
Changes of the diversity of the bacteriocenosis in the digestive tract of fish under the impact of heavy metals

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The effect of a mixture of five metals (Cu, Zn, Ni, Cr, Fe) on the bacteriocenosis of the digestive tract of rainbow trout (*Oncorhynchus mykiss*) was investigated. The results obtained indicated the mixture to have a negative effect on the quantitative composition of bacteriocenosis of the digestive tract of rainbow trout. Viable counts of bacteria decreased in the digestive tract after exposure to the mixture. Under its influence the qualitative composition of bacteriocenosis in the digestive tract of rainbow trout changed. The mixture reduced the diversity of bacteria in the digestive tract of fish. Bacteria of the genera *Flavobacterium*, *Micrococcus* and of the family *Enterobacteriaceae* disappeared from the bacteriocenosis of the digestive tract.

Key words: fish, digestive tract, bacteria, heavy metals

INTRODUCTION

Numerous toxic pollutants such as heavy metals and organic compounds are being released to the environment as a result of increasing industrialization. Heavy metals are stable and persistent environmental contaminants. Metals in aquatic environments are present in ionic forms or hydrated and in complexes with a variety of naturally occurring organic compounds. Metal toxicity depends on molecular form and speciation in solution [17]. Considerable evidence shows that the free ionic forms of several trace metals (*e.g.*, copper, cadmium, lead, zinc) are the forms most toxic to biota [8]. Metal toxicity depends on pH, suspended particles, redox potential, water hardness, organic and inorganic compounds and temperature [10].

The effects of heavy metals on the environment can be assessed by toxicity bioassays. These can be divided into two categories: (i) – tests using eucaryotic organisms, which include fish, invertebrates, and (ii) – tests that employ microorganisms or enzymes [10].

There are a lot of data on the toxic effect of separate heavy metals on hydrobionts [1, 7, 22]. It has been determined that sensitivity of fish to toxicants depends on a number of factors such as species, stage of development, age, genetic structure, etc. [13]. However, fish in early stages of development are most sensitive to toxic substances [9].

The microbial populations within the digestive tract of fish are rather dense, within numbers of microorganisms much higher than those in the surrounding water, indicating that the digestive tract provides favorable ecological niches for these organisms [4]. However, the gastrointestinal microflora of fish appears to be simpler than that of endotherms. While the digestive tract of endotherm is colonized mainly by obligate anaerobes, the predominant bacterial genera/species isolated from most fish guts have been aerobes and facultative anaerobes [4, 16]. Typical numbers of bacterial populations in fish intestines are 10^8 aerobic and facultative anaerobic heterotrophic bacteria g^{-1} and approximately 10^5 anaerobic bacteria g^{-1} [20, 21].

In salmonids, the gastrointestinal flora of juvenile and adult stages has been investigated by several authors [2, 14, 23]. The intestinal microflora of freshwater and seawater fish harbors different microorganisms [16, 23]. The intestinal microflora of freshwater salmonids is mainly composed of the bacterial genera *Aeromonas*, representatives of the family *Enterobacteriaceae*, *Flavobacterium*, *Pseudomonas* [23].

Microorganisms of the digestive tract comprise an obligatory part of all the trophic relations. Certain microorganisms exhibit the sensitivity resembling animal sensitivity to toxic substances, while other microorganisms are resistant to a toxic substances. Animals sensitive to toxic substances are able to feed on microorganisms resistant to a toxicant and exhi-

bit no response to toxic substances. Conversely, toxic substances may be responsive for the death of sensitive microorganisms in the digestive tract of animals when the concentration of the toxic substance is not dangerous to the animal, while the animal may die from a disorder of the activity of the digestive system [15].

Nevertheless, it is clear that elevated levels of heavy metals can alter the qualitative as well as the quantitative structure of a microbial community [5]. Unfortunately, we know very little about the environmental concentrations of metals able to cause these changes.

The aim of this study was to evaluate the effect of a heavy metal mixture on the aerobic and facultative anaerobic bacterial populations in the digestive tract of rainbow trout.

MATERIALS AND METHODS

Experiments on the possible effects of a heavy metal mixture (HMM) on the qualitative as well as quantitative composition of the bacteriocenosis of the digestive tract of fish were carried at the Institute of Ecology of Lithuania. The fish under study were yearlings of rainbow trout (*Oncorhynchus mykiss*) 17–18 cm of total length and 42–48 g of total weight. The fish were kept in holding tanks (1000 l capacity) under laboratory conditions before the beginning of experiments. They were fed of cattle spleen and commercial fish food. Physical and chemical characteristics of the water ranged between: water temperature 9–12°C, oxygen concentration 9–10 mg/l, pH 6.8–7.5, average total hardness 250 mg/l CaCO₃; alkalinity approximately 200 mg/l HCO₃⁻. The fish were transferred from holding tanks to 20 l aquaria for the experiments. They were kept there until they acclimated to the new environment and started swimming freely and feeding. Water in the aquaria was changed daily. The following concentrations of metals in the model mixture tested were taken as 100%: Cu – 0.002; Cr – 0.02; Ni – 0.05; Zn – 0.06; Fe – 0.3; Pb – 0.03; Cd – 0.003; Mn – 0.09 mg/l. The heavy metal concentration in 100% mixture was close to the mean annual levels of heavy metals found in discharges from the Vilnius City into the Neris River. The fish were exposed to a 100% concentration of heavy metal mixture for 48 and 96 h.

Microbiological investigations of the digestive tract of rainbow trout were carried out by methods described in [11]. Each set of experiments involved 5 fish. The fish were killed by physical destruction of brain, the surface sterilized with 95% ethanol and opened aseptically. The contents were squeezed, weighed and placed in physiological NaCl solution. At least three dilutions expected to give bet-

ween 30 and 300 colony forming units were plated on two solid media. The media chosen were beef agar (for isolation of heterotrophic bacteria) and milk agar (for proteolytic bacteria). Incubation occurred aerobically at 20 °C for 7 days. Bacterial colonies appearing on each plate were counted and the count of viable bacteria per g wet weight of intestinal contents was obtained. Proteolytic bacteria were identified according to the zones of protein (casein) hydrolysis on milk agar.

After incubation the bacterial colonies were divided into types according to colonial characteristics (shape, size, elevation, structure, surface, edge, color and opacity). The number of colonies of each recognizable type was counted in all the agar media. Three representatives of each colony type were streaked onto beef agar and purified by repeated sub-culturing on the same medium. Each strain was examined for cell morphology and biochemical capabilities. The results from the characterization were used as a basis for identifying the strains according to a modified [19] scheme of Shewan et al. [18] and Bergey's Manual of Systematic Bacteriology [3].

RESULTS AND DISCUSSION

The results of this study support the contention that a dense bacterial population occurs in the alimentary tract of rainbow trout, with numbers of bacteria higher than those obtained in rearing water (Table 1). It was revealed that in the bacteriocenosis of the digestive tract of control rainbow trout heterotrophic bacteria were predominant. The viable counts of heterotrophic bacteria amounted 10⁶ cells g⁻¹ of the digestive content (wet weight). Proteolytic bacteria in the digestive tract of control rainbow trout were less abundant (Table 1). It was established that the exposure to a mixture of heavy metals had an injurious effect on the quantitative composition of bacteriocenosis in the digestive tract of rainbow trout (Table 1). After 48 h of exposure to a 100% heavy metal mixture the abundance of heterotrophic bacteria in the digestive tract of fish decreased 7 times compared to control, while the abundance of proteolytic bacteria decreased 60 times. After a 96-h exposure the abundance of heterotrophic bacteria in the digestive tract of rainbow trout decreased further. The proteolytic bacteria disappeared. A decrease of viable counts of heterotrophic and proteolytic bacteria was observed in the water after exposure to a heavy metal mixture.

Experiments with Atlantic salmon to assess the effects of dietary exposure to copper and cadmium have shown that salmon exposed for four weeks to 35 and 700 mg Cu/kg diet had significantly elevated

Table 1. The viable count of bacteria in the digestive tract of rainbow trout and water after exposure to 100% heavy metal mixture

Time of exposure	Bacterial abundance (log g ⁻¹)			
	heterotrophic		proteolytic	
	digestive tract	water	digestive tract	water
Control	7.79 ± 0.71	3.47 ± 0.41	7.20 ± 0.44	4.47 ± 0.50
After 48 h of exposure	6.94 ± 0.61	3.00 ± 0.34	5.39 ± 0.28	4.00 ± 0.29
After 96 h of exposure	6.68 ± 0.53	3.00 ± 0.73	0	3.95 ± 0.73

intestinal copper concentrations compared to control fish [12].

A total of 100 strains of aerobic and facultative-anaerobic bacteria were isolated from the digestive tract of rainbow trout under aerobic conditions. These were composed of *Aeromonas*, *Pseudomonas*, *Enterobacteriaceae*, *Flavobacterium*, *Micrococcus*. Among them, *Pseudomonas* (23%), *Aeromonas* (37%) and *Enterobacteriaceae* (28%) were predominant. These bacteria were isolated from the digestive tract of rainbow trout regularly and in great numbers.

A comparative study conducted on the microflora associated with gills, intestine, liver and kidney of healthy rainbow trout taken at two different hatcheries in the North-West of Spain had shown that the main bacterial groups isolated from fish in both rearing facilities were *Aeromonas*, *Pseudomonas-Xanthomonas*, *Enterobacteriaceae* and Gram (+) cocci.

Members of *Vibrio*, *Flavobacterium-Cytophaga* and *Moraxella-Acinetobacter* were also detected [4].

Features of bacteria isolated from the digestive tract of control rainbow trout with their identification are shown in Table 2. Two strains of bacteria of the family *Enterobacteriaceae* were isolated from the contents of the digestive tract of rainbow trout. Both isolates reduced nitrate and one of them produced H₂S and acid from glucose, sucrose, lactose, arabinose and liquified gelatin. Bacteria of the genera *Aeromonas* and *Pseudomonas* also reduced nitrates, liquified gelatin and produced H₂S and acid from glucose and sucrose. These bacteria are oxidase-positive, while *Enterobacteriaceae* are oxidase-negative. The qualitative composition of bacterial flora in the digestive tract of rainbow trout was effected by HMM. Some differences were evident in the group of dominating bacteria from the digestive tract

Table 2. Morphological and biochemical characteristics of bacteria isolated from the digestive tract of control rainbow trout

Morphological and biochemical characteristics	Bacterial family and genera					
	<i>Enterobacteriaceae</i> (1)	<i>Aeromonas</i>	<i>Enterobacteriaceae</i> (2)	<i>Pseudomonas</i>	<i>Flavobacterium</i>	<i>Micrococcus</i>
Cell shape	rods	rods	rods	rods	rods	cocci
Motility	+	+	+	+	-	-
Oxidation/fermentation	F	F	F	A	A	A
Gram reaction	-	-	-	-	-	+
Catalase	+	+	+	+	+	+
Oxidase	-	+	-	+	+	-
Nitrate reduction	+	+	+	+	+	-
H ₂ S production	+	+	-	+	-	-
Gelatinase	+	+	-	+	-	-
Acid from: glucose	+	+	+	+	+	+
sucrose	+	+	+	+	-	+
lactose	+	-	-	+	-	-
arabinose	+	+	+	-	-	-

“+” –positive reaction; “-” – negative reaction; A – aerobic; F – facultative anaerobe.

Table 3. Morphological and biochemical characteristics of bacteria isolated from the digestive tract of rainbow trout after a 48-h exposure to a mixture of heavy metals

Morphological and biochemical characteristics	Bacterial family and genera			
	<i>Enterobacteriaceae</i> (2)	<i>Aeromonas</i>	<i>Pseudomonas</i>	<i>Micrococcus</i>
Cell shape	rods	rods	rods	cocci
Motility	+	+	+	–
Oxidation / fermentation	F	F	A	A
Gram reaction	–	–	–	+
Catalase	+	+	+	–
Oxidase	–	+	+	–
Nitrate reduction	+	+	+	–
H ₂ S production	–	+	+	–
Gelatinase	–	+	+	–
Acid from: glucose	+	+	+	+
sucrose	+	+	–	+
lactose	+	–	+	–
arabinose	+	+	+	–

Table 4. Morphological and biochemical characteristics of bacteria isolated from the digestive tract of rainbow trout after a 96-h exposure to a mixture of heavy metals

Morphological and biochemical characteristics	Bacterial family and genera	
	<i>Aeromonas</i>	<i>Pseudomonas</i>
Cell shape	rod	rod
Motility	+	+
Oxidation/fermentation	F	A
Gram reaction	–	–
Catalase	+	+
Oxidase	+	+
Nitrate reduction	+	+
H ₂ S production	+	+
Gelatinase	+	+
Acid from: glucose	+	+
sucrose	+	–
lactose	–	+
arabinose	+	+

of rainbow trout after exposure to HMM for 48 h. After exposure of fish to HMM for 48 h, one strain of bacteria belonging to the family *Enterobacteriaceae* which produced H₂S and liquified gelatin was not detectable in the digestive tract. Bacteria of the ge-

nus *Flavobacterium* disappeared, too (Table 3). Diversity of bacteria in the digestive tract of rainbow trout was reduced even more after a 96-h exposure to HMM (Table 4). Bacteria of the genera *Micrococcus*, *Flavobacterium* and family *Enterobacteriaceae* disappeared from the digestive tract of fish. In our case, bacteria of the genera *Flavobacterium*, *Micrococcus* and of the family *Enterobacteriaceae* exhibited the highest sensitivity to HMM.

The effects of heavy metals and other trace elements were tested on the fermentative activity of the rumen microflora and growth of functionally important rumen bacteria [23]. The bacteria that most frequently exhibited the highest sensitivity were *Bacteroides*, *Ruminococcus*, *Eubacterium*.

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**ŽUVŲ VIRŠKINAMOJO TRAKTO
BAKTERIOCENOZIŲ ĮVAIROVĖS KAITA VEIKIANT
SUNKIESIEMS METALAMS**

S a n t r a u k a

Tirtas sunkiųjų metalų modelinio mišinio poveikis vaivorykštinio upėtakio (*Oncorhynchus mykiss*) šiųmetukų bakteriocenozėms. Nustatyta, kad dėl sunkiųjų metalų poveikio pakinta tiek kiekybinė, tiek kokybinė virškinamojo trakto bakteriocenozė sudėtis. Heterotrofinių bakterijų gausumas sumažėjo 7 kartus, paveikus žuvis 100% koncentracijos sunkiųjų metalų mišiniu 48 val., o proteolitinių gausumas – 60 kartų, palyginti su kontrole. Po 96 val. sunkiųjų metalų poveikio tirtų žuvų virškinamajame trakte išnyko proteolitinės bakterijos, o heterotrofinių bakterijų gausumas dar labiau sumažėjo.

Ištyrus vaivorykštinių upėtakių virškinamojo trakto bakteriofloros kokybinę sudėtį nustatyta, kad vyrauja gramneigiamos genčių *Pseudomonas*, *Aeromonas* ir šeimos *Enterobacteriaceae* bakterijos. Taip pat iš upėtakių virškinamojo trakto išskirtos *Flavobacterium* ir *Micrococcus* genčių bakterijos, bet jų gausumas nedidelis. Paveikus žuvis sunkiųjų metalų mišiniu 96 valandas, jų virškinamajame trakte pakinta kokybinė bakteriofloros sudėtis. Iš virškinamojo trakto išnyko *Flavobacterium*, *Micrococcus* genčių ir *Enterobacteriaceae* šeimos bakterijos.

Raktažodžiai: hidrobiontai, virškinamasis traktas, bakterijos, sunkieji metalai