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Composition of psychrotrophic microflora of water and biofilter filler in recirculation aquaculture system on trout farm

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ABSTRACT

The advancement of RAS technology and advantages over flow-through systems has led to increasing of Recirculating Aquaculture Systems (RAS) use. All the key biological mechanisms involved in RAS need to be better understood, especially those determining the development of bacterial populations and their interactions with fish. This study presents new knowledge on compositions *of psychrotrophic* bacteria community in various parts of RAS for rainbow trout growing, which constitute essential tools for system management. *Pseudomonas, Alcaligenes, Acinetobacter, Aeromonas, Enterobacteriaceae*, and also a small number of gram-positive bacteria and cocci were identified as dominant taxa of psychrotrophic bacteria in RAS biotopes. Microorganisms of the genera *Pseudomonas, Alcaligenes, Acinetobacter* and *Aeromonas* are the autochthonous microflora which forms an active microbial fiofilm on biofilter filler.
Keywords: recirculation aquaculture systems, rainbow trout, biological filter, psychrotrophic bacteria, biofilm.

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INTRODUCTION

Strict environmental restrictions aimed at minimizing pollution from fish breeding plants and aquaculture farms in Northern Europe served as the impetus to rapid technological development of recirculation aquaculture systems (RAS) [1]. Growing public demand for a healthy tasty and affordable food is stimulating the"boom" in this industry [2]. Rainbow trout farming in RAS belongs to a higher form of industrial fish farming and is an intensive system of managed and controlled fish growing. The main difference between growing of rainbow trout in RAS and growing fish outdoors in open ponds is a limited territory which has to provide favorable conditions both for growth of fish and functioning of recycled water treatment system [3]. Microflora as a part of biocenosis formed in different biotopes of the RAS plays an important role in these processes. It takes part in regeneration of recycled water and is an integral part of biological filter to which the biofilm is attached or exist in form of sludge [4, 5]. Rainbow trout growing takes place in RAS, where optimal water temperature for fish have to be approximately 8–10 °C. This temperature is favorable mostly for psychrotrophic microorganisms [6]. Thus, the proper functioning of the biofilter in a RAS happens due to active development of psychrotrophic microflora. Some researchers have hypothesized that each RAS biofilter should have a unique microbial community composition shaped by operational controls and components implemented in the RAS [7, 8].

The objective of this study was to deepen study the composition of psychrotrophic microflora in main biotopes of RAS for rainbow trout farming. Such new information, coupled with the already available literature data, will contribute to allow reaching the possibility to control and manage the community of psychrotrophic microflora in a RAS.

MATERIALS AND METHODS

The study was conducted on full-system rainbow trout farm of Ukrainian Eastern Fish Company "Mzha". The samples for study of RSA microflora were selected from 6 major biotopes of RSA: water microflora from the well at the entrance to incubator (biotope 1); water microflora after biological filter from incubator (biotope 2); microflora of filler for biological filter through which water comes from the incubator (biotope 3); water microflora from well at the entrance to pool of commodity fish (biotope 4); water microflora after biofilter from RSA modules for growing of fry fish and keeping of commodity fish (biotope 5); and microflora of filler for biological filter in which water comes from RSA modules for growing of fry fish and keeping of commodity fish (biotope 6).

The degassing of water which comes from the well into a pool for commodity fish was conducted by means of diffuser, degassing of water for incubator was conducted in a cooling tower.

Psychrotrophic microorganisms from water samples were cultivated on meat peptone agar with incubation for 10 days at a temperature 6.5 °C. Taxonomic analysis of psychrotrophic microorganisms was conducted after the separation of pure culture and its distribution on cocci, gram-positive and gram-negative bacteria by means of microscopy.

Gram-negative bacteria belonging to the family *Enterobacteriaceae* and genus *Acinetobacter* were determined by the cytochromeoxidase test (described by Kovach). Taxonomic analysis of cytochromeoxidase-positive gram-negative bacteria was conducted using Bergey's Manual of Systematic Bacteriology.

By means of catalase test coccus microflora was distributed on the families *Micrococcaceae* and *Streptococcaceae*. Genera *Micrococcus* and *Staphylococcus* were separated in the family *Micrococcaceae* by the ability to glucose fermentation. Genera *Streptococcus* and *Enterococcus* were separated in the family *Streptococcaceae* using Bergey's Manual of Systematic Bacteriology. *Pseudomonas aeruginosa* was identified by cultivation on selective media with 0.2 % N-Cetylpyridinium chloride.

RESULTS AND DISCUSSION

The taxonomic analysis of RSA microflora in different biotopes showed the presence of microorganisms belonging to genera *Pseudomonas, Alcaligenes, Acinetobacter, Aeromonas, Staphylococcus, Micrococcus* and family *Enterobacteriaceae*, and also gram-positive bacteria and cocci. *Pseudomonas* and *Acinetobacter* were

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the main genera revealed in all the RSA biotopes. Microorganisms of genera *Staphylococcus* and *Micrococcus* were revealed only in one biotope – biotope 1 (water from well at the entrance to incubator with degassing in cooling tower). Their proportions were 5.5 and 11.1% respectively (Figure 1).

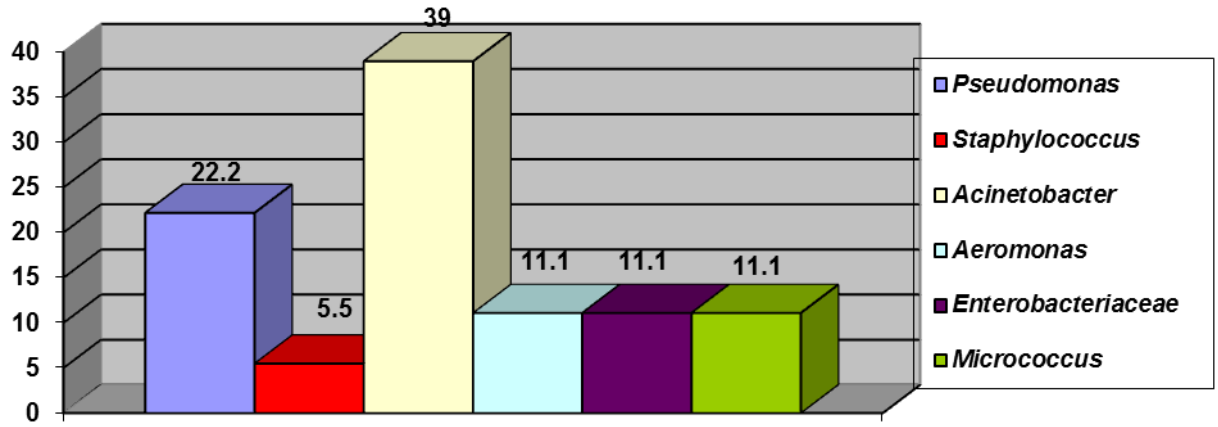

Figure 1. Composition of psychrotrophic microflora in biotope 1 (%).'

As a rule microorganisms of these genera are separated from human and animal organism. At the same time they are able to exhibit psychrotrophic properties and adaptation to the water temperature thanks to labile enzyme systems. They have the ability to change the type of nutrition and metabolism as well [7].

The proportion of dominant bacteria *Acinetobacter* and *Pseudomonas* together in biotope 1 was higher than 61 %.

In water samples from incubator after biofilter we have observed the increasing of *Pseudomonas* and *Aeromonas* bacteria number. The differences between their proportions in biotope 1 and biotope 2 were 19.9 and 6.5 % respectively (Figure 2). Besides, missing in previous point microorganisms of the genus *Alcaligenes* (17.6 %) and gram-positive bacteria (5.9 %) were found in water after biofilter.

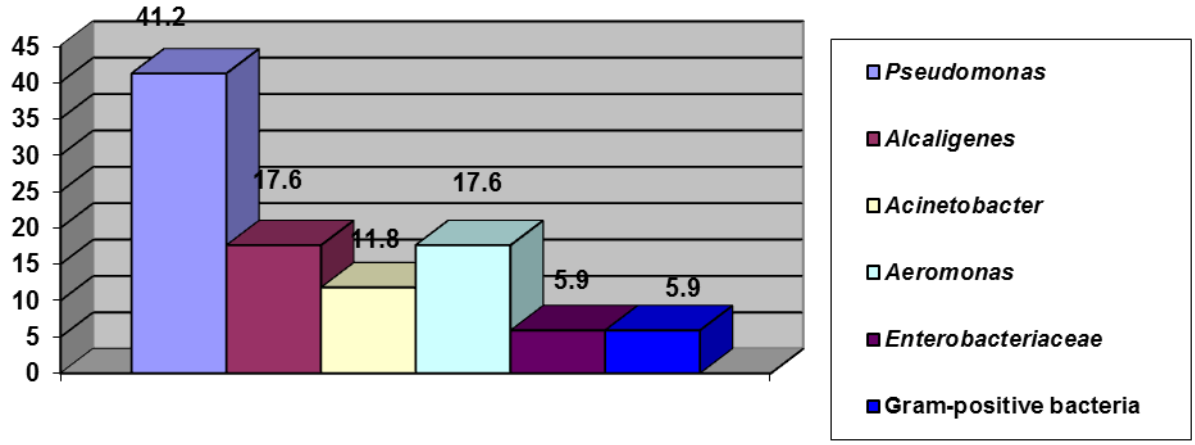

Figure 2. Composition of psychrotrophic microflora in biotope 2 (%).

The proportions of bacterium of genus *Acinetobacter* and family *Enterobacteriaceae* in water after biofilter were respectively 27.2 and 5.2 % less than in water of biotope 1.

The increase of microorganisms of genera *Pseudomonas* and *Alcaligenes* in water after biofilter can be associated with their release from surface of the biofilter filler, where their number amounted to 75.0 and 12.5 % respectively (Figure 3).

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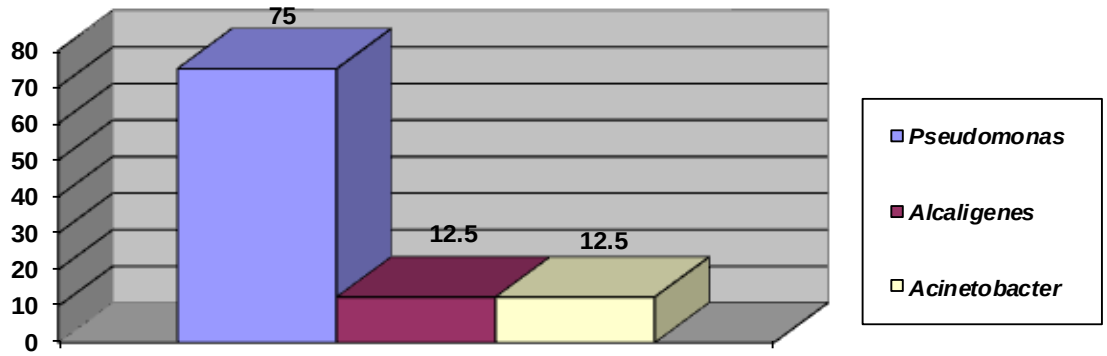

Figure 3. Composition of psychrotrophic microflora in biotope 3 (%).

A small amount of genus *Acinetobacter* microorganisms (12.5%) on the surface of biofilter filler may indicate their destruction and transition to active silt.

Thus, *Pseudomonas, Alcaligenes, Acinetobacter* and *Aeromonas* are the main genera of psychrotrophic microflora in water and biofilter filler in RSA incubator. They meet regularly and in large quantities.

Pseudomonas was the dominant genus of microorganisms in water from well at the entrance to pool with commodity fish (biotope 4). The proportion of this bacteria in common amount of psychrotrophic microflora was 63 % (Figure 4).

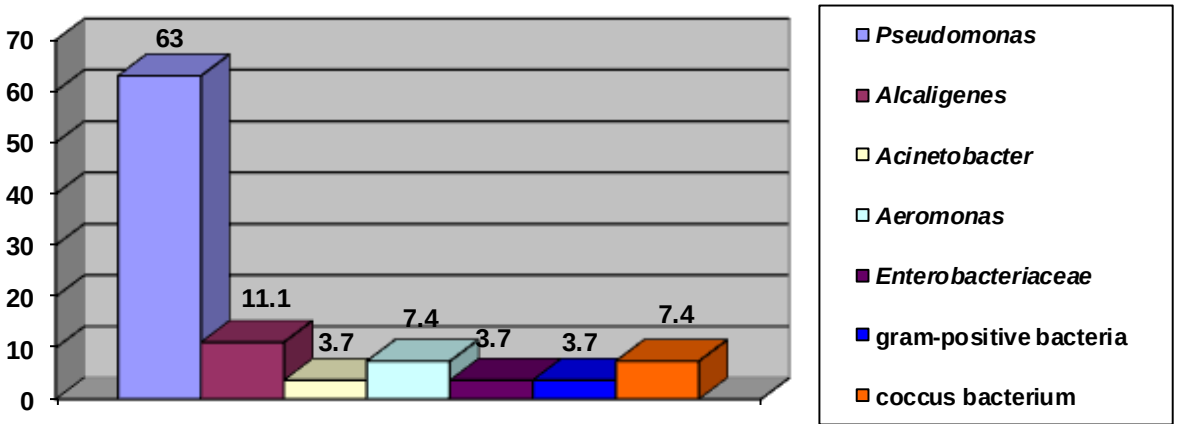

Figure 4. Composition of psychrotrophic microflora in biotope 4 (%).

The lowest amounts (3.7 %) were characterized for representatives of genus *Acinetobacter,* family *Enterobacteriaceae* and gram-positive bacteria. As noted above, the degassing of water here was conducted by means of diffuser.

The composition of psychrotrophic microflora in water after biofilter from RSA modules for growing of fry fish and keeping of commodity fish (biotope 5) was significantly different from that of the biotope 4 (Figure 5): 7.4 times more of *Enterobacteriaceae*, 4.1 times less of *Acinetobacter,* 3.5 times less of *Pseudomonas,* 2.0 times more of *Aeromonas*, 1.6 times more of gram-positive bacteria.

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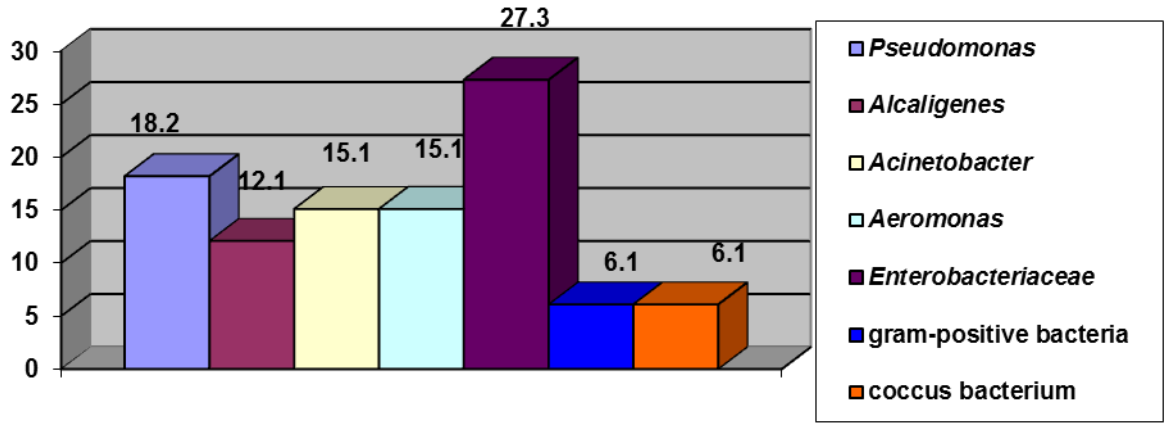

Figure 5. Composition of psychrotrophic microflora in biotope 5 (%).

Microbiocenosis of biotope 6 (filler of biofilter reactor) contained all the psychrotrophic bacteria revealed in previous biotope excluding biotope 1 (Figure 6).

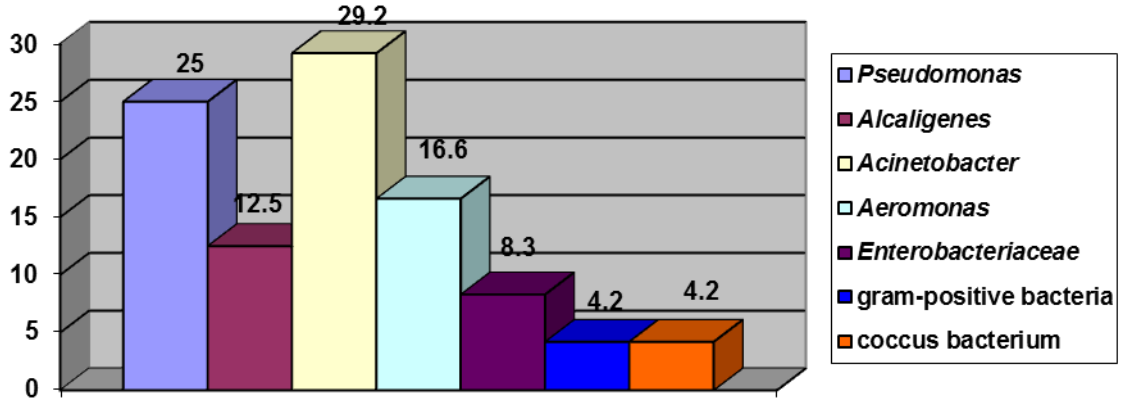

Figure 6. Composition of psychrotrophic microflora in biotope 6 (%).

Microorganisms of the genera *Pseudomonas, Alcaligenes, Acinetobacter* and *Aeromonas* are the autochthonous microflora of this biotope. They colonize biofilter filler and form the biofilm. Obviously, the effective functioning of the RSA modules will be associated with the existence of biofilm bacteria. Presence in biotope of gram-positive bacteria, coccus bacteria and microorganisms of the family *Enterobacteriaceae* in our opinion related to the life activity of fish. They are the allochthonous microflora in this biotope and transiently pass through it.

The results indicate that not all bacteria that get into the biofilter present in the composition of biofilm. Most of them are located in the biofilter temporarily in state of the plankton.

The efficiency of biological filter depends of microorganisms' ability to degrade organic and inorganic substances accumulating in water during fish growing. That is why it is necessary to create the conditions for rapid formation of biofilm containing autochthonous psychrotrophic bacteria from water taken in the wells.

CONCLUSION

In this study, we revealed data that deeper explored the microbial community composition for a production-scale freshwater RAS, expanding our understanding of the complexity of these systems. The practical implications for these essentially preliminary findings have yet to be elucidated. However, it is clear that the continued study of psychrotrophic microflora in different RAS biotopes is required and should be encouraged in order to control and manage the community of these bacteria in a RAS.

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