

**ASPECTS REGARDING THE PROFILE OF INTESTINAL
MICROBIOTA ON WILD POPULATIONS OF STERLET
(*ACIPENSER RUTHENUS*, LINNAEUS, 1758)**

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The aim of the present research was to examine the profile of intestinal microbiota of sterlet from the Danube River. Genomic DNA was extracted from each gut fish and polymerase chain reaction (PCR) was used to amplify the conserved 16S ribosomal RNA gene. Using Denaturing Gradient Gel Electrophoresis (DGGE) of PCR amplified 16S rDNA to characterise the variability of bacterial populations, the results showed different microbial profiles for 50% of fish. These results demonstrate the potential of PCR-DGGE-based analysis for identification of gut microbiota and could contribute to a better understanding of fish ecology.

Keywords: bacterial community, sterlet, PCR, DGGE

Introduction

Denaturing Gradient Gel Electrophoresis (DGGE) is a new approach in microbial ecology; it is based on the analysis of bacterial genetic information without cultivation of the microorganisms. This molecular technique separates double-stranded DNA fragments of identical length according to the melting property of each unique DNA fragment. DGGE can be used for the analysis of PCR-amplified 16S rDNA sequences obtained from community DNA extractions. The resulting diversity patterns can be analysed and compared. The major advantage of this technique is that it allows the direct determination of bacterial genetic diversity (Rombaut *et al.*, 2001). Also, PCR-DGGE has the advantage of not requiring previous knowledge on microbial populations (Muyzer, 1998).

The microbial community of the intestine is more densely populated than the external environment, suggesting the provision of better ecological niches, favorable for the growth of microorganisms (Denev *et al.*, 2009). The importance

of intestinal bacteria in the nutrition and well-being of the host has been established for several animals and was recently demonstrated also for fish. It is known that the microbiota of fish contribute to important key functions, such as development, immunity and xenobiotic metabolism (Bates *et al.*, 2006; Navarrete *et al.*, 2012).

The intestinal microbiota of fish is comparatively less dense and diverse than humans and other endotherms (Trust *et al.*, 1974). The gastrointestinal tract of many fish species is made up of stomach, pyloric caeca and intestine. Thus, it may be expected that the bacterial microbiota along the gastrointestinal tract would be different, showing regional specialization (Sun *et al.*, 2011). It was demonstrated that herbivore microbiotas contained the most phyla (14), carnivores contained the fewest (6), and omnivores were intermediate (12) (Ley *et al.*, 2008).

The aim of our research is to characterise the bacterial profile of fish gut, using molecular techniques. As fish model, we chose the sterlet (*Acipenser ruthenus*) because of its importance for Romanian fishing and aquaculture sector.

Material and Methods

Fish sampling

Microbial communities were studied on 6 fish (A-F) from the Danube River. The fish have been caught out of area Chiscani-Gropeni, Braila county. Immediately after the catch, all fish were measured and it was weighed, observed that fish A, C, F were lighter than fish B, D, E (Table 1).

Table 1. Length and weight of fish samples

Name	Fishing area	Sampling	Length (cm)	Weight (g)
A	Chiscani Gropeni Braila	15.01.2012	42	310
B	Chiscani Gropeni Braila	15.01.2012	44	360
C	Chiscani Gropeni Braila	15.01.2012	38	300
D	Chiscani Gropeni Braila	15.01.2012	45	390
E	Chiscani Gropeni Braila	15.01.2012	48	350
F	Chiscani Gropeni Braila	15.01.2012	43	340

The skin was disinfected with alcohol (70%) before opening the fish and removing the gastrointestinal tract out of the fish using sterile instruments. The whole gut of each fish was kept on ice in sterile individual Petri dishes, during the transportation to the laboratory. Upon arrival at the laboratory, gut contents were separately collected in sterile Eppendorf tubes under a laminar flow. The samples were frozen at -80°C until analysis.

DNA extraction

DNA extraction of gut contents was performed using the DNA Stool Mini-Kit (Qiagen). The product concentration was measured by a NanoDrop ND-1000 spectrophotometer according to manufacturer's instructions. All DNA were analyzed by 1% agarose gel electrophoresis for 30 minutes at 100V. DNA

extracted from a microbial population can be used to identify the genetic diversity of the dominant populations by PCR and DGGE (Muyzer *et al.*, 1993).

PCR reaction

To avoid unspecific amplifications, the 16S rRNA gene fragments were amplified by a semi-nested PCR. There were used conserved 16S rDNA bacterial domain specific primers 27f (GTTTGATCCTGGCTCAG) and 1369r (GCCCGGGAACGTATTCACCG) for the first PCR, 968gc (CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGAACGCGAAGAACTC) and 1369r (GCCCGGGAACGTATTCACCG) for the second PCR. The PCRs were performed as follows: sample DNA was denatured at 98°C for 1 min and amplified by 20 cycles of 98°C of the first PCR containing the template, 1x Reaction Buffer, 200µM of dNTPs and 1 µl of Phire Hot Start II DNA polymerase (Finnzymes). The second PCR was performed using 1 µl of the first PCR product in a 50 µl reaction mixture containing the same reagents as the first PCR. The conditions of the second PCR were identical to the first reaction. The final PCR product was analysed on a 1% agarose gel and visualized by UV illumination after SYBR Safe (Invitrogen) staining. All PCR products were analyzed by 1% agarose gel electrophoresis for 30 minutes at 100V.

DGGE gels

Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified V6-V8 regions of the bacterial 16S rRNA gene was used to obtain a molecular fingerprint of the microbial communities in all different types of samples. Electrophoresis and staining of the gels were performed as reported previously. Briefly, assays were performed in a BioRad© DCODE Universal Mutation Detection System™. Analysis of the amplicons was performed on 8% polyacrylamide gels containing a urea-formamide gradient from 30% to 60% (a 100% urea-formamide solution consists of 7 M urea and 40% [vol/vol] formamide). PCR samples were applied to gels in aliquots of 10 µl per lane. Electrophoresis was performed in 0.5% Tris-acetate-EDTA buffer (pH 8.0) at 60°C and 85 V for 16 h. The gels were silver stained and air dried, after which it was examined. The gel was scanned at 400 dpi.

Results and discussion

Several authors have used molecular methods in the study of fish intestine (Ringø *et al.*, 2000, Ringø *et al.*, 2006, Holben *et al.*, 2002, Kim *et al.*, 2007). Microbial diversity plays a critical role in the functioning of gastrointestinal systems (Nakay, 2019). Gel electrophoresis fingerprints is a useful tool for comparing complex microbial community profiles from different environmental samples (Merrifield *et al.*, 2009; Dilly *et al.*, 2004). DGGE is undeniably a valuable approach in screening complex ecosystems on a large scale and in analyzing various environmental samples in a reduced amount of time (Diarrassouba, 2011)

The result of amplification of genomic DNA is shown in Figure 1. All of these PCR products (400 bp) showed 100 % sequence similarity to 16S rDNA sequences of different bacterial species.

Denaturing gradient gel electrophoresis analysis showed the microbial communities presented in all gut samples (Figure 2).

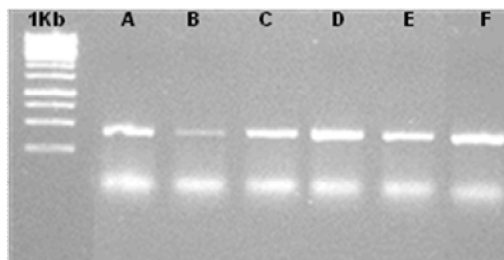


Figure 1. PCR products for all samples. The letters (A-F) indicate biological replicates

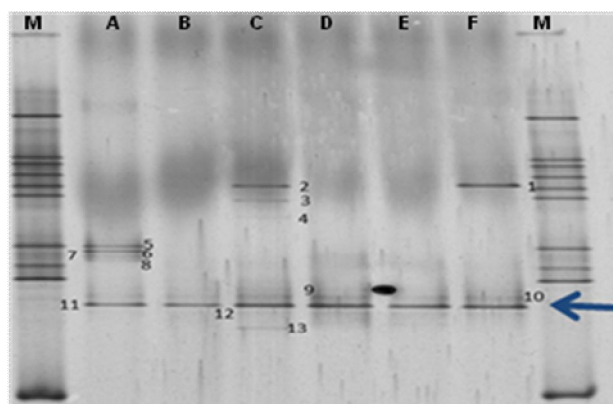


Figure 2-Denaturing gradient gel electrophoresis profiles. The letters A-F indicate the amplicons of 16S rDNA from the six fish that were analysed. The arrow indicates the position of bands present in all samples. M-marker

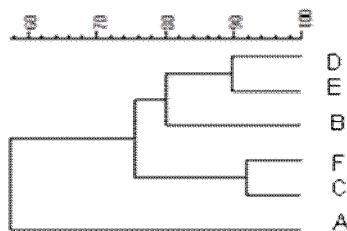


Figure 3. UPGMA dendrogram showing similarity of DGGE profiles of microbial communities in all gut samples of the study. Dendrogram was determined by calculating similarity indices of the densiometric curves of the profiles compared by using the Pearson product-moment correlation. The total similarity was 57, 29%. The letters (A-F) indicate biological replicates

The profile of DGGE for V6-V816S rRNA genes amplicons indicates from all gut samples distinct bands that appear or disappear (presumed species). The different bacterial species are present in samples ranged between 1-13. The bands number

10 and 11 are common for all samples. The bands number 5,6,7,8 are present only in fish A and the bands number 3,4,13 are present in fish C. The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram, determined by calculating similarity indices of the densitometric curves of the profiles compared by using the Pearson product-moment correlation, clearly shows that samples tend to cluster per weight, respectively the small fish B, D, E are closer and the big fish F, C and A are grouping apart (Figure 3). The presence or absence of bands are correlated with fish weight. The results indicate that 50% of fish had own personal and unique microbial profile, respectively fish A, C, F and the other 50% had almost identical microbial profile, respectively fish B, D, E. In our case the gut microbiota changed over time (for small fish) and tends to be stable (for big fish). Also, Ring (2003) and Gomez (2008) demonstrated that gut microbiota often varies with age, weight.

The sterlets main source of food is benthic organisms; they commonly feed on crustaceans, worms, and insect larvae. Frequently they eat fish eggs and fish larvae, also. This means that sterlet is almost a carnivore fish. However, it is difficult to compare all the available results from different researches. There are a lot factors that influence the profile of microbiota: sampling from different parts of the gut, different fish species, sampling location, type of feed, research methods, etc.

Conclusions

The application of molecular tools to intestinal microbiology has greatly facilitated the study of the complex microbial community in the fish gut. A not very complex profile was observed in the gut of fish. DGGE analysis of the bacterial DNA, isolated from gut, indicated that the bacterial profile is different between specimens but also they have some common bacterial populations. According to the bacterial profile determined, sterlet seems to be closer to the carnivore fish species. These results can contribute to a better understanding of fish ecology.

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