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The immune response of a warm water fish orange-spotted grouper (*Epinephelus coioides*) infected with a typical cold water bacterial pathogen *Aeromonas salmonicida* is AhR dependent

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ABSTRACT

The present study reported the first pathogenic *Aeromonas salmonicida* (SRW-OG1) isolated from the warm water fish orange-spotted grouper (*Epinephelus coioides*), and investigated the function of Aryl hydrocarbon receptor (AhR), a ligand-dependent transcriptional factor which has been recently found to be closely associated with immune response in mammals and *E. coioides*. Our results showed that AhR was activated by an unknown ligand in the spleen, intestine and macrophages. Meanwhile, *ahr1a* and *ahr1b* were significantly increased in the spleen, intestine and macrophages, whereas *ahr2* was only increased in the intestine, which indicated that the contribution of AhR2 to the immune response may be less than that of AhR1a and AhR1b. Some key genes involved in the macrophage inflammatory response, bacterial recognition, and intestinal immunity were significantly up-regulated in the SRW-OG1 infected *E. coioides*. Nevertheless, declining macrophage ROS production and down-regulation of related genes were also observed, suggesting that SRW-OG1 utilized its virulence mechanisms to prevent macrophage ROS production. Furthermore, AhR inhibitor 3′, 4′-DMF and the silence of *ahr1a* or *ahr1b* significantly rescued the increased *IL-1β* and *IL-8* induced by SRW-OG1 infection, which proved that the induction of *IL-1β* and *IL-8* in *E. coioides* macrophages was mediated by AhR. However, *BPI/LBP*, ROS production and related genes were not affected by AhR. The survival rate and immune escape rate of SRW-OG1 in the *ahr1a/ahr1b* knocked-down and 3′, 4′-DMF treated macrophages were significantly increased compared with those in wild type macrophages. Taken together, it was preliminarily confirmed that *ahr1a* and *ahr1b* played an important role in the immune response against *A. salmonicida* SRW-OG1.

1. Introduction

Aeromonas salmonicida is a facultative anaerobic Gram-negative brevibacteria widely distributed in the environment (Jin et al., 2020). It is one of the oldest known bacterial pathogens of fish, which can infect both marine and freshwater fish (Jin et al., 2020), including salmonid species and a variety of non-salmonid fish (Connors et al., 2019). To date, infection of rainbow trout (*Oncorhynchus mykiss*) (Findlay and Tatner, 1996; Hoover et al., 1998; Long et al., 2015), carp (*Cyprinus carpio*) (Nakayama et al., 2017), turbot (*Scophthalmus maximus* L.) (Coscelli et al., 2015), Atlantic salmon (*Salmo salar*) (Weeksperkins and Ellis, 1995), goldfifish (*Carassius auratus*) (Connors et al., 2019) with *A. salmonicida* has been reported. The furunculosis caused by *A. salmonicida* has been a major reason of mortality for cultured fish worldwide and antibiotic usage in commercial aquaculture (Braden et al., 2019). *A. salmonicida* is divided into five subspecies: subsp. *salmonicida*, subsp. *achromogenes*, subsp. *masoucida*, subsp. *smithia* and subsp. *pectinolytica* (Gulla et al., 2016; Schwenteit et al., 2011) *A. salmonicida* subsp. *salmonicida* causes typical furunculosis of salmonid fish, while the *A. salmonicida* subsp. *achromogenes* strains cause atypical furunculosis of various fish species (Gudmundsdóttir and Björnsdóttir, 2007). The typical furunculosis caused by *A. salmonicida* subsp.

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