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Analysis of gene expression in nodavirus-inoculated Senegalese sole using a new Openarray® platform

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Abstract

Nervous necrosis virus (NNV) is the causative agent of the viral encephalopathy and retinopathy, a disease that affects cultured Senegalese sole (Solea senegalensis). A NNV reassortant (Ss160.03), combining genomic segments from red-spotted grouper nervous necrosis virus (RGNNV) and striped jack nervous necrosis virus (SJNNV) genotypes, has been previously isolated from Senegalese sole, being highly virulent to this fish species. The RNA-Seq technology has been used in a previous study to comparatively analyse Senegalese sole transcriptomes in two organs (head kidney and eye/brain) after infection with two NNV virus with different levels of virulence to that fish species, a highly virulent reassortant isolate (wSs160.03) and a less virulent mutant reassortant obtained by reverse genetics (rSs160.03247þ270). To validate previous RNA-Seq results, a 112- assay OpenArray® platform (Thermofisher) has been designed. This platform included 89 genes chosen according to transcriptomic changes observed by RNA-Seq (covering PRRs, type I IFN response, signal transduction, inflammation, virus responsive genes, and apoptosis), 17 genes selected based on their previously described relation with the immune response against fish viral infections, and 6 control genes (including 3 endogenous genes and 3 viral genes). A total of 63.25% differentially expressed genes (DEGs) detected by RNA-Seq were validated by the OpenArray designed, showing similar expression levels and a 100% expression tendency accuracy. Furthermore, this tool brings new information about the infection process that was not shown by the RNA-Seq analysis, such as the expression profiles of mda5, ifng, c9, c3, mx, ifit-1, myd88, tbkbp1, and ube1 genes in different samples at 48 h post-infection (pi). Moreover, a consistent decrease in the number of DEGs was observed at 72 hpi, confirming that 48 h is an adequate time point to study innate immune response of sole against NNV infection. In conclusion, this molecular platform has been confirmed as a good tool for further studies on the sole immune response against NNV mutant infections, which will contribute to the knowledge of the mechanisms of the pathogen-host interaction.

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keywords: Solea senegalensis, Reassortant Nervous Necrosis Virus, Open- Array®, differentially expressed genes (DEGs), Immune response.

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