sequenced to assess taxonomical composition and structure of different epithelia 6 h and 72 h after a short bacterial bath infection (1 h). We also assessed the immune status of fish after bath infection through qPCRassays. Alpha diversity measurements (inverse Simpson and Shannon index) suggest that the different microbiotas are highly diverse but predominated by few taxa and that the bacterial infection does not affect these indices. On the other hand, beta diversity measurements showed a potential early infection through gills after 6 h,then affecting skin and caudal fin microbiotas. Negative binomial generalized linear model (nb-GLM) highlighted the increase of different opportunistic pathogens such as Aeromonas, Pseudomonas, etc 6 h after bath infection. These observations are consistent with immune assays (cytokines, humoral response, gene expressions) showing a response of the immune system after 6 h followed by a disorder in its functioning. This study suggests that furunculosis not only impair immune system in rainbow trout but also induce dysbiosis leading to the increase of opportunistic pathogens in the bacterial community.

Keywords: Aquaculture, Microbiota, pathogen infection, immune system, *Oncorhynchus mykiss*

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O-040.

TLR5M and TLR5S play opposite roles in NF-κB pathway in *Vibrio parahaemolyticus* flagellin stimulation in orange-spotted grouper, *Epinephelus coioides*

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Abstract
A family of Toll-like receptor (TLRs) acts as primary sensors, which detect various microbial components, activate the host innate immune response to eliminate invading pathogens. In mammals, nuclear factor (NF)-κB signaling pathway is critical to the inflammatory cytokines and effectors expression. However, in fish, the bidirectional regulation of TLRs on NF-κB pathway is poorly understood. Here, we investigated the potential molecular mechanism of orange-spotted grouper (*Epinephelus coioides*) TLR5M (*Ec*TLR5M) and TLR5S (*Ec*TLR5S) regulating the NF-κB pathway after *Vibrio parahaemolyticus* flagellin stimulation.

*Ec*TLR5M, a member of the conserved TLR protein family, is involved in specifically recognizing flagellin and activating the NF-κB pathway. After knockdown of *Ec*TLR5M in grouper spleen (GS) cell line, the phosphorylation of IκBα and the expression of downstream cytokines, such as interferon (IFN) -γ2, interleukin (IL) -6 and tumor necrosis factor (TNF) -α, were all significantly suppressed. The overexpression of *Ec*TLR5M induced not only the activation of NF-κB pathway, but also mRNA expression of *Ec*TLR5S.

*Ec*TLR5S, consisting of 17 extracellular leucine-rich repeat domains, is located in the cytoplasm and involved in flagellin recognition. Knockdown of *Ec*TLR5S enhanced the phosphorylation of IKKα/β and IκBα, promoted NF-κB p65 nuclear transport, and augmented the cytokines IFN-γ2, IL-6 and TNF-α mRNA expressions after flagellin stimulation. Consistently with these observations, over-expression of *Ec*TLR5S negatively regulated the NF-κB pathway activation. We verified that the N-terminal (aa 1-254) and C-terminal (aa 514-643) of *Ec*TLR5S are the major functional domains of negative regulation by deletion mutation.

Taken together, *Ec*TLR5M is identified as a positive regulator, activating the NF-κB signaling after flagellin recognition. And for the first time, *Ec*TLR5S is demonstrated as a negative regulator that suppresses flagellin-induced activation of NF-κB, suggesting an important role for *Ec*TLR5S in control of innate immunity homeostasis.

Acknowledgements: Supported by Science and Technology Planning Project of Guangzhou, China (201607010043), Guangdong Provincial Natural Science Foundation (2018A030313156) and Qingdao National Laboratory for Marine Science and Technology (2017-4A03).
Keywords: NF-κB; TLR5M; TLR5S; flagellin; cytokine

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O-041.

Bacterial outer membrane vesicles of *Aeromonas salmonicida* induce a proinflammatory immune response in vitro and in vivo

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Abstract
High mortality rates after bacterial infections cause huge annual losses for the aquaculture industry. As treatment with antibiotics is not an alternative, bacterial vaccines for intramuscular or intraperitoneal injection were developed resulting in protection but also in inflammatory granulomas and stress. Here we propose the design of a modular vaccine based on outer membrane vesicles (OMVs) of the bacterial fish pathogen *Aeromonas salmonicida* (*A. salmonicida*.). The simple preparation, the safety due to their non-replicative nature as well as the composition of natural surface exposed membrane antigens in their native confirmation are the advantages of such a vaccine design. In the present project, the innate immune response to OMVs in comparison to bacterial stimulation was characterized using a peritoneal model for rainbow trout (*Oncorhynchus mykiss*). The distribution, recruitment and kinetics of myeloid cell populations in peritoneum, blood, spleen and head kidney were compared using lineage marker specific monoclonal antibodies. Additionally, the monocyte/macrophage cell line RTS-11 was used to characterize the mRNA profile response of phagocytes to OMVs and *A. salmonicida* bacterial particles.
First results indicate similar patterns of cellular responses in vivo either by stimulation with OMVs or with bacteria in regards to cell kinetics as well as to the induction of pro-inflammatory genes.
Next steps will include engineering of recombinant *A. salmonicida*, which produce of OMVs, presenting the immunogenic G-proteins of Viral-hemorrhagic-septicemia-virus (VHSV), Infectious-hematopoietic-necrosis-virus (IHNV) and Spring-viraemia-of-carp-virus (SVCV).Those OMVs will be used to analyze the innate immune response against bacterial and viral pathogens in regard to induction of protective immune memory.

Keywords: *Aeromonas salmonicida*, outer membrane vesicles, *Oncorhynchus mykiss*, innate immune response, vaccine

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O-042.

Impact of sea lice (*Caligus rogercresseyi*) infection levels on skin transcriptome in Atlantic salmon (*Salmo salar*)

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