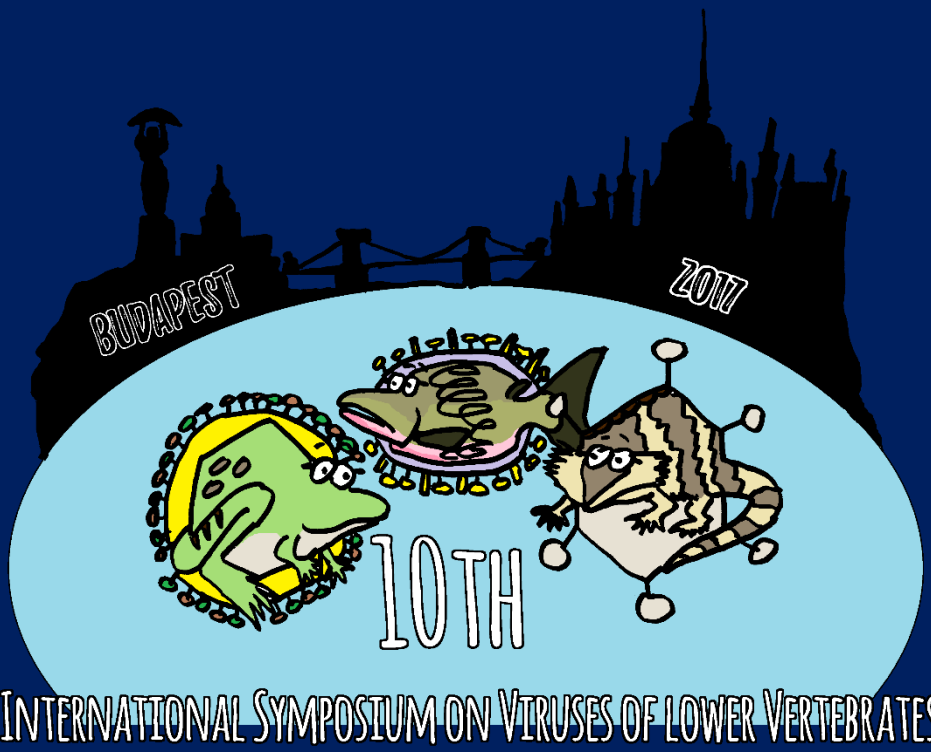

INTERNATIONAL SYMPOSIUM ON VIRUSES OF LOWER VERTEBRATES

ABSTRACT BOOK

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Implications of granzyme B in the immune response of gilthead seabream against nodavirus (NNV)

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Nodavirus (NNV; *Nodaviridae* family, *Betanodavirus* genus), is one of the most threatening virus for teleost fish and causes the viral encephalopathy and retinopathy (VER) disease that alters brain and retina structure and function. Among marine fish, the European sea bass (*Dicentrarchus labrax*), a very relevant species in Mediterranean aquaculture, is one of the most susceptible species, while the gilthead seabream act as a reservoir of the RGNNV strain and does not suffer the disease. Thus, comparative studies between sea bass and seabream specimens are worthy elucidating the differences in fish immunological response and their relation with susceptibility. Amongst the immune parameters, the cell-mediated cytotoxicity is very important to fight and clear the viral infections. This cytotoxic activity is mainly mediated by the perforin and granzyme proteins but they have been scarcely characterized in fish. Our aim was to characterize the granzyme B (GrB) of gilthead seabream and its potential implication against the NNV infection. Thus, seabream *grb* gene was sequenced. In *in vitro* experiments consisting on cytotoxic assays using seabream leucocytes as effectors and naïve or NNV-infected cells as targets we evaluated the transcription of *grb* as well as the GrB activity. In *in vivo* experiments where seabream specimens were infected with NNV we evaluated the *grb* transcription as well as the presence and distribution of GrB^+ cells in the brain. Our results show that seabream leucocytes under *in vitro* cytotoxic activity increased *grb* transcription and GrB activity in both cell lysates and supernatants, which was in some cases further increased when the target cells were infected with NNV. In the *in vivo* infection trial, seabream showed a significant up-regulation in the *grb* mRNA levels in the brain but a down-regulation in the head-kidney. Similarly, the immunohistochemical study revealed that the number and areas of GrB^+ cells was increased in the brain upon NNV challenge. In conclusion, our data demonstrate the importance of the GrB, at both mRNA and protein levels, in the gilthead seabream immune response against NNV infections and could represent one of the mechanisms involved in the NNV clearance. (Support: Grants AGL2013-43588-P and AGL2016-74866-C3-1-R (MINECO and FEDER) and 19883/GERM/15 (*Fundación Séneca de la Región de Murcia*, Spain) are gratefully acknowledged).

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