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Conference Abstract

Detection of fish pathogen *Saprolegnia parasitica* in environmental DNA samples by droplet digital PCR

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Abstract

Oomycetes are fungal-like microorganisms parasitic towards a large number of plant and animal species. Genera from order Saprolegniales, such as *Saprolegnia* and *Aphanomyces*, cause devastating infections of freshwater animals. *Saprolegnia parasitica* is a widely distributed oomycete pathogen that causes saprolegniosis, a disease responsible for significant economic losses in aquaculture, as well as declines of natural populations of fish and other freshwater organisms. Despite its negative impact, no monitoring protocol for *S. parasitica* has been established to date. Thus, we aimed to develop a droplet digital PCR (ddPCR) assay for the detection and quantification of *S. parasitica* in environmental DNA samples.

Saprolegnia parasitica-specific primers were designed to target internal transcribed spacer region 2 (ITS 2), based on the alignment of ITS sequences of *S. parasitica* and a range of *Saprolegnia* spp., as well as other oomycetes. The specificity of primers was tested using genomic DNA of *S. parasitica* (as positive control) and DNA of non–*S. parasitica* oomycete isolates, as well as trout/crayfish DNA (as negative control). The primers specifically amplified a segment of the ITS region of oomycete pathogen *S. parasitica*, while no

amplification (i.e. no positive droplets) was obtained for closely related *Saprolegnia* spp. (e.g. *Saprolegnia* sp. 1 and *S. ferax*) and other more distantly related oomycetes. Next, the limit of detection (LOD) of the assay was established by using serial dilutions of the *S. parasitica* genomic DNA. The determined sensitivity of the assay was high: LOD was 15 fg of pathogen's genomic DNA per µL of the reaction mixture.

Assay performance was further assessed with environmental DNA samples isolated from water from the trout farms and natural environments, as well as (ii) biofilm from the host surface (swab samples). Water samples were collected from 21 different locations in Croatia, while swab samples were collected from *S. parasitica* host/carrier species: (i) skin and eggs of the rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) and brown trout (*Salmo trutta* Linnaeus, 1758), and (ii) cuticle of signal crayfish (*Pacifastacus leniusculus* Dana, 1852) and narrow clawed crayfish (*Pontastacus leptodactylus* Eschscholtz, 1823). Samples were classified into agent levels A0 to A6, depending of the number *of S. parasitica* ITS copies per ng of total DNA.

Saprolegnia parasitica was detected in 76 % of water samples (16/21) and the range of pathogen's ITS copies in positive samples was between 0.02 and 14 copies/ng of total DNA (agent levels A1 to A3). Regarding the swab samples, *S. parasitica* load was significantly higher in diseased trout than in those with healthy appearance: 9375 vs 3.28 *S. parasitica* copies/ng of total swab DNA (average agent level A6 vs. A2, respectively). Despite the fact that none of the sampled crayfish had signs of infection, the pathogen was detected in all tested cuticle swabs. Swabs of *P. leniusculus*, a known *S. parasitica* host, had significantly higher *S. parasitica* load than swabs of *P. leptodactylus*, *S. parasitica* carrier: 390 vs 83 *S. parasitica* copies/ng (agent level A5 vs. A4, respectively).

In conclusion, our results demonstrate the applicability of the newly developed ddPCR assay in monitoring and early detection of *S. parasitica* in aquaculture facilities and natural freshwater environments. This would help in a better understanding of *S. parasitica* ecology and its effects on the host populations.

Keywords

crayfish biofilm, ddPCR detection, eDNA, freshwater environment, saprolegniosis, trout biofilm

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