Short communication

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Pathogen identification and epidemiological assessment in Recirculation Aquaculture System based on Illumina sequencing technology

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ABSTRACT. Microbial polymorphism in water, on the skin, and internal organs of juvenile trout, reared in Recirculation Aquaculture System (RAS) of a trout farm, was analyzed by 16S rDNA amplicon Meta Sequencing (Illumina sequencing technology). The microbiome pattern of water from different locations indicated the perfect work of RAS. *Flavobacterium* was identified as the only pathogen in the ulcers and internal organs of the diseased fish larvae. We proposed that pathogenic *Flavobacterium* sp. entered a trout farm through contaminated eggs. Metagenomic analysis of the microbial community is a sensitive and informative indicator of the epidemiological situation in RAS. In terms of amplicon metasequencing, modern NGS technologies become a powerful instrument for microbial monitoring in fish aquaculture.

Keywords: Recirculation Aquaculture System, epidemiological assessment, pathogen identification, amplicon metasequencing, Illumina sequencing technology

1. Introduction

RAS technology has steadily developed over the past 30 years and is widely used for broodstock management, in hatcheries, and increasingly for production of fingerlings. The taxonomic structure of the microbial community in RAS is an extremely sensitive and informative indicator of the health status of fish (Bentzon-Tilia et al., 2016; Dittmann et al., 2017). The community includes both microbiomes of fish and recirculating water objects. Here, using a specific trout farm as an example, we present the results of a fast and efficient analysis of epidemiological assessment and pathogen identification in RAS based on 16S rDNA amplicon Meta Sequencing (Illumina sequencing technology).

2. Material and methods

The sampling for microbiome analysis was carried out on March 27, 2019, at Recirculation Aquaculture System of trout farm (the Angara River, below the dam of the Irkutsk hydroelectric power plant). Water samples were filtered through polycarbonate filters with a pore diameter of 0.22 μm (filter diameter 25 mm, Millipore). They were collected from the water supplied to the system from the river, several pools with fish and the outlet of biofilter after the UV irradiation. Fragments of internal organs (liver and spleen) were aseptically taken from diseased and visually healthy fish as well as fragments of necrotic tissue from individuals with ulcers on the outer integument. All samples were fixed immediately after collection with 200 μL DNA / RNA Shield reagent (Zymo Research); further storage and transportation were carried out at room temperature. Totally, 18 samples of biological material were analyzed to identify the pathogen and assess the epidemiological situation in the aquarium complex. The V3-V4 variable regions of the 16S rDNA were amplified using the F515 / R806 (GTGCCAGCMGCCGCGGTAA / GGACTACVSGGGTATCTAAT) primers specific for a wide range of microorganisms, including bacteria and archaea (Bates et al., 2011) and sequenced using Illumina technology.

3. Results and Discussion

3.1. Water in pools

Flavobacteria as monodominants (24.4%) were identified only in water of trays with larvae right after

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hatching. In the remaining pools with fingerlings, high bacterial diversity was found with the top 10 most common bacteria, which fraction varied from 1.6 to 13.2%. The composition of aquatic microbial communities in the pools was similar to the water that entered the system from the Angara River. The water in the outlet of biofilter after purification and the UV treatment was essentially different and dominated by *Deinococcus* (83.8%). These microorganisms are common to the aquatic community and known by their resistance to the UV irradiation.

It should be noted that even in small quantities, the "typical" pathogen of freshwater farms, *Aeromonas hydrophila*, was not found in the aquatic microbial communities. However, some heterotrophic bacteria could be pathogens for fish, including secondary pathogens, which do not cause infectious diseases. Their development on the outer covers of injured or stressed individuals with low immunity still leads to a deterioration of the fish appearance. In complex cases, they cause the death of fish.

3.2. Identification of the pathogen in fish larvae

Flavobacterium was determined as the major bacterium of the diseased fish microbiome. Its fraction in ulcers on the outer integuments and organs of the fish was up to 99.6%. It might be identified as a pathogen that caused larvae fish mortality. There were no accompanying bacteria, or their fraction in total microbiome was less than 5%. Among the internal organs, the spleen was apparently first affected. In diseased fish, the fraction of flavobacteria in the spleen and liver was 99.3–99.6% and 17.0–97.9%, respectively. In a healthy larva, flavobacteria were detected only in the spleen, and their fraction was 2.4%.

Notably, during the period of mortality, flavobacteria were identified as the only pathogen, without accompanying opportunistic microorganisms. Pathogenic *Flavobacterium* sp. was found in high fraction only in the aquatic community of trays, where larvae were incubated right after hatching. Its content decayed to zero after biofilter and decreased substantially in other pools. The internal organs of the fish are seeded with flavobacteria in the trays and further, with low immunity, high stocking density and other unfavorable factors, the immune system of the fish cannot cope with the pathogen, which leads to mortality in the pools. We can assume that this pathogen enter a trout farm only through contaminated eggs.

5. Conclusions

The results indicated the perfect work of RAS. Pathogenic *Flavobacterium* sp. entered a trout farm through contaminated eggs. The metagenomic analysis of the microbial community becomes a sensitive and informative indicator of the epidemiological situation in RAS.

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