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Environmental Microbiota Related to Bacterial Carriage in Cultivated Trout (*Onchorhyncus mykiss* and *Salvenilus fontinalis*)

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Abstract. The recent introduction and spreading of new pathogens, along with the global climate change, has contributed to a considerable decrease in trout production. The increase of fish production presumes the avoidance and control of limiting noxious factors (Purser and Forteath, 2003; Sedgwick, 1990; Shepherd and Bromage, 1992). The presence of potentially pathogenic bacteria in fish of different age categories (n=10/ basin) and level of bacterial contamination of culture waters (12 basins) on a commercial trout farm with mixed population of rainbow and brook trout was monitored by insemination on McConkey agar and use of ID 32 E, API 20NE and API Staph galleries. Both fish and culture waters were higly polluted with bacteria, but the highest bacterial load was found in commercial trout, suggesting the vulnerability of this age category. The pottentially pathogenic bacteria for both fish (*Aeromonas hydrophila*) and humans (*E. coli*) indicated technological failure and potential fecal polution of the waters, increasing the risk for handlers and consumers.

Keywords: trout farms, bacteria, *Onchorhyncus mykiss*, *Salvenilus fontinalis*, basins

INTRODUCTION

Infectious diseases (Cahill, 1990) represent a limiting factor for the development of aquaculture. The study of diseases in fish is based on vast knowledge on constraints exerted by the aquatic vital environment, in connection with the physiology of survival in such an environment and the numerous pathogens that can cause infections and induce the immune response of the host (Boujard et al., 2002; Hardy et al., 2000; Del Rio-Rodriguez et al., 1997; Suomalainen et al., 1999). The increase of fish production presumes the avoidance and control of limiting noxious factors (Purser and Forteath, 2003; Sedgwick, 1990; Shepherd and Bromage, 1992).

The aquatic environment represents an almost inexhaustible reservoir of food bioresources that can be used by humans, still one of the most exposed to microbial pollution. The increase of fish production presumes the avoidance and control of limiting noxious factors (Purser and Forteath, 2003; Sedgwick, 1990; Shepherd and Bromage, 1992). The recent introduction and spreading of new pathogens, along with the global climate change, has contributed to a considerable decrease in trout production. Emerging pathologies in rainbow trout culture include viral diseases, e.g. infectious haematopoietic necrosis (IHN), bacterial diseases, such as lactococcosis and visceral flavobacteriosis, and parasitical diseases, e.g. proliferative kidney disease (PKD). Higher mortality rates in trout fry and fingerlings are generally induced by visceral flavobacteriosis and IHN, while the main losses in large trout during the warm season are due to lactococcosis and PKD. A good sanitary management of fish farms is essential for avoiding or limiting losses caused by emerging pathologies (Kvitt et al., 2002). Transmission of zoonotic agents to humans, through the consumption of cultured

fish is not too widespread in Europe but transmission of bacteria by means of deficient management of trout cultures, improper cleaning of the basins or inappropriate quality of the water used for cultivation of fish, could represent a potential hazard for fish farmers and fish processors, as well as for people preparing fish meals. Therefore, the development and refinement of methods to minimize the side effects of prophylaxis and environmental impacts of disease outbreaks and treatment are therefore increasingly important tasks (Bergh, 2007).

The research aimed to investigate the extent of pollution with bacteria in fish and culture waters from basins from a commercial trout farm, the epidemiological importance of such a microbial source, by identifying the role of the water in the disease chain as well as the zoonotic potential of the isolated fish specimens.

MATERIAL AND METHODS

Biological samples (n=10/basin) were obtained during winter and summer seasons from a farm with mixed cultivation of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Live fish of different age categories were collected from the culture basins, kept in plastic bags with the original basin water for transportation to the laboratory and euthanatized according to the method proposed by Yanong et al. (2007). Three measuring depth (0.1 m, 1 m and 1.7 m) were considered when sampling water from the 12 basins on the farm. Samples were taken from the water source and the evacuation channel as well. The transportation time of the samples to the laboratory did not exceed 30 min.

The samples from the gills and the hepatopancreas of the fish as well as water samples were inseminated on McConkey agar plates, incubated for 48 h at 37^0C. Following preliminary microscopy, the isolated colonies were identified by use of biochemical tests (ID 32 E, API 20NE and API Staph galleries, (bioMérieux, Inc., France). For biochemical tests all the bacterial cultures were standardized at a density of 0.5 on the McFarland scale. As adjacent tests, the oxydase test (Bactident®Oxidase test - Merck, Germany), nitrate reduction, mobility, glucose oxidation and fermentation were carried out as well. The taxonomic identification was given by the APILAB Plus software. Supplementary tests, such as.

RESULTS AND DISCUSSION

The health of the fish raised on farms mainly depends on water quality, including the level of oxygen, its contamination with chemical or biological pollutants (Svobodová et al., 1993) but also on management techniques. Within the framework of preserving the health of artificially grown fish and not only, the national Program for surveillance, prevention and control of animal diseases, of transmissible diseases, animal protection and environment protection in Romania included for 2009 several diseases of fish, mainly of salmonids. Thus, surveillance and control for viral hemorrhagic septicemia, infectious haematopoietic necrosis, infectious pancreatic necrosis, bacterial kidney disease (*Renibacterium salmoninarum,* Faisal and Eissa, 2009), furunculosis in salmonids (*Aeromonas salmonicida,* Beaz-Hidalgo et al., 2010; Dalsgaard et al., 1994), yersiniosis (*Yersinia ruckeri,* Tobback et al., 2010), and infection with *Chondrococcus columnaris* in trout were mentioned. Moreover, numerous observations have led to speculation that microorganisms, particularly bacteria, might be at least partly responsible for some mortality of salmon eggs incubating in hatchery (Bell et al., 1971), therefore, the importance of incubation or growing waters as a microbial contamination source is utmost.

Pathogenic microorganisms could be more easily spread in water partly due to the lower oxygen content and abundant presence of organic matter. The environmental influence on bacterial disease development is more pronounced in the aquatic medium, while the incubation period varies (Svobodová et al., 1993). Numerous bacteria causing diseases in fish own a zoonotic potential, their consumption therefore raising the risk of disease in human populations. Transmission of *Streptococcus iniae, Vibrio vulnificus* and *Mycobacterium marinum* by means of improper manipulation of infected fish, could represent a potential hazard for fish farmers and fish processors, as well as for people preparing fish meals (Ghittino et al., 2003).

Investigating the environment (culture waters) of farmed trout would lead to a better understanding of both fish biology and health risk for humans as risk categories (farmers, managing workers, processors) and consumers of farmed fish (Mooney et al., 1996).

The results of bacteriological exams carried out during the experiment were presented in tables 1 (fish) and 2 (culture waters).

Tab. 1.

Bacteria isolated from fish of different age categories

Isolated bacteria	**PC 0**	**PC 1 and PF 1**	**Reproduction**	**Total**
Serratia liquefaciens	4	15	2	21
Pantoea spp.	8	2		10
Enterobacter cloacae	2	4		6
Rahnella aquatilis		2	3	5
Bacillus mycoides	5			5
Staphylococcus spp	1	4		5
Serratia rubidaea	2		2	4
Serratia marcescens	2	2		4
Citrobacter freundii		2	2	4
Clostridium spp	1	2		3

Legend: PC 0 – rainbow trout ray; PC1 – yearling rainbow trout, PF1-yearling brook trout (commercial)

Tab. 2.

Bacterial species isolated from water (centralised)

Isolated bacteria	**Sampling depth**			**Total**
	0.1m	**1m**	**1.7m**	
Aeromonas hydrophila	17	7	4	28
Serratia fonticola	6		1	7
Staphylococcus spp	1	2	2	5
E. coli	1	2	1	4
Klebsiella planticola	1		1	2
Citrobacter freundii	1			1
Serratia marcescens			1	1

The most frequently isolated bacteria from fish of all age categories was *Serratia liquefaciens*. It was the most important pathogen in commercial trout but it was also found in

early stages. As fish advanced in age (reproductive category) the number of this species decreased probably due to an increasing immune response.

The bacterial load, as genera, although with no specific pathogenicity for the investigated species, isolated from fish was higher than the one isolated from culture waters. Nevertheless, in culture waters some specific fish pathogens such as the most frequent *Aeromonas hydrophila* and also potentially zoonotic pathogens (*E. coli, Clostridium spp., Staphylococcus spp.*) were found.

There was little correspondence of isolated bacterial species in fish and culture waters. Supposingly, the depth of the water, due to decreased temperature, would diminish the number of bacteria, but it was not the case in this study. The lack of cleannes of the basins and accumulation of feed residues at the bottom could increase the number of bacterial isolates.

The obtained results indicated a high bacterial pollution of the culture waters, with little correspondence to bacterial load of the fish in terms of isolated species, that suggested a lowered immunity in the younger age categories.

CONCLUSIONS

- Both fish and culture waters were higly polluted with bacteria, but the highest bacterial load was found in commercial trout, suggesting the vulnerability of this age category.
- The pottentially pathogenic bacteria for both fish (*Aeromonas hydrophila*) and humans (*E. coli*) indicated technological failure and potential fecal polution of the waters, increasing the risk for handlers and consumers.

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