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In vitro assessment of Senegalese sole (*Solea senegalensis*) immune responses against different *Tenacibaculum maritimum* strains

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Currently, little is known about *T. maritimum* evading strategies and many aspects regarding the host-pathogen interaction are still not fully elucidated. Hence, the present study aimed to assess Senegalese sole cellular immune responses following stimulation with either live or UV killed *T. maritimum* through both functional (e.g. superoxide anion and nitric oxide production, leucocytes killing capacity) and gene expression approaches. Senegalese sole head-kidney leucocytes were isolated and exposed to several live or inactivated *T. maritimum* strains during 4 h, 12 h, 24 h and 48 h. Results from the present study did not reveal significant changes in superoxide anion and nitric oxide production in leucocytes exposed to different bacterial strains. UV killed *T. maritimum* strains induced higher nitric oxide production by leucocytes in contrast to the lower superoxide anion release induced by live strains. Moreover, lactate dehydrogenase activity was assessed and results suggested some evidence for necrotic cell death induction mainly during the first 4 h following bacterial inoculation. Regarding gene expression, stimulation with live strains induced an increase in interleukin 1β (IL1β), hepcidin antimicrobial peptide (HAMP1), cyclooxygenase 2 (COX2) and g-type lysozyme (gLYS) transcripts at 4 h, which decreased similarly until 48h. Although interleukin 10 (IL10) expression levels presented a similar pattern, an upregulation was observed at 48 h post stimulation. Moreover, the expression levels of IL1β, COX2, HAMP1 and IL10 from host cells stimulated with inactivated bacterial strains increased more than those from leucocytes exposed to live bacteria. In conclusion, the downregulation of inflammatory and iron regulating genes as well as the extensive destruction of phagocytes were considered important tools in bacterial pathogenesis. In addition, further increases in cellular mediate immune parameters beside, upregulation of inflammatory genes following stimulation with UV killed strains gave insight into the potential use of those strains in vaccine preparation.

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