Biodiversity of gut microorganisms in aquacultured African catfish

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Abstract. The article examines the intestinal microbiome of young and adult catfish *Clarias gariepinus* raised under aquaculture conditions. Genomic DNA was isolated from the large intestine of fish. The study was conducted using the 16S Metagenomics protocol, and the library pool was sequenced on Illumina MiSeq. The microbiota is characterized by a diverse set of both aerobic and anaerobic bacteria, the qualitative and quantitative composition of which is strictly individual for each individual. The microbiota of young catfish consisted mainly of opportunistic gram-negative bacteria *Pseudomonas*, as well as gram-negative anaerobic bacteria *Cetobacterium somerae*. In adult catfish, higher biodiversity and the appearance of opportunistic gram-positive bacteria of the genus *Streptococcus* in the dominant genera were revealed; the species *Propionibacterium acnes* was also common. The study results indicate that the diversity of the gut microbiome of the catfish *Clarias gariepinus* increases over time.

1 Introduction

The volume of aquaculture production has been growing steadily in recent years, with an annual growth rate of approximately 8.5% [1-2]. One of the most popular objects is the Clarian (African) catfish due to its unpretentiousness in cultivation, the ability to be kept in pools with a high stocking density, as well as tasty dietary meat. Industrial conditions for raising fish can influence the microbiocenosis of their intestines through the use of factory feed, the process of water purification through biofilters, increased fish stocking density, strictly controlled conditions (temperature, pH) [3], the absence of pollutants that can have a negative impact on various aspects of fish life [4]. N. Kh. Sergaliev and co-authors, in the course of studying differences in the structures of microbial communities between the phases of water treatment, identified a specific microbial community characteristic of settling tanks - Pseudomonas, Cetobacterium, Lactococcus - during the "artificial wintering" period, and unclassified prokaryotes, Xanthomonadaceae and Flavobacterium - during temperature optimum in the RAS system [5]. Compared to the enormous contribution of fish species to overall vertebrate diversity, fish gut microbiota is still far

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from being fully understood and exploited [6]. A review of phylogenetic and statistical analyzes of 25 16S rRNA studies found that salinity, trophic level, and possibly host phylogeny determine the composition of fish gut bacteria [7]. Previous analyzes have focused on factors such as habitat [3, 8-10], species characteristics [11], age-related changes in the intestinal environment, and changes in dietary patterns [12]. Information on the intestinal microbiome of African catfish of different ages has not been found in the scientific literature available to us, so the purpose of this work was to analyze the composition of the microbial community of the gastrointestinal tract, in particular, the large intestine of juvenile and adult fish of the species *Clarias gariepinus*.

2 Materials and methods

The objects of our study were two groups of catfish *Clarias gariepinus*:

Group 1 - juveniles (average weight 85.3±4.88 g, body length 210.0±7.16 cm) and group 2 - adults (average weight 652.6±15.91 g, body length 430.2 ±4.79 cm) grown under aquaculture conditions (pools with a volume of 2.5 m3 using a closed water supply system).

Genomic DNA from the large intestine of fish was isolated using the DNA-EXTRAN-2 kit (Syntol, Moscow). The library pool was sequenced on Illumina MiSeq (read length - 250 bp on both sides of the fragments) using MiSeq Reagent Kit v2 (500 cycles). FASTQ files were obtained using bcl2fastq v2.17.1.14 Conversion Software (Illumina).

Automated data analysis was performed using Illumina MiSeq Reporter software (16S Metagenomics protocol). The results of high-throughput sequencing of amplicon samples obtained using primers specific to the V3-V4 region of the 16S rRNA gene were analyzed:

forward: 5'-CCTACGGGNGGCWGCAG
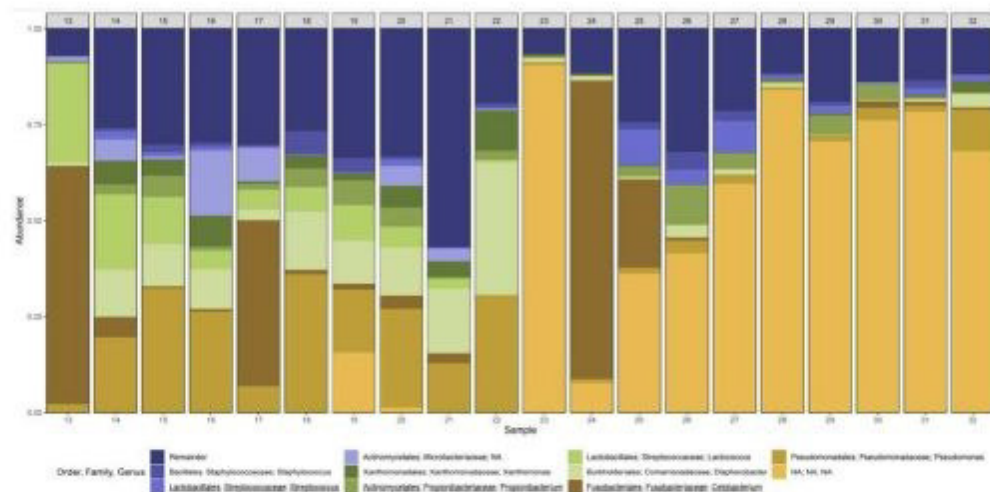reverse: 5'-GACTACHVGGGTATCTAATCC

3 Results and Discussion

As a result of sequencing, paired-end reads of 250 bp in length were obtained. Analysis of the microbiome composition was carried out in the QIIME2 environment [13]. At the first stage, adapter sequences, as well as specific primer sequences, were removed from the reads. Error correction was then performed using DADA2, the forward and reverse reads were merged, and then checked for the presence of chimeric sequences.

Taxonomic classification was performed using a naive Bayes classifier trained on the greengenes database. Visualization in the form of bar charts and heat maps is shown in Figures 1 and 2.

The microbiota of the first group includes representatives of 23 genera and 24 species. Analysis of the data obtained showed that in this group the most common microorganisms are opportunistic gram-negative aerobic bacteria of the genus *Pseudomonas* (from 2 to 31%): *P. moraviensis, P. koreensis*. The bacteria *Pseudomonas aeruginosa*, which poses a danger not only to fish but also to humans, was found in only one individual.

In two individuals from the first group, the dominant is the gram-negative anaerobic bacteria *Cetobacterium somerae*, which are capable of producing cobalamin - 36 and 55%. The next most represented are aerobic microorganisms of the genera *Diaphorobacter* and *Lactococcus* (mainly represented by the probiotic bacteria *L. raffinolactis* - 5.9%). Further, it should be noted the presence of gram-negative aerobic microorganisms *Xanthomonas oryzae*, which are found in most individuals in quantities from 3 to 8.5% and are classified as phytopathogenic, as well as gram-positive anaerobic bacteria of the genus *Propionibacterium: P. acnes* (on average 2.6%).

Fig. 1. Results of the taxonomic classification of intestinal microorganisms of two groups of catfish (samples with numbers 13-22 - group 1, samples 23-32 - group 2).

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In the second group (in adult catfish), representatives of at least 27 genera and 40 species of microorganisms were identified in the microbiota, taking into account the fact that a significant part of the microbial community could not be identified for certain reasons. Of the established genera, the leading positions are occupied by the bacteria Pseudomonas moraviensis and *Cetobacterium somerae* (1.9 and 9%), and the species *Propionibacterium acnes* (2.4%) is also common. *Diaphorobacter* and *Lactococcus* form minor groups (occurrence less than 1%). Another difference in the microbiota of adult catfish is the appearance in the dominant genera of opportunistic gram-positive bacteria of the genus *Streptococcus*: *S. tigurinus* (5.9%), S. *pseudopneumoniae* (0.59%), S. *sanguinis* (0.12%), S. *infantis* (0.11%).

In Figure 2 you can see a large number of minor groups of microorganisms, such as *Enhydrobacter*, *Psychrobacter*, *Veillonella*, *Fusobacterium*, *Actinomyces*, *Acinetobacter*, *Corynebacterium*, *Staphylococcus*, *Prevotella*, *Micrococcus*, *Sphingomonas*, etc. You can also note that bacteria of the minor groups *Rhizobiales*, *Microbacteriaceae*, *Ruminococcaceae* and *Comamonadaceae* are found in slightly greater numbers in juveniles than in adults.
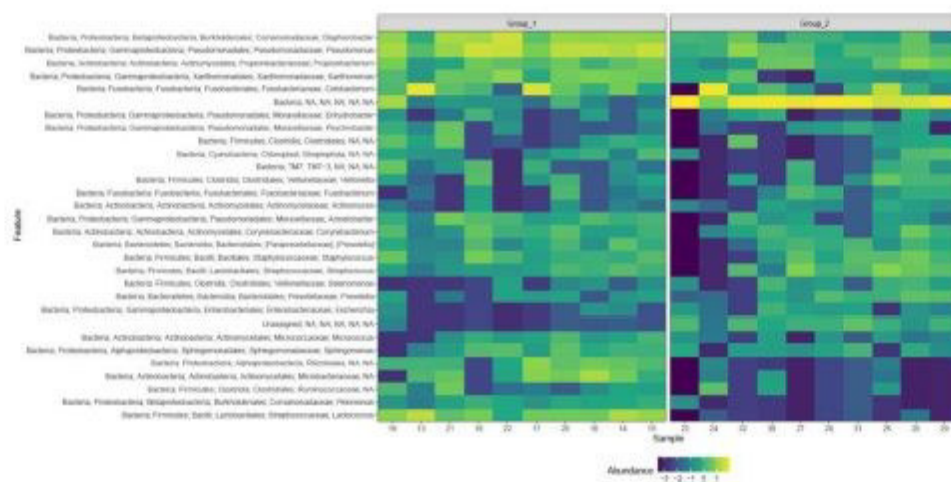
Fig. 2. OTE heat map of intestinal microorganisms of two groups of catfish.

In the process of analyzing species diversity in adult catfish, interesting facts were established: each individual in the intestine has three or four species of bacteria that were not included in the highest 8 taxonomic classifications of the remaining catfish of this group. Thus, for the first individual these are *Sporosarcina psychrophila*, *Arthrobacter crystallopoietes* and *Arthrobacter rhombi*; for the second – *Propionigenium modestum*, *Clostridium alkalicellulosi*, *Erysipelothrix muris* and *Acetobacterium fimetarium*; for the third – *Rothia mucilaginosa* and *Streptococcus sanguinis*; for the fourth - *Corynebacterium sundsvallense*, *Cupriavidus gilardii*, *Chelatococcus daeguensis* and *Escherichia albertii*, etc.

Alpha diversity analysis (Shannon index) between groups is shown in Figure 3.
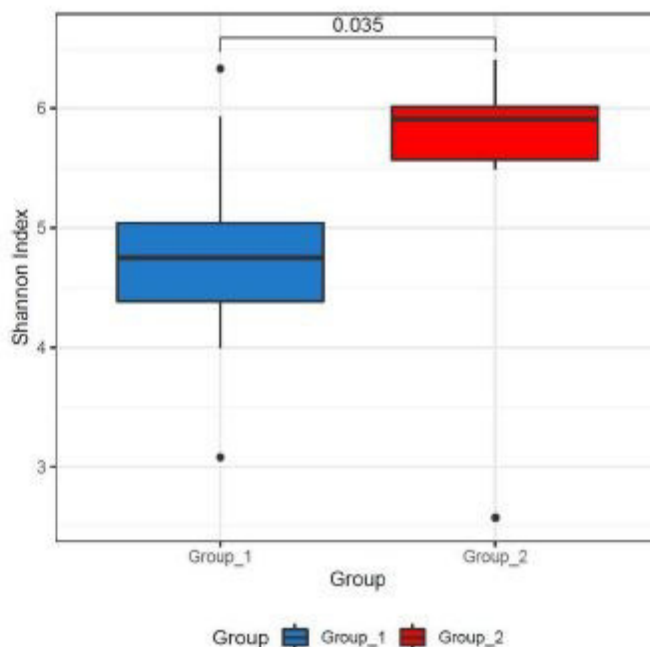
Fig. 3. Assessment of biodiversity of catfish intestinal microbiomes according to Shannon.

As can be seen from Figure 3, the biodiversity of intestinal microorganisms in the group of large catfish is approximately 1.5 times higher than that of small ones. The Shannon index fluctuates in the range of 5.6-6.0 and 4.3-5.1, respectively, i.e. Despite the fact that African catfish are kept in pools with a closed water supply and factory-fed feed, the diversity of their intestinal microbiome increases over time.

4 Conclusion

The microbiota of the hindgut of *Clarias gariepinus* grown under aquaculture conditions was studied for the first time. It is characterized by a diverse set of both aerobic and anaerobic bacteria, the qualitative and quantitative composition of which is strictly individual for each individual, but the dominant families are approximately the same, regardless of age: in juveniles - *Pseudomonadaceae, Comamonadaceae, Fusobacteriaceae, Streptococcaceae, Microbacteriaceae*; in adult catfish – *Fusobacteriaceae, Propionibacteriaceae, Streptococcaceae, Pseudomonadaceae, Veillonellaceae*.

The total number of microorganisms in adult African catfish is approximately 4 times greater compared to juveniles, and the species composition of bacteria is also significantly greater, which indicates a direct relationship between the age of the fish and the biodiversity of the intestinal microbiota.

Acknowledgement

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