Cent. Eur. J. Biol. • 8(12) • 2013 • 1265-1271
DOI: 10.2478/s11535-013-0236-3

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Central European Journal of **Biology**

Changes in microbiota of rainbow trout caused by sediments contamination

Research Article

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Received 09 August 2012; Accepted 03 July 2013

Abstract: The abundance, composition and hydrocarbon-degrading bacteria, as possible biomarkers of contamination with oil hydrocarbons, of autochthonous and alochtonous microbiota of the digestive tract of rainbow trout have been estimated. The samples of the bottom sediments for microbiological tests have been collected and a response of natural bacterial communities in the digestive tract of rainbow trout and nutritional changes has been investigated. Experimental fish have been fed with a mixture of three substances with the aim to assess the influence of hydrocarbon-degrading bacteria contained in the sediments on the microbiota of rainbow trout's digestive tracts. The abundance values of rainbow trout intestinal heterotrophic bacteria were found to change depending on alochtonous microbiota of different bottom sediments given to the experimental fish with food *in vitro*. According to the results of our research, it is likely that the changes in the abundance values of the microbiota of the digestive tract of fish and in the proportions of functional groups of the bacteria allow us to determine changes in the functional activity of bacteria depending on food composition. Any relative increase or decrease of abundance or activity of alochtonous microbiota allows the prediction of toxic effects of the contaminants on animals and the environment.

Keywords: *Hydrocarbon-degrading bacteria • Fish • Digestive tract • Toxic effect*

1. Introduction

It is determined that crude oil which reaches the deepest marine water layers is being degraded by microbial populations very slowly due to a high pressure, and therefore the most resistant fractions of crude oil and its products survive for many years and even decades. A complete destruction without hydrocarbon-degrading microorganisms is impossible [1].

Biological degradation of hydrocarbons is actually due to the oxidation-reduction reaction where hydrocarbons release electrons and the acceptor of the electrons is reduced (joins electrons to itself). Aerobic microorganisms use only molecular oxygen (O_2) as an acceptor of electrons, whereas anaerobic microorganisms use compounds, such as nitrates, iron oxide, sulphates and carbon dioxide, as acceptors of electrons. Hydrocarbons are more rapidly degraded by aerobic microorganisms; therefore, they are often applied to clean up hydrocarbon contaminants in the environment [2].

An anthropogenic effect on aquatic organisms as filters firstly manifests through the changes in the qualitative and quantitative composition of microbiota of the digestive tract: disturbance of the balance of bacterial communities, weakening of the ferment functions of bacterioflora, and the change in evolutionary ratios between macro- and microorganisms. Crude oil and its products getting into a water ecosystem have different effects on autochtonous bacteria: they can be toxic to some microorganisms while other microorganisms can use them as the only source of carbon and energy. In such a way the ecosystem of the digestive tract fills up with hydrocarbon-degrading bacteria [3-8].

The formation of the microorganism populations in the digestive tract of fish was found to take place directly through the surrounding environment. Microorganisms compete for the feeding substrates, and with an occurrence of new substrate in the environment, quantitative and qualitative changes take place in the endo-system of the macroorganisms.

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Therefore, intensification of fermentation activity of bacteria of one or even several functional groups and at the same time inhibition or even elimination of other groups can take place. This causes changes in the metabolites excluded by the microorganisms, which affect the physiological status, productivity and the immune system of an animal. The narrower the spectrum of the qualitative composition and the abundance of microorganisms, the less are the possibilities for the microorganisms to adapt to the changing feeding substrates [9-11]. According to Nayak [9] the common bacterial colonizers in the gastrointestinal tract of freshwater and marine fish include *Vibrio, Aeromonas, Flavobacterium, Plesiomonas, Pseudomonas, Enterobacteriaceae, Micrococcus, Acinetobacter, Clostridium, Fusarium and Bacteroides,* which may vary from species to species as well as the environmental conditions.

Earlier we established that in the intestinal tract of the fish from the Curonian Lagoon (the Baltic Sea basin) oil hydrocarbons are degraded by: *Aeromonas allosacharophila, Aeromonas eucrenophila, Aeromonas media* and *Pseudomonas flavescens* [12].

Given the above we can state that crude oil hydrocarbon-degrading bacteria (HDB), as microbiological biomarkers of the contamination of the digestive endosystem of aquatic animals with crude oil and its products, allow evaluation and prediction of a toxic effect of the contaminants on animals and the environment because normal microbiota of the digestive tract of animals is a primary target for the substances to get into the animal's organism from the environment. Therefore investigation of bacterial communities of the digestive endosystem of aquatic animals is of prime importance when settling ecotoxicological, physiological and other biological problems with the purpose of maintaining a normal microbiota in the animal's organism [4-7].

A possible effect of water ecosystem sediments on the changes in the bacterial communities of the digestive tract of aquatic animals has not been sufficiently investigated. As most biogenic substances and contaminants get into marine waters from rivers and occasionally from semi-closed lagoons, the ecotoxic effect of the sediments should be estimated in the zones of river deltas. The Curonian Lagoon is a water basin linking the Nemunas River with the Baltic Sea and accepting and settling wastes from the Nemunas and other rivers. The Lagoon has two sources of the maximal concentration of crude oil hydrocarbons: the Nemunas River mouth reaches and the Klaipėda Port aquatic area. All wastes getting into small water bodies sooner or later accumulate in the ultimate wastewater basin, the Curonian Lagoon, and later get into the Baltic Sea [13,14].

The Curonian Lagoon is a shallow freshwater body which though separated from the Baltic Sea by a narrow spit, still belongs to the Sea basin. The Curonian Lagoon is a junction reservoir receiving the flow of the Nemunas River and acting as a sedimentation basin. Thus the aim of the work is to assess the effects of the ecological risk of the sediments of different habitats on the bacterioflora of the digestive tract of the fish *in vitro*.

2. Experimental Procedures

Microbiological investigations have been carried out at the Laboratory of Genotoxicology of the Institute of Ecology of Nature Research Centre, Vilnius, Lithuania. Rainbow trout (*Oncorhynchus Mykiss*) adults were obtained from the Žeimena Fish Hatchery (Švenčionys District, Lithuania) and kept in holding tanks of about 2,000 L capacity supplied with flow-through artesian aerated water at the Laboratory of Ecology and Physiology of Hydrobionts of the same Institute. Physical and chemical water characteristics were: 8–10°C water temperature, pH 7.0–7.6, 9–10 mg L^{-1} oxygen concentration and 250 mg/l ($CaCO_3$) average total hardness.

The samples of the bottom sediments were collected using a dredge of a special construction, allowing selective collection at the surface (0–3 or 0–5 cm) or at deeper (5–0 or 10–5 cm) layers. Water samples have been collected using samplers of various designs at selected sampling stations. The sediments and water samples have been placed in glass or plastic vessels, transported immediately to the laboratory and analyzed or prepared for the biotests during the same day.

The total body mass of fish ranged from 50.1±1.6 to 52.8±1.5 g. Toxic effects of the bottom sediments on adult fish have been investigated according to the method suggested by Pereira [15], with negligible changes (concerning the amount of the sediments supplement) to this method. The amount of the tested sediments added to the usual fish food (*i.e.* sediments supplement) was lowered to 1/3 of the total fish food weight. During the experiments (14 days) the collected samples of the bottom sediments were kept in a refrigerator at -20°C. The tested fish were fed daily until satiety with a mixture of three substances: bovine spleen (1/3), commercial DANA FEED fish food (1/3) and sediments supplement (1/3). Before fish feeding all the constituents have been attentively and carefully mixed. The control fish were fed with a mixture of 2 substances: bovine spleen (1/2) and commercial DANA FEED fish food (1/2).

The samples of the bottom sediments for the microbiological tests have been collected according to NATO/CCMS Pilot Study Programme [16]. They were collected from the Avandelta near the Atmata River (northern branch of the Nemunas Delta), from the Atmata River after the inflow of the Minija River (at Nida), and from the Aklas Stream (artificial channel).

For the assessment of the changes in the microbiota of the digestive tract of rainbow trout, we have examined 10 individuals per each separate habitat and control fish. All the fish were washed with distilled water, the surface having been cleaned with wool pads wetted with ethyl alcohol. That was done to prevent outside microbiota from contaminating the digestive system of the fish; the digestive system was aseptically prepared.

The populations of aerobic and facultative anaerobic heterotrophic bacteria in the digestive system of fish have been counted using a dilution plate technique [17]. The surface of the rainbow trout specimens was sterilised with 95% ethanol and then fish were dissected to remove their digestive tract. The content of the digestive tract was removed onto sterile Petri dishes. Each experiment set involved ten specimens of the fish. All digestive tract samples were weighed and placed into a test tube, and then nine volumes of diluents were added. The tenfold dilution was done serially. The least dilutions (0.1 ml), expected to give 30 to 300 colony-forming units (CFU), were plated in triplicate on solid media. Incubation was carried out aerobically at 20ºC for seven days, except for MacConkey agar plates, which were incubated at 37ºC for 7 days. The number of CFU in the digestive tract of the trout has been established on five media: tryptone soya agar (OXOID) was chosen for isolation of the total heterotrophic bacteria (THB); milk agar for proteolytic bacteria (PB) as a separate group of THB, PB were identified according to the zones of protein (casein) hydrolysis on milk agar; MacConkey agar (OXOID) was used for total coliform bacteria (TCB); starch agar with 4% (soluble) starch for amilolytic bacteria (AB) as a separate group of heterotrophic bacteria were determined according to the zones of starch agar under the action of iodine solution; the Voroshilova-Dianova agar with crude oil for HDB as a separate group of THB and the same agar without crude oil was used as a control. Bacterial colonies appearing on each plate have been counted, and a CFU per 1 g (wet weight) of the digestive tract content was obtained [18,19]. All data were analysed using standard statistical methods. Mean values, standard deviations and 95% confidence intervals have been assessed.

The average values and mean square deviations were calculated [20].

3. Results

The data of the microbiological analysis carried out in this work show that abundance of heterotrophic bacteria in fish can vary within a wide range. Having examined the microbiota of the digestive tract of the control fish *in vitro,* we distinguished THB, PB, AB, coliform bacteria (CB) and HDB; however, the spectrum of abundance values of bacteria was wide and depended on the feeding of trout. The most abundant were THB and PB, the latter being characteristic of carnivorous fish [21,22]. Less abundant were AB, and the least abundant were TCB and HDB (Figure 1). The least abundance values of all examined functional groups of HB were found in the food of the investigated fish (spleen) (Figure 2) as compared with the control and experimental fish and the silt of different habitats (Figures 1, 3-8).

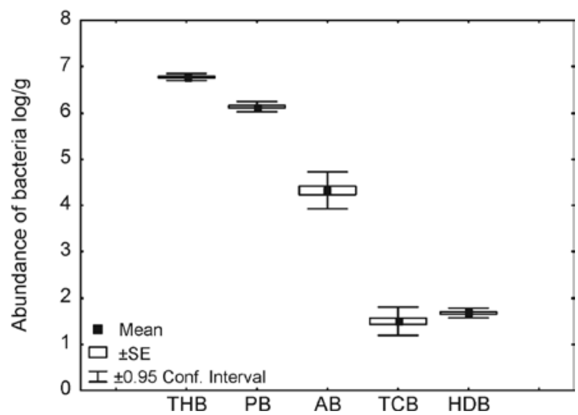

Figure 1. Abundance of bacteria in the digestive tract of the control fish having been fed a mixture of substances (bovine spleen (1/2) and commercial DANA FEED fish food (1/2)) for 14 days

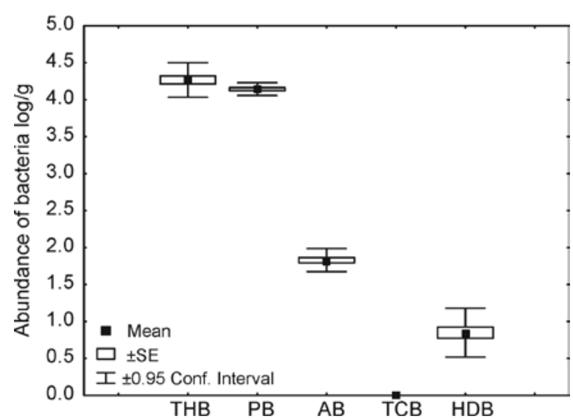

Figure 2. Abundance of bacteria in the bovine spleen.

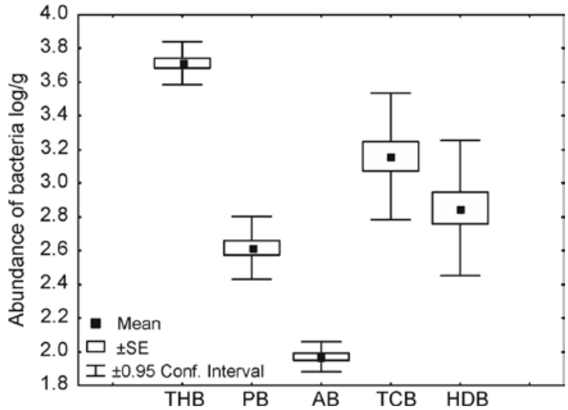

Figure 3. Abundance of bacteria in the sediments from the Avandelta near the Atmata River.

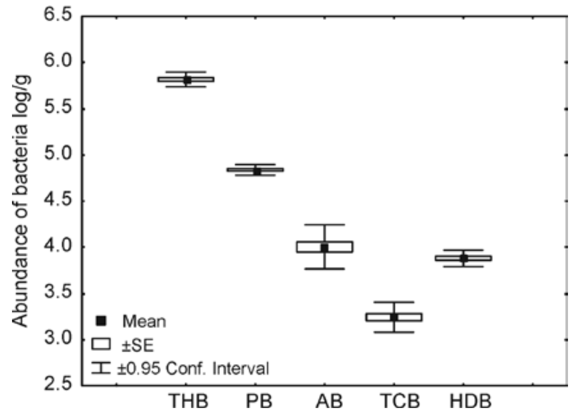

Figure 4. Abundance of bacteria in the digestive tract of trout whose food contained sediments from the Avandelta near the Atmata River.

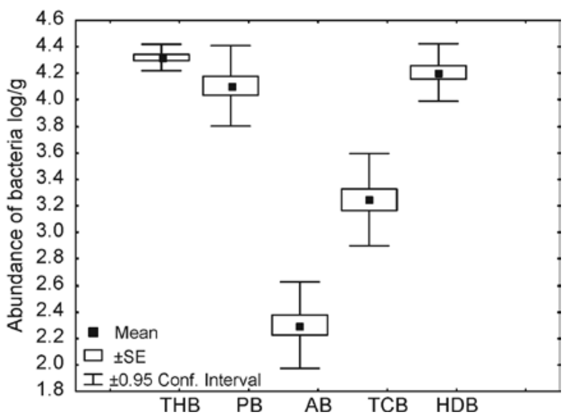

Figure 5. Abundance of bacteria in the sediments from the Atmata.

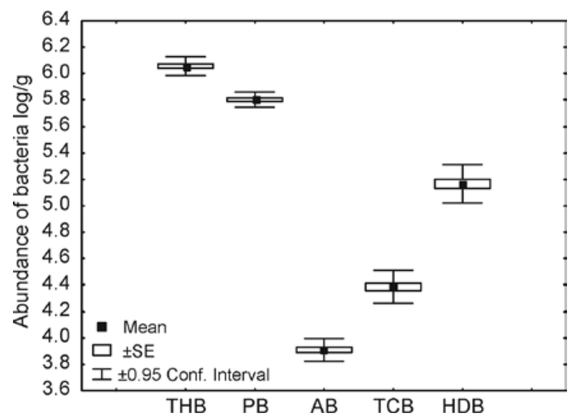

Figure 6. Abundance of bacteria in the digestive tract of trout whose food contained sediments from the Atmata.

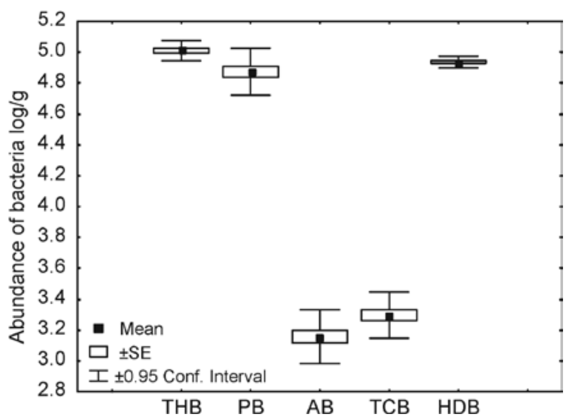

Figure 7. Abundance of bacteria in the sediments from the Aklas Stream.

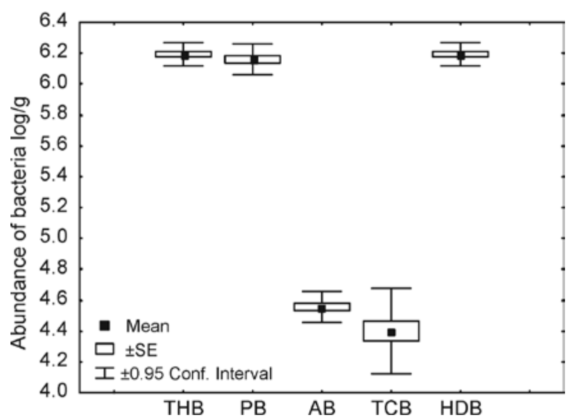

Figure 8. Abundance of bacteria in the digestive tract of trout whose food contained sediments from the Aklas Stream.

The average densities of HDB in water samples of the Curonian Lagoon ranged from 0.68 to 9.70×10^3.

In our experiment we proved that the abundance of HDB in the sediments (2.68 $\log_{10}$ n g^{-1}) (logarithm of number per gram of intestine weight) (Figure 3) and HDB (3.91 $\log_{10}$ n g^{-1}) (Figure 4) in the microbiota of the digestive tract of trout which ate the food containing sediments from the Avandelta near the Atmata River was not high; however, their proportion in THB was considerable compared with the control group (Figure 1). The abundance of HDB in the fish from the Avandelta near the Atmata River was the lowest compared with the fish from the Atmata River (Figures 5, 6) and the Aklas Stream (Figures 7, 8).

The data of our experiment revealed the following possible changes in the autochtonous microbiota of the digestive tract of the trout which ate the food containing sediments from the Atmata River: a considerable decrease of THB and PB and abundance of HDB and TCB (Figures 5, 6). The conditions and the processes of an uncontrollable entry and settlement of alochtonous microbiota in the animal endosystem might contribute to the environmental contamination with different contaminants where xenobiotic-degrading bacteria start dominating in the microbiota thus ousting natural functional groups of the microorganisms formed in the course of evolution. This could considerably decrease the feeding activity of aquatic animals and lead to the weakening of one trophic chain at the biocenotic level or the disruption of the entire network of trophic links. The greatest changes were registered in the trout which ate the food containing sediments from the Aklas Stream (Figure 7).

On the other hand it seems that associations of alochtonous bacteria formed in the endosystem of the investigated fish can help the animal's organism neutralise the toxic effect of contaminants (Figure 8).

Comparing the data percentages of HDB in the THB of bacterial communities in the digestive tract of the trout from the groups whose food contained sediments from the Avandelta (21.2%), the Atmata River (61.9%) and the Aklas Stream (83.64%) showed that the sediments from the Avandelta had the smallest effect on the changes in HDB compared with the sediments from the Atmata River and the Aklas Stream. The sediments from the Atmata River and the Aklas Stream most probably had more aromatic hydrocarbons which get accumulated through trophic chains. Besides, the concentration of organic substrate (crude oil and its hydrocarbons) regulates the intensity of bacteria growth.

According to the microbiological investigations the greatest amount of HDB and THB was found in the digestive tract of the trout whose food contained sediments from the Aklas Stream (Figures 7, 8).

4. Discussion

Bacteria get into the digestive tract of animals from the surrounding environment with food. The abundance of microorganisms in the digestive tract of aquatic animals evince that ferments synthesised by the organism are insufficient for a complete degradation of food substrates [9-11,21,22].

It is known that not all microorganisms that get into the animal's digestive tract with food or from water settle in the digestive tract. Some of them get adapted in the digestive tract and others are consumed by the ferments isolated by microorganisms. The source of nutrition of the microflora of the digestive tract is the animal's food which is digested with participation of the ferments secreted by the animal. As a result humus is forming, the composition whereof determines microorganism communities in the digestive tract. Bacteria compete for food in the digestive endosystem. The digestive endosystem contains micro-organisms which use for their nutrition the substances isolated by other microorganisms during metabolism [24-31].

Researchers have already proved that abundance of the HDB and their proportion in THB is rising when the concentration of crude oil and other hydrocarbon contaminants increases. Thus, in conclusion we can say that hydrocarbon-degrading microorganisms most probably reflect the degree of contamination of an ecosystem [32,33].

These microbiological changes in the digestive tract of fish are early indicators of the contamination with crude oil and its products [1,34,35].

A comparison of the proportion of HDB in THB showed that sediments from the Avandelta (21.2% of HDB in THB) had the smallest effect on the changes of HDB in the microbiota of the digestive tract of the fish compared with the effect of the sediments from the Atmata River (61.9% of HDB in THB) and the Aklas Stream (83.64% of HDB in THB). The greatest amount of HDB and THB was found in the digestive tract of the rainbow trout which ate food containing sediments from the Aklas Stream. HDB usually do not exceed 10% of THB in freshwater ecosystems (if the method of counting grown bacteria colonies is used). When this quantity exceeds 10%, it is suggested to consider it as an indication of permanent pollution with oil products [28].

The petroleum HDB most rapidly adapt to the environmental changes. By using petroleum hydrocarbon as the only source of carbon, HDB perform biodegradation of the pollutants, and when biodegradation products appear other functional groups of bacteria utilize the newly appeared products [34-36].

The changes in the abundance values of the microbiota of the digestive tract of fish and in the proportions of functional groups of the bacteria allow us to determine changes in the functional activity of bacteria depending on food composition. Any relative increase or decrease of abundance or activity of alochtonous microbiota possibly allows the prediction of toxic effects of the contaminants on animals and the environment.

Acknowledgements

The author would like to thank the reviewers for their helpful comments on this article.We express our sincere thanks from the Institute of Ecology of Nature Research Centre to Dr. Liongina Mickėnienė for the assistance in carrying out the experiment and Dr. Aleksandras Rybakovas for the presentation of the investigated material. Also grateful thanks are due to Dr. Zita Milda Vosylienė and Professor Anolda Četkauskaitė (Vilnius University) for the assistance in organising and carrying out the experiment and for their valuable comments.

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