefore, the antioxidant genes expression would be more significant. The immune challenge results revealed that the relative expression of AcGRX1 in the head kidney and spleen has similar significant upregulation at early time points. Immune stimulants activate major immune pathways and ultimately, the cells undergo inflammation or apoptosis. In order to counterbalance the excessive stress condition in cells, preventive anti-oxidatant enzymes such as GRX1 must be activated. Therefore, the elevated mRNA expression of GRX1 might observe during the immune stimulation. These results suggest that AcGRX1 might play a role in reduction of oxidative stress in fish during pathogenic invasion.

PP-19

Molecular characterization and immune responses of thioredoxin domain-containing 5 from *Haliotis discus discus*.

DS Liyanage[*], WKM Omeka[*], Tae hyug Jeong[*,**] and Je hee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background of This Study** : Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen and readily react with macromolecules such as DNA and RNA. ROS comprise free radicals like $O_2\bullet^-$, •OH, and non-radicals such as H_2O_2 and O_2. ROS are involved in several signaling pathways related to cell proliferation, metabolism, cell survival, antioxidants, anti-inflammatory reactions, ion homeostasis, and DNA damage response.

◎ **Methods** : In this study, Thioredoxin domain-containing 5 (TXNDC5) cDNA sequence was examined from disk abalone transcriptomic database and characterized to understand the role of disk abalone TXNDC5 better. The cDNA and protein sequence of TXNDC5 characterized. The *in-silico*, spatial, and temporal expression patterns in hemocytes and gills response to bacteria (*Vibrio parahaemolyticus*, *Listeria monocytogenes*), viral hemorrhagic septicemia virus (VHSV), and PAMPs (Poly I:C and lipopolysaccharides) were observed. Further abalone *TXNDC5* expression was examined in different developmental stages.

◎ **Results & Discussion** : Thioredoxin is a highly-conserved protein, can be found in bacteria to higher-level eukaryotes. Other than the normal metabolic processes, in response to the different oxidative stresses like radiation, metal ions, and pathological conditions, reactive oxygen species (ROS) are produced. Higher ROS levels lead to damage to the cell components, and thioredoxin acts as an active regulatory enzyme in excessive ROS levels. Evolutionary identification showed TXNDC5 protein belongs to the thioredoxin superfamily. TXNDC5 containing 1179 bp ORF and produce 392 amino acids. Identity and similarity analysis proved that the sequence matched to *Conus magus* species with 53.37 percent sequence identity. Conserved thiol-disulfide Cysteine residue containing two Cys-X-X-Cys motifs can be found in abalone TXNDC5. Quantitative real-time PCR indicated that hemocyte and gill tissues with higher TXNDC5 mRNA expression levels protect the host from invading pathogens. Immune-challenged abalone hemocytes and gills were observed at different time points by qPCR, and results showed upregulated expression of TXNDC5. As similar to spatial expression results, the immune challenge also showed due to protects the host from invading pathogens. Therefore, the abalone TXNDC5 gene can be used as an immunologically important gene.

PP-20

Molecular characterization and immunological functional analysis of red-spotted groper (*Epinephelus akaara*) interleukin-10

A.M Erandi[*,**], J.R.P Jayasingha[*,**], Sarithaa Sellaththurai[*,**] and Je hee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : In this study, Interleukin-10 (IL-10) gene was cloned and characterized from Red-spotted grouper (*Epinephelus akaara*), which is a highly demanded candidate fish species for aquaculture in the Asia Pacific region. IL-10 is an anti-inflammatory cytokine that plays important role in infection by the down-regulating immune response to pathogens hence prevents chronic inflammation and tissue damage in the host. The open reading frame of EaIL-10 is 564 bp in length encodes a putative 187 amino acids protein with N terminal signal peptide of 22 amino acids. The calculated molecular weight of EaIL-10 was 21.71 kD with an isoelectric point of 5.83. The sight of this study was the characterization and identification of the potential immunological role of Red-spotted groper Interleukin-10.

◎ **Methods** : Unchallenged healthy grouper were used to synthesis cDNA after the extraction of RNA from the different tissues to determine the relative transcription levels of EaIL10. Immune challenge experiment was performed with polyinosinic: polycytidylic acid (poly I:C), lipopolysaccharide (LPS), nervous necrosis virus (NNV) as an immune stimulant and respectively synthesized cDNA was used to perform the qPCR analysis.

◎ **Results & Discussion** : The biological functional structure of IL-10 is a homodimer. The 3D structure of EaIL-10 consists of six α-helices. The structurally important four conserved Cys residues are responsible for the two disulfide bridges within the chain. The phylogenetic analysis clusters EaIL-10 with fish-specific homologs. Quantitative real-time PCR of tissue distribution was observed that EaIL-10 were ubiquitously expressed in all examined tissues, the highest expression was in the spleen followed by the intestine. The involvement of IL-10 in Grouper's immune responses was demonstrated by investigating the mRNA expression profiles of IL-10 in the spleen following intraperitoneal injection of Poly I:C, LPS and NNV. According to post-injection mRNA expression of EaIL-10 in the spleen, significantly higher expression was observed until 12 hours with tested stimuli. This result beneficent to understand the involvement of IL-10 in the resolution of inflammation in fish.

◎ **Reference** : L. Grayfer, J.W. Hodgkinson, S.J. Hitchen, M. Belosevic: Characterization and functional analysis of goldfish (*Carassius auratus L.*) interleukin-10, Mol. Immunol, 48:563–571, 2011.

PP-21

Functional characterization and expression analysis of antibacterial genes, C-type and G-type (like) lysozymes in response to immune challenge in clownfish (*Amphiprion clarkii*).

Ga eun Kim[*], Han chang Sohn[*,**], W. M. Gayashani Sandamalika[*], W.K.M. Omeka[*] and Je hee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : The innate immunity is the most important defense system in fish species dealing with diversity spectrum of pathogens. Lysozymes are enzyme factors of the innate immune, which exists in organisms like invertebrates, vertebrates, plants, bacteria, and phages. They protect from bacterial infections and kills the bacteria. Therefore, this immune study and analysis of AcLysC and AcLysG-like can be used as fundamental research materials in further studies.

◎ **Methods** : In this study, for the better understanding of lysozyme functions in *Amphiprion clarkii*, two lysozymes; c-type lysozyme (AcLysC) and g like-type lysozyme (AcLysG-like) were characterized and identified with molecular, transcriptional and functional techniques. To characterization of AcLysC and AcLysG-like, they were cloned and purified recombinant proteins. For protein assays, we conducted antibacterial, lytic (pH, temperature), SEM information.

◎ **Results & Discussion** : The cDNA sequence of AcLysC and AcLysG-like were 595 bp and 1370 bp consisting of 429 bp and 570 bp of open reading frame respectively. AcLysC had a signal peptide (Met1-Ala17). However, AcLysG-like possesses no signal peptide sequence. The in-silico analysis, such as multiple sequence alignment and phylogenetic tree of AcLysC and AcLysG-like genes were analyzed to confirm the orthologous relationship with other species. The tissue distribution of AcLysC and AcLysG-like was determined by the elongation factor 1- beta gene in all 12 sampled tissues (liver, head kidney, skin, brain, heart, spleen, gill, kidney, intestine, blood, muscle, stomach). It was used as a control and detected by real-time PCR (qPCR). In addition, immune-stimulating agents LPS, Poly I:C, and Vibrio harveyi were used to analyze the amount of mRNA expression per immune response time. The AcLysC and AcLysG-like, cloning in to the pMAL-c5x vector and recombinant proteins were purified with amylose affinity chromatography method. In protein assay, After that, the optimized pH and temperature conditions were checked to conduct an activity experiment of the recombinant protein. Antibacterial activity was confirmed using various fish pathogens, and cell wall destruction of Micrococcus luteus by the recombinant protein was observed through scanning electron microscopy (SEM).

◎ Reference : J. Nilojan, S.D.N.K. Bathige, R. Kugapreethan, W.S. Thulasitha, B.H. Nam, J. Lee: Molecular: transcriptional and functional insights into duplicated goose-type lysozymes from *Sebastes schlegelii* and their potential immunological role. Fish Shellfish Immunol, 67:66-77, 2017.

N. Umasuthan, S.D.N.K. Bathige, S.R. Kasthuri, Q. Wan, I. Whang, J. Lee: Two duplicated chicken-type lysozyme genes in disc abalone *Haliotis discus discus*: Molecular aspects in relevance to structure, genomic organization, mRNA expression and bacteriolytic function. Fish Shellfish Immunol, 35:284-299, 2013.

J. Ko, Q. Wan, S.D.N.K. Bathige, J. Lee: Molecular characterization, transcriptional profiling, and antibacterial potential of G-type lysozyme from seahorse (*Hippocampus abdominalis*). Fish Shellfish Immunol, 58:622-630, 2016.

L. Callewaert, C.W. Michiels: Lysozymes in the animal kingdom, J. Biosci, 35:127-160, 2010.

PP-22

Molecular characterization and expression analysis of mitochondrial antiviral signaling protein (MAVS) from red spotted grouper, *Epinephelus akaara*.

W.M. Gayashani Sandamalika[*] and Je hee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Mitochondrial antiviral signaling protein (MAVS), involves in the RIG-I and MDA5 downstream signaling pathways and act as an essential antiviral signaling protein in the innate immune system leading to the production of type I interferons and inflammatory cytokines. In this study, our objective is to identify MAVS from red-spotted grouper and characterize it at the molecular and transcriptional level to elucidate its immune responses.

◎ **Methods** : *Epinephelus akaara* cDNA library was constructed and MAVS homolog was identified and referred to as (EaMAVS). The molecular features of EaMAVS were analyzed using several bioinformatics tools. Healthy grouper fish with an average body weight of 70?g were used to collect 12 types of tissues including the blood, head kidney, spleen, liver, muscle, gills, intestine, kidneys, brain, skin, heart, and stomach, and used for the tissue distribution analysis. Another set of fish was subjected to an immune challenge experiment using Lipopolysaccharide (LPS), poly I:C, and Nervous necrosis virus (NNV). After the challenge, the blood and spleen were collected in a time-course manner and used for the temporal expression analysis of EaMAVS. Spatial and temporal expression analysis was carried out using real-time quantitative PCR.

◎ **Results & Discussion** : EaMAVS consists of 574 amino acids with 52.76 kDa of predicted molecular mass. The theoretical pI calculated as 4.6. According to sequence alignment, EaMAVS contains a conserved caspase activation and recruitment domain (CARD) at the N-terminus and a proline-rich region, and a C-terminal membrane targeting the transmembrane domain. In the constructed phylogenetic tree EaMAVS closely clustered together with *Epinephelus coioides*. In the analysis of tissue-specific expression, the highest expression of EaMAVS was observed in the blood. Moreover, we found that the transcription of EaMAVS in blood and spleen was significantly upregulated when the fish were stimulated with LPS, poly I:C, and NNV, suggesting its involvement as antiviral signal protein in the grouper immune system.

◎ **Reference**: C. Vazquez and S. M. Horner: MAVS Coordination of Antiviral Innate Immunity. J. Virol., 89(14):6974-6977, 2015.

R. Krishnan, J. K, Z. Mushtaq, K. U. Shyam, and P. P. Kurcheti: Antiviral activity of transiently expressed mitochondrial antiviral signaling adapter, MAVS orthologue from Asian seabass, Fish Shellfish Immunol., 76:183-186, 2018.

R. Krishnan, P. Girish Babu, K. Jeena, G. Tripathi, and K. Pani Prasad: Molecular characterization, ontogeny and expression profiling of mitochondrial antiviral signaling adapter, MAVS from Asian seabass Lates calcarifer, Bloch (1790). Dev. Comp. Immunol, 79:175–185, 2018.

PP-23

Transcriptional modulation of the stimulator of interferon genes (STING) of *Amphiprion clarkii* under pathogenic invasion

Jayamini Harasgama[*,**], Thilina Kasthuriarachchi[*,**], Tae hyug Jeong[*,**], Qiang Wan[*,**] and Je hee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Stimulator of interferon genes (STING) is an endoplasmic reticulum (ER) associated signaling molecule, essential for controlling the transcription of a number of host defense genes including type I interferons and pro-inflammatory cytokines upon pathogenic invasions. Once activated by aberrant DNA species or cyclic dinucleotide, STING stimulates the transcription of imperative innate immune genes to mount a particular immune response against the invading pathogens. Therefore, STING and STING associated signaling is considered as an important immune parameters in eliminating pathogenic invasions. Considering the significantly important features as an immunomodulator, the current study has focused on structurally and functionally characterize the *A.clarkii* STING protein using bioinformatics and gene transcription experiments.

◎ **Methods** : In the current study, the sequence of *A.clarkii* STING was identified from the cDNA library (AcSTING) and in-silico analysis of the protein was carried out using appropriate bioinformatics tools and software. cDNA samples synthesized from twelve different tissues were subjected to quantitative real-time PCR in determining the tissue-specific expression of the gene. The immune challenge experiment was carried out by immunizing grouped health redlip mullet with (LPS), poly I: C, *Vibrio harveyi* and PBS (control). In different time points, blood and spleen tissues were collected, mRNA was extracted and cDNA was synthesized. The expression of TGF-β1 was then analyzed by subjecting the cDNA into qPCR.

◎ **Results & Discussion** : Complete ORF of the AcSTING consisted of 1170 base pairs encoding 389 amino acids. Conservation of the imperative amino-acids within the fish species could be seen by the multiple sequence alignment of STINGs. Phylogenetic analysis demonstrated the highest evolutionary relationship of AcSTING with other fish counterparts. Tissue-specific expression analysis demonstrates the highest expression of AcSTING in the stomach followed by the heart tissues of the fish. The challenge experiment demonstrates that AcSTING transcription has significantly upregulated in blood and spleen tissues at 6 hours of exposure to pathogens and further significant upregulation was observed in V. harveyi challenged fish. Results of the current experiment suggest that the transcriptional modulation of AcSTING plays an important role in the pathogenic attack in the fish.

However, more detailed assays will further validate the involvement of this imperative immune gene in fish under pathogenic invasion.

PP-24

Characterization of a SOCS 3 and 3-like from red-lip mullet (*Liza haematocheila*): demonstration of mRNA upregulation in response to immunostimulants

Jeong eun Kim[*], Chae hyeon Lim[*] and Je hee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Cytokines are important inflammatory biomarker. They activate the Janus kinases-signal transducers and activators of transcription (JAK/STAT) signaling pathway to express inflammation related gene. However, excessive cytokine signaling lead chronic inflammation, or tumorigenesis. Suppressors of cytokine signaling (SOCS) are involved dysregulatory function of cytokines and expressed through JAK/STAT pathway. Among the member of SOCS, SOCS3 interacts directly with cytokine receptor and JAKs to regulate cytokine signaling. In this study, we aimed to characterize SOCS3 from red-lip mullet (LhSOCS3).

◎ **Methods** : In order to analyze the in-silico, we used bioinformatic tools such as NCBI conserved domains, EMBOSS needle, Clustal Omega and Molecular Evolutionary Genetics Analysis (MEGA 7.0) software to identify the conserved domains, pairwise alignments, multiple alignments and phylogenetic tree, respectively. To investigate temporal transcriptome levels, mullets were challenged with live pathogen (*Lactococcus garvieae*; *L. garvieae*) and mimic of dsRNA virus (polyinosinic:polycytidylic acid; poly I:C). Relative expression of *LhSOCS3* and *LhSOCS3-like* was determined using the Livak method.

◎ **Results & Discussion** : In redlip mullet, SOCS3 gene is duplicated: *LhSOCS3* (SOCS3 from redlip mullet) and *LhSOCS3-like*. Two copies of LhSOCS3 have shown a difference between coding sequence. However, these genes have conserved LhSOCS3 and LhSOCS3-like specific domain and shared highest similarity from *Amphiprion ocellaris*. According to the phylogenetics tree, LhSOCS3 and LhSOCS3-like split over two branches of Fish and Mammalia due to PEST region. We observed mRNA expression patterns upon immune stimuli. Immune challenge by *Lactococcus garvieae* in the gill and liver resulted a later-systemic response, then by poly I:C resulted a response at early stage. Collectively, these data suggested that *LhSOCS3* and *LhSOCS3-like* are the key physiological regulator and plays an important pathological role in immune homeostasis.

PP-25

Genomic characterization of caveolin-1 from yellowtail clownfish (*Amphiprion clarkii)* and its expression profiling in response to immune stimulants

Kishanthini Nadarajapillai[*,**], Cheong Uk Park[*,**], Subothini Ganeshalingam[*] and Je hee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Caveolin-1 (Cav-1) is a flask shape of invagination present in plasma membranes and serves as a major structural component of caveolae. Cav-1 is involved in various biological functions for instance; endocytosis, cholesterol trafficking, transcytosis, signal transduction, and immunity. To date, three caveolin members have been identified in mammals, Cav-1, Cav-2, and Cav-3. In this study, we examined the molecular features and the expression analysis of the Cav-1 from *Amphiprion clarkii*.

◎ **Methods** : The cDNA sequence of AcCav-1 was identified from the *A. clarkii* cDNA database. In-silico analysis was carried out by using different bioinformatic tools. The spatial distribution of AcCav-1 was analyzed by collecting the different healthy *A.clarkii* tissues. Temporal expression was evaluated by immune challenged with poly I:C, LPS, and *Vibrio harveyi*. After post-injection, spleen tissue were collected at different time points and qPCR was carried out.

◎ **Results & Discussion** : The cDNA of Cav-1 from *Amphiprion clarkii* (AcCav-1) was 2606 bp and is comprised of 546 bp of ORF. The protein is deduced with 181 amino acids and a molecular weight of 20.73 kDa. It had a predicted isoelectric point of 5.48. The phylogenetic tree revealed that AcCav-1 is closely related to teleost fish orthologs and clustered together with vertebrates. It shares the highest identity (99.4 %) and similarity (100 %) with *Amphiprion ocellaris*. AcCav-1 was highly expressed in the skin followed by the spleen. Significant up and down regulations were observed in spleen tissues for all stimulants throughout the experiment. This result suggests Cav-1 might have a role in the immune system.

◎ **Reference:** R.G.W. Anderson: The caveolae membrane system. Annu. Rev. Biochem, 67:199–225, 1988.

C.J.G. de Almeida: Caveolin-1 and Caveolin-2 can be antagonistic partners in inflammation and beyond. Front. Immunol, 8:1–15, 2017.

PP-26

Insilico analysis and transcriptional profiling of IRF3 from yellow tail clownfish

W.K.M Omeka[*], D.S Liyanage[*] and Je hee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Interferons (IFN) are multifunctional cytokines that act against viral and bacterial infections. Interferon regulatory factors (IRF) are transcriptional factors that induce the IFN expression. Among 11 IRFs, IRF3 considered as II interferons inducing protein upon viral infections. *Amphiprion clarkii* is one of popular tropical ornamental fish that has higher demand worldwide. In this study, we characterized the IRF3 gene from *A.clarkii* (AcIRF3) and explored its role in host immunity.

◎ **Methods** : Several bioinformatic tools were used to identify the signal peptide, N-linked glycosylation sites, domain organization of AcIRF3 sequence. Pairwise and multiple sequence alignments were obtained by Clustal Omega and Emboss needle tools. Phylogenetic tree was reconstructed by MEGA7 using NJ method with 5000 bootstraps. Temporal expression analysis of AcIRF3 in head kidney was carried out by using lipopolysaccharides (LPS), polyinosinic:polycytidylic acid (poly I:C) and *Vibrio harveyi*. The qPCR expression was analyzed by Livak method.

◎ **Results & Discussion** : The nucleotide sequence of AcIRF3 was 1395 bp long and deduced protein consisted with 382 amino acids. Sequence contained IRF superfamily domain and IRF3 domain. According to the pairwise alignment, AcIRF3 shared highest identity (98.27%) and similarity (99%) with *Amphiprion ocellaris*. As depicted in multiple sequence alignment, AcIRF3 share conserved characteristics with fish species. As shown in phylogenetic tree, IRF3 clade to fish IRF3 group and closely clustered with IRF3 from *A.ocellaris*. The AcIRF3 transcripts were ubiquitously expressed in all the tested tissues. According to the tissue specific expression profile, AcIRF3 was predominantly expressed in blood followed by brain. In the head kidney, AcIRF3 expression was highly elevated for poly I: C, LPS and *V.harveyi*. Altogether, results in this study suggests that AcIRF3 may actively involve in host immune responses.

PP-27

Identification, expression profiling and functional characterization of B-cell lymphoma-2 (Bcl-2) from yellowtail clown fish (*Amphiprion clarkii*)

K.P Madushani, K.A.S.N Shanaka and Je hee Lee

Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea
Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : *Amphiprion clarkii* which belongs to family Pomacentridae is a highly popular ornamental fish in Korea. However, recently it has been greatly hindered by frequent pond stresses like destructive diseases. Bcl-2, a key regulator of an apoptosis which is involved in immune responses has not well been investigated in anemonefish including *A. clarkii*. Hence present study *A. clarkii* Bcl-2 (AcBcl-2) was molecularly and functionally characterized at the sequence and transcriptional level.

◎ **Methods** : Bcl-2 was identified in yellowtail clownfish transcriptomic database. In silico analysis was performed to characterize the sequence structures domains may responsible for the suspected antiviral defense. Expression pattern was analyzed against different pathogenic stimuli by qPCR. Viral transcription was detected against VHSV infection in AcBcl-2 transfected MKD cells. Cell viability assay was performed to verify the results of the viral stimulations.

◎ **Results & Discussion** : In silico study of *A. clarkii* Bcl-2 (AcBcl-2) showed that this protein contains 208 amino acids with 23 kDa molecular weight and p.I of 5.04. Unchallenged tissue expression analysis with qPCR was shown a significant increase in the expression of AcBcl-2 in the brain. In the immune challenge results, the expressions of the AcBcl-2 were significantly upregulated after exposure to Poly I:C, LPS and *Vibrio harveyi*. Late expressional upregulation of AcBcl-2 was observed in both blood and spleen of V. harveyi challenged samples. LPS and the *V. harveyi* had a similar expression pattern. Obtained expression patterns of AcBcl-2 indicate its involvement in the stress response and apoptosis regulation. The pattern of the expression of AcBcl-2 after the immune challenge varied in the case of extent and time-span of up-regulation, indicating there may be distinct apoptotic signalling in response to different forms of cell injury caused by these stimuli. Furthermore the significant reduction of antiviral system related gene expression and the enhanced cell viability under VHSV infection proved that the antiapoptotic nature of the Bcl2Collectively these research findings disclose the importance of Bcl-2 in the innate immunity of clark's anemonefish.

◎ **Reference** : Feng, C.Y., Rise, M.L.: Characterization and expression analyses of anti-apoptotic Bcl-2-like genes NR-13, Mcl-1, Bcl-X1, and Bcl-X2 in Atlantic cod (Gadus morhua). Mol. Immunol, 47:763-784, 2010.

PP-28

Genomic characterization, tissue distribution and immune response of clusterin from *Hippocampus abdominalis* (big-bellied seahorse)

Sarath Wijerathna[*,**], Kishanthini Nadarajapillai[*,**], H.M.V.Udayantha[*], Suk kyoung Lee[*,**] and Je hee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Clusterin (CLU) is a glycoprotein consists of two chains (α- and β-clusterin) which is involved in several physiological and pathological states of cells. CLU has multifunctional activities such as extracellular chaperone function, immune modulation, and lipid transportation. Besides, CLU plays its role in various cell signaling pathways related to several diseases such as oxidative stress, proteotoxic stress, cell death and survival.

◎ **Methods** : The present study was carried out to characterize the homology of CLU found in *Hippocampus abdominalis* (designated as HaCLU). Immune response was evaluated by challenging with lipopolysaccharide (LPS) (Gram negative bacterial ligand), Polyinosinic:polycytidylic acid (Poly I:C) (Mimic of viral double stranded RNA), *Edwardsiella tarda* (Gram negative bacteria), and *Streptococcus iniae* (Gram positive bacteria). PBS was used as the control.

◎ **Results & Discussion** : The present study was carried out to characterize the homology of CLU found in *Hippocampus abdominalis* (designated as HaCLU). Immune response was evaluated by challenging with lipopolysaccharide (LPS) (Gram negative bacterial ligand), Polyinosinic:polycytidylic acid (Poly I:C) (Mimic of viral double stranded RNA), *Edwardsiella tarda* (Gram negative bacteria), and *Streptococcus iniae* (Gram positive bacteria). PBS was used as the control.

HaCLU contains an open reading frame (ORF) of 1389bp and its encoded 462 amino acids with a molecular weight of 51.28 kDa and the estimated isoelectric point of 5.41. In silico analysis shows the signal peptide of HaCLU exist in the region of first 29aa, while, ∞- and β-clusterin domains were in 34-227aa and 228-455aa regions respectively. Pairwise sequence analysis results illustrated that HaCLU has 97.2% of identity and 98.9% of similarity with the amino acid sequence of CLU of *Hippocampus comes*. qPCR experiment which was done to investigate the tissue distribution pattern of HaCLU revealed that the highest expression was in the Liver tissue, followed by heart, spleen, and brain tissues. Furthermore, immune response was investigated by analyzing the mRNA expression of HaCLU in the liver tissue of *H. abdominalis* upon stimulation with LPS, Poly (I:C), *Edwardsiella tarda*, and *Streptococcus iniae* intraperitoneally. The qPCR results unveiled that HaCLU is upregulated in the liver tissue after the immunization with all the stimulants.

Taken together, this study exposed HaCLU may involve in immune regulation against pathogenic infection in *H. abdominalis*.

◎ **Reference** : CLU; Clusterin precursor; Homo sapiens (Human); CLU gene & Protein, (n.d.), 2021.

S.R. Matukumalli, R. Tangirala, C.M. Rao, Clusterin: Full-length protein and one of its chains show opposing effects on cellular lipid accumulation. Sci Rep., 7:41235, 2017.

PP-29

Genomic characterization and expression regulation in response to immune stimulants of B-cell lymphoma-2 isoform 1 (Bcl-2) from *Amphiprion clarkii*

Sarithaa Sellaththurai, Su mi Jung and Je hee Lee

Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea
Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Apoptosis have an essential role in immune response and many physiological process. BCL-2 family proteins generally localized to the mitochondrial outer membrane and regulate cell death (apoptosis). Intrinsic apoptosis pathway is required for preventing cancer and normal embryonic development. Moreover, BCL-2 proteins play critical roles in normal cell physiology. The different members of the Bcl-2 family share one or more Bcl-2 homology domains, required for their ability to regulate each other. The current work identified and characterized Bcl-2 isoform 1 from *Amphiprion clarkii* (AcBcl-2) and analyzed the immune response upon immune challenge experiment.

◎ **Methods** : In silico analysis was performed using various bioinformatics tools. An equal amount of tissue samples collected from five healthy *A. clarkii* for tissue distribution analysis and five challenged fishes at each time point were used for challenge experiment analysis. Expression analysis were carried out by real-time PCR.

◎ **Results & Discussion** : The cDNA sequence of AcBcl-2 exist the ORF of 690 bp, which encoding 229 amino acids. The molecular weight and pI were predicted as 25.72 kDa and 5.79, and signal peptide was not observed. Bcl-2 super family was revealed from protein sequence of AcBcl-2. AcBcl-2 showed 100 %, 97.8 %, 89.2 %, and 88.8 %, identity with that of *Amphiprion ocellaris*, *Stegastes partitus*, *Oreochromis niloticus*, and *Maylandia zebra* Bcl-2 protein sequences, respectively. Phylogenetic analysis showed the vertebrate ancestral origin of AcBcl-2 and was subclustered with Bcl-2 of *Amphiprion ocellaris* and *Acanthochromis polyacanthus*. The mRNA transcript of AcBcl-2 was detected in all tested tissues and the highest expression was observed in muscle. The expression modulations of AcBcl-2 mRNA in gill tissue was observed with all three stimulants (*Vibrio harveyi*, polyinosinic: polycytidylic, and lipopolysaccharide) at the time post immune challenge (6 h, 12 h, 24 h, 48 h, and 72 h). The above results demonstrates the potential role of AcBcl-2 in innate immunity of *A.clarkii*.

◎ **Reference** : K.L. Banta, X. Wang, P. Das, A. Winoto: B cell lymphoma 2 (Bcl-2) residues essential for Bcl-2's apoptosis-inducing interaction with Nur77/Nor-1 orphan steroid receptors. J. Biol. Chem., 293:4724-4734, 2018.

PP-30

Expression and bioinformatics analysis of two isoforms of toll-like receptor-5 (TLR5) in rockfish (*Sebastes schlegelii*)

K.A.S.N Shanaka, K.P Madushani and Je hee Lee

Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea
Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Although mammals possess only the membrane-bound forms of TLR, soluble forms are reported in fishes. Correspondingly, TLR5, which detects bacterial flagellin, present either as a membrane or a cytosolic form in fish. In this study, economically important Rockfish soluble TLR5 (SssTLR5) and membrane-bound (SsmTLR5) forms were identified and characterized. Since TLR5 holds paramount importance in innate immunity, the findings of this study hold paramount importance in fish vaccine development.

◎ **Methods** : Nucleotide sequences of SssTLR5 and SsmTLR5 were identified from a Rockfish transcriptomic database. Bioinformatic analysis was conducted to identify the specific domains and sequences of both proteins. The expression pattern was analyzed with RTq-PCR.

◎ **Results & Discussion** : SssTLR5 and SsmTLR5 are proteins with 637 and 891 amino acids, separately. Whereas molecular mass and the pI of SssTLR5 are 71 KDa and 7.0 and for SsmTLR5 are 102 KDa and 5.4, respectively. The characteristic toll/interleukin-1 receptor (TIR) domain was only found in the SsmTLR5. Unchallenged tissue expression analysis found both isoforms strongly expressing in gills. Immune challenge experiment with distinct immune stimulants including, poly I:C and LPS unveiled significant downregulation of each isoform. However, an immune challenge with Gram-positive *Streptococcus iniae* revealed a significant upregulation of both proteins. Results revealed the ability of the two TLR5 isoforms of rockfish is responsible for Gram-positive bacteria detection. Since SssTLR5 lacks the TIR domain, signal propagation and the subsequent activation of the innate immune pathways are still enigmatic. Therefore, more studies are required to further characterize soluble form. In a conclusion finding of this research discloses the importance of TLR5 in innate immunity.

PP-31

Molecular characterization and transcriptional expression analysis of peroxiredoxin 1 from *Epinephelus akaara*

Subothini Ganeshalingam[*], W.M.Gayashani Sandamalika[*], Chae hyeon Lim[*], Sarithaa Sellaththurai[*,**] Kishanthini Nadarajapillai[*,**] and Je hee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Peroxiredoxins (Prxs) are non-seleno peroxidases that present in all living organisms and primarily involved in the cellular redox homeostasis during aerobic respiration. Besides their roles in oxidative stress regulation, cellular receptor signaling, inflammatory modulation, tissue repairing and protection against cell death are quite distinct. The most studied roles of Prx1 are immunological. In the present study, we investigated the molecular and transcriptional roles of Red-spotted grouper Prx1 (EaPrx1).

◎ **Methods** :The in-silico analysis of EaPrx1 was done using several online bioinformatic tools. The healthy, red-spotted grouper fish were purchased and reared under suitable conditions. The immune challenge experiment was conducted for poly I:C, LPS and Nervous Necrosis Virus (NNV) and the qPCR was done for the spatial and temporal expression analysis.

◎ **Results & Discussion** : The in-silico analysis revealed that the ORF region of EaPrx1 possesses 597 base pairs, encoding 198 amino acids. The predicted molecular weight, theoretical isoelectric point and instability index were 22.08 kDa, 6.42 and 19.12 respectively. EaPrx1 predominantly localized in the cytosol that lacks signal peptide. The polypeptide chain consists of a single thioredoxin-like superfamily domain and two conserved C- terminal and N-terminal catalytic cysteine residues. The multiple sequence alignment revealed the conserved VCP signature motifs that are responsible for peroxidative active site formation. Pairwise sequence alignment revealed that the EaPrdx1 had the highest identity similarity with *Epinephelus lanceolatus* (as 99.5%, 99% respectively), followed by *Gymnodraco acuticeps* (98.5% for both). The phylogenetic analysis confirmed that EaPrx1 is evolutionarily closely related to *E. lanceolatus* and clustered with other teleosts Prx1. The tissue distribution analysis showed that the EaPrx1 had the greatest expression level in the spleen tissue. In the spleen, EaPrx1 expression was significantly upregulated for Poly I:C and LPS stimulants compared to NNV. For Poly I:C, the upregulation was nearly begun after 6 hrs of the post-injection (p.i.) and the highest level was obtained at 12-24 hrs p.i. and after 48 hrs of p.i. the downregulation pattern was noticed. Besides, for LPS, the peak upregulation was observed after 12 hrs of p.i. and its stands until 24 hrs of p.i. later, after 48 hrs of p.i. a significant downregulation pattern was

observed. For NNV, a slight upregulation was observed after 24 hrs of p.i. and after 48 hrs of p.i. the downregulation was recorded. Altogether this study suggests that EaPrx1 may actively involve in the host immune responses.

PP-32

The expression of the *Wnt2b* gene is critically regulated during pathogenic invasion in redlip mullet (*Planiliza haematocheila)* liver tissue.

T.D.W. Kasthuriarachchi, J. C. Harasgama, Qiang Wan, and Je hee Lee

Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea
Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : The Wnt signaling pathway is a conserved pathway involved in essential developmental, regeneration, and cell fate functions. Recent research are enlightening the involvement of Wnt signalling in immune functions as well. Wnt signalling is one of the key players in macrophage polarization and resolving inflammation other than wound healing. Studies in mammals showed Wnt2b is upregulated in inflammation due to pathogenic invasion. However, the studies on Wnt signaling in teleost are currently lacking. The present study has designed to molecular characterize the Wnt2b ligand in redlip mullet and investigate its expression regulation patterns during the pathogenic invasion.

◎ **Methods** : A nucleotide sequence for the Wnt2b gene was identified from the redlip mullet transcriptomic database. Bioinformatics tools and software were used in sequence characterization. Five redlip mullets were dissected, and 12 different tissues were sampled, including the liver. Groups of healthy redlip mullets were challenged with LPS, Poly I: C, and *Lactococcus garvieae*. The liver of fish (n=5) was sampled in 0, 6, 12, 48 hours after the IP injection. qPCR was used to investigate the transcriptional modulation of the PhWnt2b gene in tissues of healthy fish and challenged fish.

◎ **Results & Discussion** : The PhWnt2b gene consists of 1176bp, and the predicted polypeptide sequence is 391AA. The intestine shows the highest expression of PhWnt2b than other tissues. The digestive tract is one of the primary entering routes of pathogens. That may cause induction of inflammation and expression of inflammation mediating genes in the intestine. PhWnt2b is downregulated in liver tissue by all three PAMP's. LPS is a cell wall component of Gram-negative bacteria, and poly I: C is a mimic of viral RNA. The *L. garvieae* is Gram-positive bacteria in which causes green liver syndrome in redlip mullets. The effect of these immune stimulants on the expression of the PhWnt2b in liver tissue has suggested there might be a negative impact on liver tissues. Further experiments are required to elucidate the functions of the Wnt2b ligand during the pathogenic invasion in fish.

PP-33

Molecular cloning, expression profiling and functional characterization of caspase-9 in *Amphiprion clarkii*

H.M.V. Udayantha[*] and Je hee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Caspase-9 (casp9) is one of the major initiator caspases, induces apoptosis by activating the downstream intrinsic apoptosis pathway genes. Casp9 forms a multi-protein complex with apoptotic protease-activating factor-1 (Apaf-1) and cytochrome-C, called apoptosome. After that, it cleaves into large and small subunits and dimerizes by the self-priming mechanism to activate the downstream caspase-3 and caspase-7.

◎ **Methods** : In this study, we have cloned and characterized the Casp9 in the *Amphiprion clarkii* (Accasp9) together with tissue distribution and expression profiling against the LPS and poly(I:C). In addition, downregulatory pathway genes expressions were also observed after the stimulation with viral hemorrhagic septicemia virus (VHSV).

◎ **Results & Discussion** : The complete cDNA sequence (1992 bp) of Accasp9 consisted of an open reading frame of 1305 bp, encodes 434 aa. Theoretical pI and molecular weight of the AcCasp9 were 5.81 and 48.45 kDa respectively. According to the multiple sequence analysis CARD domain located at the N-terminus and large P-20, and small P-10 domains were located at the C-terminus in the AcCasp9. According to the phylogenetic analysis AcCasp9 clustered with Casp9 orthologs of different species. Although dominant expression of AcCasp9 was observed in the brain and gill with compared to the other analyzed tissues (intestine, spleen, skin, head kidney, blood, kidney, liver, stomach, muscle, and heart). The immune response of AcCasp9 was significantly elevated in the blood after the stimulation of LPS and poly(I:C). On the other hand, Casp7 expression was significantly elevated after the VHSV stimulation in Casp9 transfected FHM cells. Collectively our results suggested that AcCasp9 may be involved in the immune responses induced by the viral and bacterial stimulants in *A. clarkii*.

◎ **Reference** : M.S. Iordanov, O.P. Ryabinina, P. Schneider, B.E. Magun: Two mechanisms of caspase 9 processing in double-stranded RNA- and virus-triggered apoptosis. Apoptosis, 10:153-166, 2005.

P. Li, L. Zhou, T. Zhao, X. Liu, P. Zhang, Y. Liu, X. Zheng, Q. Li: Caspase-9: Structure, mechanisms and clinical application. Oncotarget, 8:23996-24008, 2017.

PP-34

Identification and transcriptional profile of cystatin B from big-belly seahorse

Y.K Kodagoda[*], W.K.M Omeka[*], D.S Liyanage[*], Suk kyoung Lee[*,**] and Je hee Lee[*,**]

[*]Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea

[**]Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Cystatins are a large superfamily of proteins that reversibly inhibit cysteine proteases of C1 family that required for cytosolic pathway, thereby inhibit the T-cell activation. The cysteine protease inhibitor superfamily comprises of three families: stefins, cystatins, and kininogens. Among them, cystatin B is classified under stefins which are cytosolic proteins, lacking signal peptide and disulphide bridges. Cystatin B is broadly expressed in different cell types and tissues and expected to have a specific role in neurodegenerative diseases. Although cytatin B has been biochemically distinguished, its molecular function in fish is still poorly described.

◎ **Methods** :In current study, Cystatin B ortholog was identified from Big-belly seahorse (*Hippocampus abdominalis*) using an established cDNA database and designated as HaCytB. In silico characterization was conducted using bioinformatics tools and webservers based on the identified cDNA sequence. The identified cDNA sequence of HaCytB was 873 bp long and comprise with a coding region of 297 bp. The coding sequence encodes a polypeptide comprising 98 amino acids, with a predicted molecular weight of 10.88 kDa and theoretical isoelectric point of 6.89. Multiple sequences alignment was performed using colour align conservation and presented more conserved features with fish species.

◎ **Results & Discussion** : According to the phylogenetic analysis HaCysB was distinctly cluster with homologs from fish species. The results of pairwise alignment disclosed that HaCytB possessed 95.4% of the highest identity and 96.7% of similarity with *Hippocampus comes* cystatin B sequence. Temporal and spatial expression of HaCytB transcripts were analysed by quantitative PCR technique. HaCytB was predominantly expressed in blood followed by testis at normal conditions. Upon, stimulation with LPS, Poly I:C, E.tarda and *S.inae* significantly ($p<0.05$) elevated mRNA transcript level of HaCytB in blood were detected. Collectively, these results suggest that HaCytB might contribute to the immune defence against host immune responses.

PP-35

A chemokine receptor CXCR3-2 Isoform 2 (EaCXC3-2), identified from red-spotted grouper *Epinephelus akaara*: Molecular aspects and immune defensive role upon pathogenic stress.

J.R.P Jayasingha, A.M Erandi and Je hee Lee

Department of Marine Life Science & Fish Vaccine Research Center, Jeju National University, Jeju Self-Goverming Province 63243, Republic of Korea
Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea

◎ **Background (or Objective) of This Study** : Chemokine receptor 3-2 protein is a member of the G protein-coupled receptor family, involving vital Immunophysiological functions. Red-spotted grouper (*Epinephelus akaara*) is one of the high economic important marine fish widely cultivated in Asia. Chemokine receptor 3-2 EaCXC3-2 gene was identified from *E. akaara* transcriptomic database and characterized. EaCXC3-2 protein consists of 377 amino acids which have a molecular weight of 42.54 kDa with 8.77 of an isoelectric point. This study contributes insights into the CXCR3-2 structure-function mechanism along with signal transduction cascade that leads to the up-regulation of EaCXC3-2 protein upon induction by different immune-stimulatory compounds.

◎ **Methods** : Unchallenged fish were used to synthesis cDNA after the extraction of RNA from the different tissues of healthy Grouper to determine the relative transcription levels of EaCXC3-2. Immune challenge experiment was performed with polyinosinic: polycytidylic acid (poly I:C), lipopolysaccharide (LPS), Nervous necrosis virus (NNV) as an immune stimulant and respectively synthesized cDNA was used to perform the qPCR analysis.

◎ **Results & Discussion** : EaCXC3-2 was composed of an N-terminus extracellular region, C-terminus intracellular region, seven hydrophobic transmembrane (TM) domains and three extracellular loops (ECL) and three intracellular loops (ICL) structures. Tissue distribution analysis revealed that EaCXC3-2 mRNA was predominantly expressed in the Head kidney, spleen Brain and blood whereas the lowest expression showed in liver and muscle tissues. The immune challenge experiment was disclosed significant upregulation of EaCXC protein in blood with Poly(I:C) and NNV at 6 hours post-injection (hpi). For the LPS stimulation, after 24 hpi, *EaCXC3-2* mRNA expression was significantly downregulated. Furthermore, Simulation with different pathogenic RNA inside the overexpressed cell line could lend support to our major finding in future.

◎ **Reference** : Bird S, Tafalla C.: Teleost Chemokines and Their Receptors. Biology (Basel), 4(4):756-784, 2015.

PP-36

국내 담수 자연수계 잉어봄바이러스병(Spring viraemia of carp, SVC)의 원인병원체 조사 및 계통분석

오현지* · 문성희* · 김노영* · 허영웅** · 윤정희** · 하지은** · 권세련*,** · 권준영*,**

*선문대학교 응용생물과학과

**선문대학교 수산생명의학과

◎ 연구의 배경 및 목적 : 잉어봄바이러스병(SVC)은 *Rhabdovirus carpio* (또는 SVCV)의 감염으로 인한 잉어류의 급성전염성질병이며 우리나라, 중국, 미국, 유럽 등 광범위한 지역에서 보고되고 있다. 이 병은 수온 11~17℃의 봄철에 주로 발생하며 잉어과 어류에 체색흑화, 안구돌출, 체표출혈 등의 외관변화를 유발한다. 주요 감염 장기는 신장, 비장, 아가미, 간, 뇌 등이며 감염어는 새판의 변성, 장의 염증 및 출혈 등 다양한 해부학적 증상을 나타낸다. 우리나라에서는 경북 안동과 청도에서 SVCV가 검출되었다는 보고가 있다. 본 연구에서는 내수면 수산자원보호구역 10곳(소양호, 춘천호, 남대천, 안동호, 왕피천, 남양호, 대청호, 삽교호, 나주호, 옥정호)에 서식하는 잉어과 어류를 대상으로 SVC 원인 병원체 존재 여부를 조사하였다.

◎ 실험 방법 (또는 연구방법) : 수산자원보호구역 10곳에서 채집한 잉어과 어류를 대상으로 SVCV 감염 여부를 조사하였다. 총 20마리 이상의 어류를 사용하였으며, 5그룹으로 나누어 1그룹당 4-5마리의 개체를 pooling하여 PCR 분석을 수행하였다. 잉어과 어류를 해부하여 신장과 비장을 적출하고 Ambion사 TRIzol® Reagent protocol을 사용하여 RNA를 추출하였다. 병원체의 PCR 검사 시 Enzynomics사의 TOPscriptTM One-Step RT PCR DyeMIX와 2X TOPsimpleTM DyeMIX (aliquot)-HOT를 이용하여 실시하였다. PCR primer는 병성감정지침서(국립수산과학원)에 나온 sequence를 이용하여 제작하였다. 양성이 나온 표본의 PCR 생성물은 염기서열분석 및 계통분석을 실시하였다. 계통분석은 Clustal X와 Mega X 프로그램을 사용하여 염기서열을 alignment하여 비교하고, 계통수를 그려서 시각화하였다.

◎ 결과 및 고찰 : 5년간 조사한 10개 지역 가운데 유일하게 2020년 4월에 삽교호에서 채집한 떡붕어에서 SVCV가 검출되었다. 지금까지 분리된 SVCV들의 G gene 염기서열을 삽교호에서 검출한 SVCV 염기서열과 비교하여 계통분석을 수행한 결과 1971년 Yugoslavia 분리주와 높은 염기서열 일치도를 나타냈었다. Yugoslavia (NC_002803) & Ukraine (AJ538062) 분리주는 European clade인 Id type에 속하며, 따라서 삽교호에서 검출된 SVCV도 Id type에 속할 것으로 판단된다. 이번 연구를 포함하여 국내에서는 담수 자연수계에서 세 차례 SVCV가 검출되었으며, 검출지역도 동서 지역에 두루 걸쳐있어서 확산 방지를 위해 지속적 감시가 이루어져야 할 것이다.

◎ 참고문헌 :

국립수산과학원, 2013 수산생물병성감정지침서

Goodwin AE: First Report of Spring Viremia of Carp Virus (SVCV) in North America. Journal of Aquatic Animal Health, 4:161-164, 2002.

Godahewa, G.I., Lee, S., Kim, J., Perera, N.C.N., Kim, M.J., Kwon, M.G., Jee, B.Y., Hwang,S.D., Lee, J.: Analysis of complete genome and pathogenicity studies of thespring viremia of carp virus isolated from common carp (*Cyprinus carpio carpio*) andlargemouth bass (*Micropterus salmoides*): an indication of SVC disease threat inKorea. Virus Res., 255:105-116, 2018.

PP-37

국내 담수 자연 수계에서의 Koi herpesvirus (KHV) 모니터링

김노영[*] · 문성희[*] · 허영웅[**] · 윤정희[**] · 하지은[**] · 권세련[*,**] · 권준영[*,**]

[*]선문대학교 응용생물과학과
[**]선문대학교 수산생명의학과

◎ 연구 배경 및 목적 : Koi herpesvirus disease (KHVD)는 원인 병원체 cyprinid herpesvirus 3 (CyHV-3)의 감염으로 잉어과 어류에 발병하는 전염성 질병이다. KHVD 발병 어류는 평형 상실, 피부 퇴색, 발적, 지느러미 부식 및 피부 지느러미 기저부 출혈 등의 외부 증상을 나타낸다. 주요 감염 장기는 아가미, 신장, 비장 등이며, 새변의 부식, 융합 및 곤봉화 등 해부학적 증상도 나타난다. KHV 감염개체는 증상이 없더라도 감염원이 될 수 있어 자연 수계는 물론 근처 양식장까지 전파될 수 있다. 따라서 내수면 어업의 피해 방지, 자연 수계 및 양식장의 수산생물 방역을 위해 이 질병을 지속적으로 모니터링 할 필요가 있다. 본 연구에서는 2016년부터 2020년까지 내수면 수산자원 보호구역(소양호, 춘천호, 남대천, 안동호, 왕피천, 남양호, 대청호, 삽교호, 나주호, 옥정호)에 서식하는 잉어과 어류를 대상으로 KHVD 원인 병원체 존재 여부를 조사하였다.

실험 방법 : 국내 담수 자연수계 수산자원보호구역 10곳의 잉어과 어류를 4-5마리씩 5그룹으로 나누고 신장과 비장을 잘라내어 DNA를 추출한 후 KHV 감염 여부를 조사하기 위한 PCR을 실시하였다. 질병 분석에 사용한 구체적인 방법은 국립수산과학원에서 발행한 병성감정지침서의 내용을 따랐다.

◎ 결과 및 고찰 : 본 연구에서 5년간 담수 자연 수계에 서식하는 잉어과 어류를 조사한 결과, 2017년, 2018년, 2019년에 KHV가 검출되었다. KHV의 발병 수온은 16~25℃이고 주 발생 온도는 22~24℃로 알려져 있다(국립수산과학원, 2011). 본 연구 조사 결과 지난 5년간 총 14회 KHV가 검출되었고, 이 중 8회는 26℃ 이상의 온도에서 검출되었다. 13.9℃의 낮은 온도에서도 검출되기는 하였으나 이 바이러스의 발생 가능성은 수온이 26℃ 이상 일 때 높아짐을 알 수 있었다. 2017년은 춘천호, 평창강, 안동호에서, 2018년의 경우 안동호 상류와 밀양강 하류에서, 그리고 2019에는 춘천호, 남대천, 대청호, 삽교호, 안동호에서 KHV가 검출되었다.

최근 5년간의 결과를 종합한 결과 KHV의 검출이 보고된 3개년 중 경상북도에 위치한 안동호에서 3개년 모두 검출되었다(총 6회). 또한 경상북도 밀양강에서도 KHV가 검출된 사실을 확인할 수 있었다. 이는 경북지역에서 KHV가 가장 많이 그리고 광범위하게 확산되고 있음을 시사한다. 이 지역에 대한 KHV 모니터링 및 확산 방지에 보다 많은 시간과 노력을 투자하여야 할 것으로 판단된다.

PP-38

Time-course impact of squalene-adjuvanted viral hemorrhagic septicemia virus vaccine following intraperitoneal and intramuscular injection in olive flounder (*Paralichthys olivaceus*)

Showkat Ahmad Dar, Sajal Kole and Sung Ju Jung

Department of Aqualife Medicine, Chonnam National University, Yeosu, 59626 Republic of Korea

◎ **Background (or Objective) of This Study**: For sustaining high production of olive flounder (*Paralichthys olivaceus*) aquaculture in Korea, easy-to-deliver and efficient vaccination strategies against serious pathogens, such as viral hemorrhagic septicemia virus (VHSV), is very important as it cause considerable losses to the industry. A safe and non-invasive vaccine formulation that is free from unacceptable side-effects and does not devalue the fish is needed to maintain flesh quality, as consumers prefer to eat raw flounders from aquaria. Thus, we administered the previously developed squalene-adjuvanted VHSV vaccine (vinay et al., 2013) via IP and IM injection methods and investigated the safety and persistency of the vaccine at the injection site.

◎ **Methods** : In the current study, we performed a comparative analysis of both IP and IM injection immunization routes in terms of vaccine efficacy, serum antibody responses, and immunization impact on the histology of injection site and of underlying organs at different time points.

◎ **Results &Discussion** : The clinical and histological observation of the IM and IP groups showed that our vaccine remained persistence at the injection sites for 10-17 weeks post vaccination (wpv), without causing any adverse effects to the fish. The challenge study at 3 wpv and 17 wpv revealed that the IP group was fully protected (100% RPS) and 71.4 %against VHSV challenge, whereas the RPS in the IM group was 88.9% and 92.3% compared to NVC control. Comparing the safety and efficacy aspects of the IP and IM groups, it can be inferred that the vaccine is equally efficacious when delivered via either route. However, as the vaccine residues takes 24 wpv for total metabolization, considering the persistency period (24 wpv) and both short and long-term efficacy of our vaccine. Thus, the present study recommends IM vaccination in small-sized fish and IP vaccination in table-sized fish, so as to curtail any hesitancy of customers in taking raw muscles from live fish.

◎ **Reference** : Vinay, T.N., Kim, Y.J., Jung, M.H., Kim, W.S., Kim, D.H. and Jung, S.J., Inactivated vaccine against viral hemorrhagic septicemia (VHS) emulsified with squalene and aluminum hydroxide adjuvant provides long term protection in olive flounder (*Paralichthys olivaceus*). Vaccine, 31(41):4603-4610, 2013.

PP-39

한국전통발효식품에서 분리한 probiotics의 특징 및 synbiotics 항균활성 효과

문채윤 · 허문수

제주대학교 해양과학대학 수산생명의학과

◎ 연구의 배경 및 목적 : 한국전통식품에서 probiotics를 분리하고 우수 균주를 선별하여 특정된 식품 속에 있는 섬유질 성분인 Prebiotics를 혼합하였다. 이는 장내 유익한 세균의 성장을 도모하여 유의적 변화를 일으키고 장 안의 유해세균과 유익세균의 균형을 유지시켜준다. 따라서 유해세균에 대한 항균능력이 우수한 균주를 선별하여 인공위액 및 담즙액에 대한 내성과 용혈능 등을 검토해 최종 균주를 선별한 뒤, 3종의 prebiotics와 혼합해 synbiotics로서의 항균활성 능력을 평가했다. 이는 추후 양식 산업에 일어나는 질병에 대한 예방 및 치료를 위한 응용 실험을 하는데 기초 자료로 사용될 것으로 생각된다.

◎ 실험 방법 (또는 연구방법) : 제주도 인근 시장에서 전통발효식품을 구매하여 유산균을 분리하고 항균활성을 탐색하였다. 그리고 인공 위액과 담즙을 테스트하고 우수 후보 균주를 선별하여 3종의 prebiotics와 혼합하여 항균활성을 체크하고 16S rRNA 유전자 염기서열 분석을 통해 우수 균주를 동정하였다.

◎ 결과 및 고찰 : 우수 후보 균주의 인공위액 및 담즙액에 대한 내성과 용혈능 등을 검토하여 최종 균주를 선별하였다. 최종 선별된 유산균은 3종의 prebiotics와 혼합한 synbiotics로서의 항균활성 능력을 평가하였다. 1차 항균 활성에서 C13은 인체 및 어류질병세균에서 가장 넓은 항균 스펙트럼을 보였고, β-haemolysis를 생산하지 않고 인공위액과 담즙액의 내성을 지닌 M1, K1 및 C13을 synbiotics의 항균 활성을 수행하였다. Prebiotics 3종(FOS, GOS, Inulin)과 선별된 균주가 혼합된 synbiotics에서는 이전 보다 항균 활성이 증진 또는 저해됨을 알 수 있었다. 16 rDNA 염기서열 결과, K1과 M1은 *Bacillus tequiensis* 99.72%, *Bacillus subtilis* 99.65%, *Bacillus inaquosorum* 99.72%, *Bacillus cabrialesii* 99.72%, *Bacillus stercoris* 99.58%, *Bacillus spizizenii* 99.58%, *Bacillus halotolerans* 99.58%, *Bacillus mojavensis* 99.51%로 분석되었다. 그리고 C13은 *Bacillus velezensis* 99.71%, *Bacillus nematocida* 99.36%, *Bacillus amyloliquefaciens* 99.44%, *Bacillus atrophaeus* 99.22%, *Bacillus nakamurai* 99.44%로 분석되었다.

◎ 참고문헌 :

Bergan, T., Ekstron, B, and Nord C. E., Ecological impacts of antibacterial agents: Stockholm. *Scand J Infect Dis.*, 18, 1986.

Bosscher, D., Loo, J. V, and Franck, A., Inulin and oligofructose as prebiotics in the prevention of intestinal infections and diseases. *Nutr Res Rev.*, 19:216-226, 2006.

Brinques, G. B, and Ayub, M. A. Z., Effect of microencapsulation on survival of *Lactobacillus plantarum* in simulated gastrointestional conditions, refrigeration, and yogurt. *J Food Eng.*, 103:123-128, 2011.

PP-40

Antibiofilm activity and *in vivo* efficacy of a novel peptide "Octopromycin" against multidrug resistant *Acinetobacter baumannii*

D. C. Rajapaksha[*], S. L. Edirisinghe[*], Chamilani Nikapitiya[*], Mahanama De Zoysa[*] and Ilsong Whang[**]

[*]College of Veterinary Medicine, Chungnam National University, Daejeon 34134, Republic of Korea
[**]National Marine Biodiversity Institute of Korea (MABIK), 75, Jangsan-ro 101 beon-gil, Janghang-eup, Seochun-gun, Chungchungnam-do 33662, Republic of Korea

Background of This Study : *Acinetobacter baumannii* is an opportunistic pathogen that causes nosocomial infections associated with high mortality and morbidity in intensive care units (ICU). *A. baumannii* infections are strongly related with the capability of pathogen to form biofilms on biotic and abiotic surfaces. The antimicrobial peptides (AMPs), due to broad-spectrum of activity, and low probability of development of resistance make excellent candidates as novel treatment option. Our main objective in this study was to determine the antibiofilm activity and therapeutic efficacy of novel peptide Octopromycin against multidrug resistant *A. baumannii*.

Methods : *A. baumannii* biofilm inhibition and eradication activities were determined by Crystal violet (CV) staining assay at minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of Octopromycin, 50 and 200 μg/mL respectively. *In vitro* toxicity of Octopromycin was assessed by (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) MTT (HEK293T and Raw 264.7 cells) and hemolysis (mice red blood cell) assays. *In vivo* toxicity and efficacy of Ocoptomycin were determined by zebrafish larvae exposure and *A. baumannii* infected adult zebrafish models.

Results & Discussion : Octopromycin treatments at the MIC (50 μg/mL) and MBC (200 μg/mL) showed significant ($P < 0.05$) biofilm inhibition of 44.0% and 82.3%, respectively. The biofilm eradication % of Octopromycin at MIC and MBC levels were 64.0% and 64.2%, respectively, compared to the untreated control. *In vitro* and *in vivo* safety evaluation revealed that Octopromycin was nontoxic to HEK293T and Raw 264.7 cells (< 400 μg/mL), as well as mice red blood cells (< 300 μg/mL) and zebrafish embryos (< 4 μg/mL). An *in vivo* study in zebrafish showed that the Octopromycin-treated group had a significantly higher survival rate (37.5%) than the untreated group (16.7%). Histological analysis demonstrated a reduced bacterial load and fewer alterations in spleen, kidney and gill tissues, confirming successful control of *A. baumannii* by Octopromycin. Collectively, the results indicate that the antibacterial peptide Octopromycin presents a desirable therapeutic option for prevention and control of the *A. baumannii* infections.