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Article

Gut microbiome of juvenile coregonid fishes: comparison of sympatric species and their F1 hybrids

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With 5 figures and 3 tables

Abstract: Gut prokaryotic communities of coregonid fishes reared in the aquaria of the Baikal Museum (Listvyanka sttl., Lake Baikal, Russia) for two years were identified using next generation sequencing of 16 S rDNA. We compared pelagic planctophage Baikal omul, *Coregonus migratorius* Georgi, bathypelagic bentophage lacustrine Baikal whitefish, *Coregonus baicalensis* Dyb. and their first generation hybrid crosses (♀ omul × ♂ whitefish and vice versa). The closest prokaryotic communities were determined in the omul and ♀ omul × ♂ whitefish hybrid, which differ from whitefish and the ♀ whitefish × ♂ omul hybrid. Most of the bacteria were Proteobacteria, Bacteroidetes, Firmicutes or Actinobacteria. The number of bacterial operational taxonomic units (OTUs) was 624 and declined from the omul to hybrids and to whitefish. The dominant bacterial OTU in all examined fish was closely related to the genus *Serratia* of the Enterobacteriaceae family. In total, 34 OTUs were detected in all studied fishes, consisting of 91.4 % of the total number of sequences. The highest diversity of microorganisms was found in omul, where 197 unique OTUs were detected belonging to Chlamydiae, Chloroflexi, Deinococcus-Thermus, Fusobacteria, Spirochaetes, Synergistetes, Verrucomicrobia and Candidatus Saccharibacteria. The effect of respective ecotypes on the gut microbiome diversity is discussed.

Keywords: Lake Baikal; sympatric coregonid fishes; F1 hybrids; gut microbiome diversity; next generation sequencing

Introduction

The investigation of the influence of bacteria on their host in various mutualistic communities has been of great interest during the last decade (Benson et al. 2010; El Aidy et al. 2013; Boutin et al. 2014; Lee & Hase 2014; etc.). The relationship between gut and host physiology and pathology was conceptualized over a century ago by E. Metchnikoff (Shabrov et al. 2008), who suggested that gut bacteria are essential modulators influencing homeostasis. In addition, recent research using high-throughput sequencing proposed the presence of the following groups: a "core" microbiome (Li et al. 2013), a host genetic component influencing the development of mutualistic communities (Costello et al. 2013; Lee & Hase 2014) and a high degree of interindividual variability in the composition of bacterial communities (Costello et al. 2009; Benson et al. 2010).

Inspired by the human microbiome project, which has highlighted the human intestine as a unique microenvironment in terms of microbial diversity, analogous research has been conducted for other animals, including fish (Sanchez et al. 2012; Wu et al. 2012; Amato et

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al. 2014; Ye et al. 2014, etc.). The first work was done to study the intestinal microbiota of carp (*Cyprinus carpio* L.) (van Kessel et al. 2011). It should be noted that the initial research has been devoted to the estimation of the diversity of bacterial species (van Kessel et al. 2011; Roeselers et al. 2011; Smith et al. 2012; Wu et al. 2012). A review paper by Sullam et al. (2012) analyzed all available metagenomic data on fish at the time (25 microbiomes) and presented a comparative analysis, highlighting the biotic and abiotic factors affecting their diversity. Research conducted in recent years has been more devoted to comparative studies: the analysis of the intestinal microbiome of different fish species with similar nutritional strategies whether living in the same environment (Li et al. 2014) or in different reservoirs (Sevellec et al. 2014); the evaluation of the effect of different diets on the composition of microbial communities in the same species of wild and farmed fish (Kormas et al. 2014; Carda-Diéguez et al. 2014).

These new insights, however, raised perplexing questions about the establishment of microbial communities. What drives the variation in microbial community composition observed between sympatric species adapted to different trophic niches? How does inheritance affect the presence of "core" species in microbial communities and their colonization history? There are two sympatric species, pelagic planctophage Baikal omul *Coregonus migratorius* Georgi, and bathypelagic bentophage lacustrine Baikal whitefish, *Coregonus baicalensis* Dyb., which represent a case of sympatric postglacial whitefish divergence into pelagic and benthic ecotypes due to the occupation of different trophic niches (Sukhanova et al. 2012). The main aim of the present study was a comparison of 2-year-old specimens of omul, whitefish, and their first generation hybrid crosses (♀ omul × ♂ whitefish and vice versa) reared in similar conditions in aquaria of the Baikal Museum (Listvyanka settlement, Lake Baikal, Russia). We suggest that common-garden experiment, when fishes are reared in near identical artificial habitat conditions, is a possible way to detect associations gut microbiome composition with heredity of the host.

Material and methods

Fertilization, incubation and rearing of fish

Field works were carried out in December 2010 in Chivyrkuy Bay of Lake Baikal in the spawning areas of the fish under study (the bay, monitoring station Monakhovo, river mouths Bezymyanka, Maly Chivurkuy and Lake Arangatuy). Fish were caught with gill nets of different mesh sizes. Mature specimens were collected for artificial fertilization. The fishes were assigned to the two species according to the main diagnostic characteristics (i.e., counting the gill raker number on the first left gill arch and evaluation of the mouth position). Whitefish is a benthophage that has a subterminal mouth and 25–33 gill rakers on the first gill arch (Skryabin 1969). Omul is a planktophage with a terminal mouth and 37–51 gill rakers (Smirnov & Shumilov 1974). The whitefish individuals had a typical subterminal mouth and the number of gill rakers varied from 25 to 31 (28 on average). As for omul individuals, they belonged to the littoral-pelagic morpho-ecological group, had a typical terminal mouth and the number of gill rakers varied from 40 to 49 (44 on average). Four adult individuals were randomly collected in each species. The mean fork length and body mass for omul were 362 mm (SD = 41 mm) and 495 g (SD = 193 g), respectively. The mean fork length and body mass for whitefish were 413 mm (SD = 34 mm) and 721 g (SD = 200 g), respectively. Artificial fertilization and incubation of pure omul and whitefish and hydrids (♀ omul × ♂ whitefish and vice versa) was conducted in accordance with Chernyaev et al. (1987). Eggs and semen were stripped from living fish in the field, fertilized, transported to Irkutsk and incubated till hatching (April–May 2011). Artificially fertilized eggs were obtained in four replications. The incubation of eggs and rearing of fish was performed in the Joint Instrumentation Centre "Freshwater Aquarium Complex" (FAC) of the Limnological Institute of the Siberian Branch of the Russian Academy of Sciences (SB RAS) and the Baikal Museum of the Irkutsk Scientific Centre SB RAS (Glyzina et al. 2012). The experiment was conducted in temperature-controlled aerated flow-through system with water pumped from Lake Baikal and ambient lighting with a natural photoperiod. The water quality was regularly controlled by the Certified Laboratory of Hydrochemistry and Atmosphere Chemistry LIN SB RAS. The pH ranged between 7.1–7.5. Weiss jars (0.5 L) were used for egg incubation. Water (temperature 2.0–5.0 °C) entered the Weiss jars under pressure from below and kept the eggs suspended. All egg batches were incubated in the same flow-through system and were thus subjected to compartments of the same environment. Morbid eggs or embryos were removed on a regular basis. After hatching, free-swimming larvae were transferred into 37 L aquariums (50 × 25 × 30 cm) with a thermostatically controlled submerged heaters (300 W) and the flow-through system (1.4 L min^{-1}). The temperature was kept at 6 °C for the first 4 weeks. Larvae were fed *ad libitum* with *Artemia salina* nauplii and complemented with commercial fish food (Aller Futura EX, Aller Aqua Company). After four weeks, fingerlings were transferred into 240 L aquaria (60 × 50 × 80 cm) with the flow-through system (1.4 L min^{-1}). The temperature was raised to 12 °C for 3 weeks and finally maintained at about 12 °C. Fingerlings were fed with commercial fish food (Aller Futura EX, Aller Aqua Company).

Sample collection

Two-year-old fish at the same development stage were sampled in mid April 2013. Individuals chosen for analysis were well developed and in good general shape (vs. slow growing and meagre, as observed in some specimens). They were pure omul, pure whitefish and their first generation hybrid crosses (♀ omul × ♂ whitefish and vice versa). Five specimens of each group of fish were randomly chosen for analysis. The average weights of the sampled fishes were: omul 14.3 ± 4.9 g, whitefish 31.6 ± 10.2 g, hybrid ♀ whitefish × ♂ omul 20.8 ± 4.6 g and hybrid ♀ omul × ♂ whitefish 26.7 ± 10.6 g.

Fish were killed by a sharp blow to the head and dissected in laboratory conditions. Sterile latex gloves or latex gloves wiped down with 99 % ethanol were used throughout the dissection of digestive tract samples. All nondisposable tools, blades and forceps were rinsed with ethanol and heated over an alcohol burner between samples. Fish skin was rinsed with ethanol and the ventral belly surface was opened with a sterile surgical blade and forceps. Fat deposits surrounding the gastrointestinal tract were gently removed and fragments of hindgut were individually stored in a sterile Eppendorf© tube and flash-frozen at –20 °C until further processing.

DNA isolation and high-throughput 16 S analysis

The genomic DNA of hindgut from each individual fish from the same group was extracted with the commercial kit, DNA-sorb B according to the manufacturer's protocol (AmpliSens, Moscow) and pooled together to construct a single library. In total, four libraries were analyzed.

The V3-V4 region of the 16 S rRNA genes was amplified with the primer pair 343 F (5'-CTCCTACGGRRSGCAGCAG-3') and 806 R (5'-GGACTACNVGGGTWTCTAAT-3') combined with Illumina adapter sequences, a pad and a linker of two bases, as well as barcodes on the primers (Caporaso et al. 2011). PCR amplification was performed in 50 µL reactions containing 0.7 U Phusion Hot Start II High-Fidelity and 1× Phusion GC buffer (Thermo Fisher Scientific), 0.2 µM of each forward and reverse primers, 10 ng template DNA, 2.3 mM $MgCl_2$ (Sigma-Aldrich) and 0.2 mM of each dNTP (Life Technologies). Thermal cycling conditions were as follows: initial denaturation at 98 °C for 1 min, followed by 30 cycles of 98 °C for 15s, 62 °C for 15s and 72 °C for 15s, with the final extension at 72 °C for 10 min. A total of 200 ng amplicon from each sample was pooled together and purified through MinElute Gel Extraction Kit (Qiagen). Sample libraries for sequencing were prepared according to the MiSeq Protocol (Illumina) and protocols described previously (Caporaso et al. 2011; Caporaso et al. 2012). Sample denaturation was performed by mixing 4.5 µl of combined PCR products (4 nM) and 4.5 µl 0.2 M NaOH. Denatured DNA was diluted to 14 pM and 510 µl mixed with 90 µl of 14 pM Phix library. A total of 600 µl sample mixture, together with customized sequencing primers for forward, reverse and index sequences were loaded into the corresponding wells on the reagent cartridge of the 500-cycle PE kit and run for 2 × 250 bp paired-ends sequencing on MiSeq Illumina sequencer at the SB RAS Genomics Core Facility (ICBFM SB RAS, Novosibirsk, Russia).

Bioinformatic and statistical analysis

Contigs between pairs of raw Illumina fastaq files were assembled and quality filtered with Mothur 1.31.2 (Schloss et al. 2009). Any contigs with ambiguous sites (i.e. N) were removed, as well as sequences shorter that 330 bp. De-multiplexing, quality control, chimera checking, operational taxonomic unit (OTU) binning and taxonomy assignment were performed using quantitative insights into microbial ecology (QIIME) (Caporaso et al. 2010). After OTU picking, 89246 sequences remained. To determine the statistical significance level of OTU representation at a cluster distance of 0.03 bootstrap analysis (resampling with a return) was done for all samples. The analysis of each sample was taken on the 17,978 sequences that corresponds to the sample with the lowest coverage (17,978 reads, hybrid ♀ omul × ♂ whitefish). Resampling was performed 100 times. As a result, retrieving data of OTUs at cluster distance 0.03 were obtained for each sample. On the basis of this data the most probable (modal) number of OTUs and 90 % confidence interval were determined. The rarefaction analysis as well as the Shannon, Chao1 and ACE diversity indices were calculated for each sequence library. Taxonomy for the environmental sequences was assigned to the representative sequence of each OTU using the RDP classifier. The most detailed taxonomic level assigned to an OTU's representative sequence at a confidence of greater than or equal to 0.97 was taken as the taxon of the OTU. Beta diversity was estimated by computing weighted and unweighted UniFrac distances between libraries using QIIME. Libraries were clustered based on their between-sample distances using UPGMA, and jackknifing was performed by resampling 1,000 times with replacement at a depth of 10,000 sequences per sample. In order to identify the degree of difference between the samples UniFrac indexes were estimated with «phyloseq» software in an R package (McMurdie & Holmes 2013). The input data for «phyloseq» package were the results of the Mothur calculations and source aligned DNA sequences. The clustering of samples based on the UniFrac distances was conducted with «phangorn» package in an R (Schliep 2011) using the UPGMA method. The reliability of clustering was performed using a bootstrap analysis as follows: 1) the original dataset was resampled to generate 100 replicates with Mothur software; 2) UniFrac metric values were calculated for each of the 100 replicates; 3) the resulting 100 dendrograms displaying differentiation between samples were generated using UPGMA algorithm; 4) finally, a consensus dendrogram was constructed with the «phangorn» package and bootstrap values were added on branch nodes. A Venn diagram was generated using custom Perl scripts. In the present study, data preprocessing, OTU-based analysis and hypothesis testing were performed on Mothur (Schloss et al. 2009).

Results

In total, 89,246 valid nucleotide sequences and 624 OTUs were retrieved from the four libraries through metagenome sequencing. These sequences/OTUs were assigned to 13 different bacterial phyla (Table 1).

Analysis of hindgut microbiome library diversity

In this study, between 17,978 and 36,037 sequences and 168 and 311 OTUs were retrieved from the four libraries (Table 2). The richness indices of ACE and Chao1 varied between 397 and 976, and 299 and 593, respectively. The Shannon diversity index was between 1.01 and 1.68, with the lowest value in the ♀ whitefish × ♂ omul library and the highest one in the whitefish library (Table 2). The rarefaction curves did not tend to reach the saturation plateau, except in the whitefish library (Fig. 1). The slope of the curves from the hindgut of omul and hybrids is higher compared to the curve for whitefish, indicating more diverse

Table 1. Representative bacterial phyla in four hindgut microbiome libraries obtained from omul, whitefish and their F1 hybrids.

Bacterial phylum	Sequences		OTUs	
	Total number	%	Total number	%
Proteobacteria	74174	83.11	300	48.08
Bacteroidetes	8953	10.03	42	6.73
Firmicutes	3427	3.84	120	19.23
Actinobacteria	1200	1.34	72	11.54
Spirochaetes	1095	1.22	6	0.96
Verrucomicrobia	83	0.09	7	1.12
Candidatus Saccharibacteria	53	0.06	11	1.76
Fusobacteria	47	0.05	5	0.80
Chloroflexi	23	0.03	6	0.96
Synergistetes	6	0.01	1	0.16
Acidobacteria	5	< 0.01	3	0.48
Deinococcus-Thermus	5	< 0.01	1	0.16
Chlamydiae	2	< 0.01	1	0.16
unclassified	173	0.19	49	7.85

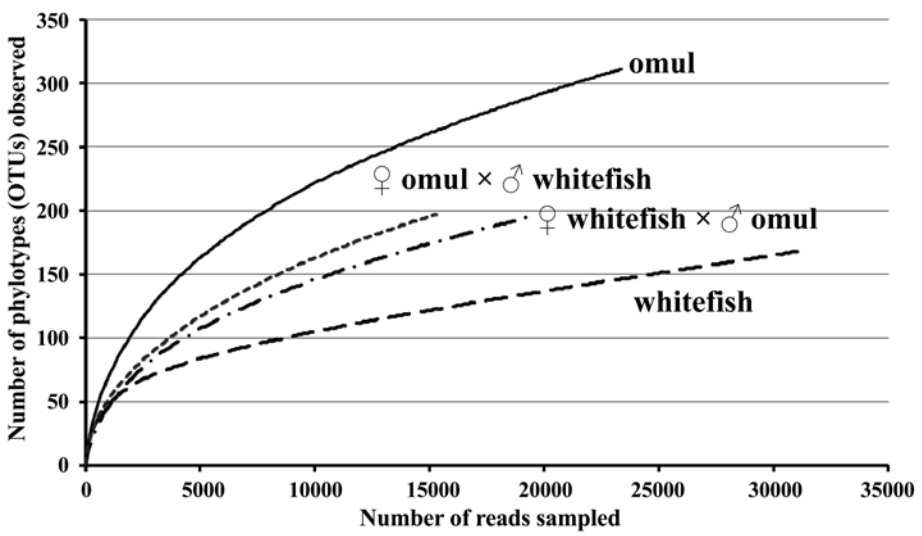


Fig. 1. Rarefaction curves of bacterial 16 S rDNA sequences for different hindgut libraries. OTUs are identified using 97% cutoffs.

communities than the microbiota from whitefish hindgut. Moreover, the curve for F1 hybrid ♀ omul × ♂ whitefish is closer to omul compared with the curve for F1 hybrid ♀ whitefish × ♂ omul which is closer to whitefish. The highest OTU number according to OTU-based alpha diversity analysis was observed in the microbiome of the omul hindgut, while the lowest one was detected in the whitefish microbiome. The estimation of most probable number of OTUs accordingly to the sample with the lowest coverage enforced these results, indicating the clustering dichotomy of omul microbiome with hybrid ♀ omul × ♂ whitefish, then hybrid ♀ whitefish × ♂ omul, while the whitefish microbiome was the most divergent (Table 2). These clustering pattern obtained with the weighted and unweighted UniFrac distances partly coincided with group definition (Fig. 2): the bootstrap analysis strongly supported the clustering OM and OM×WH.

The species rank abundance showed that 34 OTUs, including dominant ones, were shared among all hindgut libraries (Fig. 3). While 62 and 42 OTUs were shared between two and three of the libraries, the highest numbers (17, 19 and 21) were shared between the omul and F1 hybrid microbiome libraries, while

Table 2. Alpha diversity of bacteria associated with hindgut microbiome of omul, whitefish and their F1 hybrids.

Fish name	Number of sequences	Number of OTUs	Most probable number of OTUs	90 % confidence interval	Diversity indexes		
					ACE	Chao1	Shannon
Omul	28426	311	606	586–625	569	496	1.62
Whitefish	36037	168	153	143–167	976	593	1.68
♀ whitefish × ♂ omul	23094	196	350	338–368	484	382	1.01
♀ omul × ♂ whitefish	17978	197	459	442–478	397	299	1.42

the lowest (4 to 9) between the whitefish and the F1 hybrids (Fig. 3). A total of 486 OTUs occurred in only one of the four libraries. The omul, whitefish, and F1 hybrid ♀ whitefish × ♂ omul, ♀ omul × ♂ whitefish hosted 197, 96, 93 and 100 unique OTUs, respectively.

Taxonomic composition

The bacterial patterns of all hindgut microbiomes were very similar at the phylum level (Fig. 4), consisting of 7 to 9 phyla, where Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria were the most important groups, amounting to 89.8, 14.2, 5.2 and 4.2 % of the total sequences, respectively. All microbiome communities differed only in the composition of minor bacterial taxa whose abundance varied from 0.01 to 2.4 %. The minor bacterial groups of whitefish and hybrids showed relatively simple diversities, and they shared only representatives of phylum Spirochaetes. Deinococcus-Thermus, Fusobacteria and Chlamydiae were detected either in whitefish or in hybrids. More variability in minor bacterial taxa was observed between omul and hybrids. Verrucomicrobia, Chloroflexi, and Synergistetes were identified only in omul microbiome, while Acidobacteria and Candidatus Saccharibacteria were shared in omul and hybrids.

The most abundant OTU (97 % similarity) was *Serratia* which varied insignificantly among the different microbiome libraries from 60.96 to 79.48 % (Table 3). Core OTUs in the hindgut microbiome of omul included sequences mostly similar to *Porphyromonas* (6.02 %), *Achromobacter* (5.11 %), *Rhodobacter* (2.77 %), and Rhodobacteraceae (unspecified; 1.06 %). The whitefish library was dominated by sequences most closely related to Chitinophagaceae (unspecified; 6.73 %), *Achromobacter* (6.25 %), *Sediminibacterium* (5.68 %), *Brevinema* (2.22 %), *Prevotella* (1.62 %), *Caulobacter* (1.31 %) and *Sphingomonas* (1.24 %). The ten most abundant OTUs associated with hybrid libraries were very different (Table 3). The most abundant OTUs in the hybrid ♀ whitefish × ♂ omul library were those sequences similar to *Achromobacter* (4.77 %) and *Porphyromonas* (4.07 %). On the other hand, the hybrid ♀ omul × ♂ whitefish library was dominated by sequences related to *Pseudomonas* (7.49 %), *Achromobacter* (3.27 %), *Porphyromonas* (2.64 %), *Brevinema* (1.11 %) and Proteobacteria (unspecified; 1.14 %). The abundance of the top 10 dominant OTUs varied from 85.79 (omul) to 92.46 % (hybrid ♀ whitefish × ♂ omul).

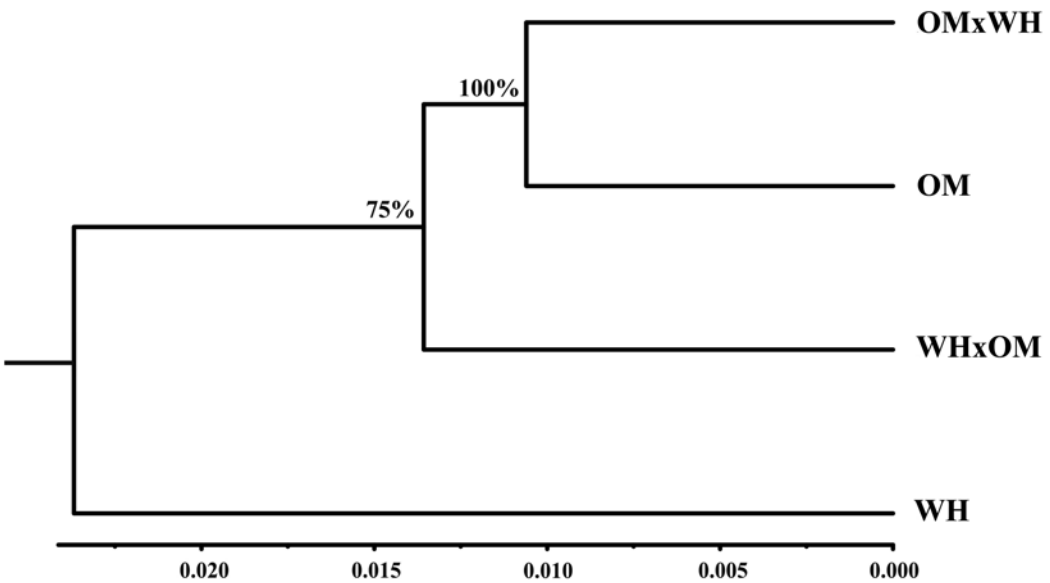
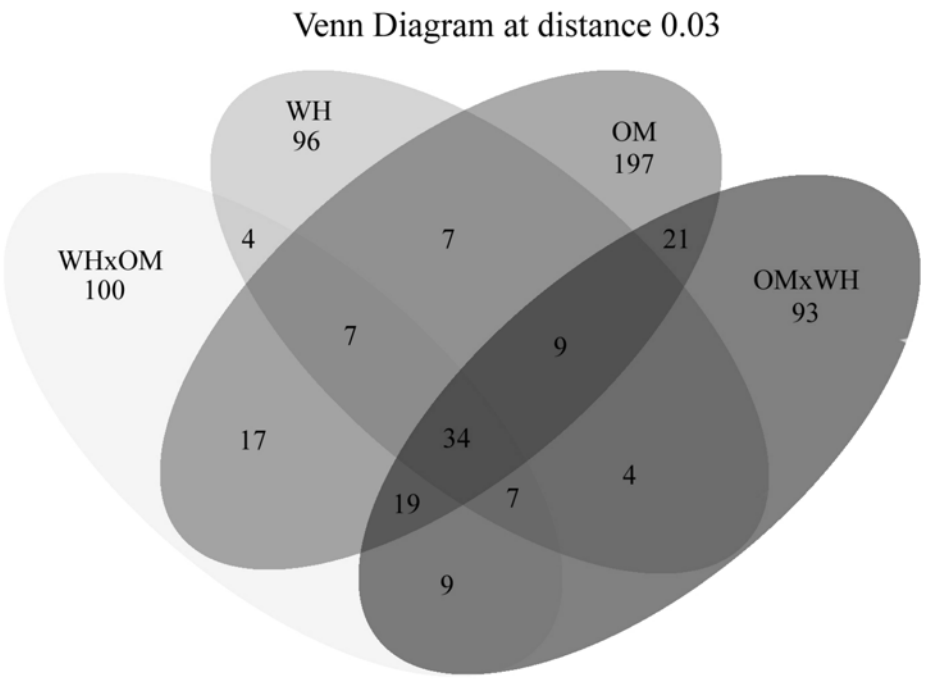


Fig. 2. Dendrogram analysis based upon UPGMA clustering hindgut microbiome libraries according to microbial community composition. Groups are defined with the weighted and unweighted UniFrac distances. OM – omul, WH – whitefish, F1 hybrids: WHxOM – ♀ whitefish × ♂ omul and OMxWH – ♀ omul × ♂ whitefish.

Table 3. Most detailed taxonomic level assigned to the 10 most abundant bacterial phylotypes associated with the hindgut microbiome libraries, listed from most to least abundant. Relative abundance (%) of each OTU is included in parentheses.

	Omul	**Whitefish**	**♀ whitefish × ♂ omul**	**♀ omul × ♂ whitefish**
1	*Serratia* (66.45) Otu001	*Serratia* (64.46) Otu001	*Serratia* (79.48) Otu001	*Serratia* (60.96) Otu001
2	*Porphyromonas* (6.02) Otu003	Chitinophagaceae (6.73) Otu009	*Achromobacter* (4.77) Otu002	*Pseudomonas* (7.49) Otu004
3	*Achromobacter* (5.11) Otu002	*Achromobacter* (6.25) Otu002	*Porphyromonas* (4.07) Otu003	*Achromobacter* (3.27) Otu002
4	*Rhodobacter* (2.77) Otu059	*Sediminibacterium* (5.68) Otu007	Proteobacteria (0.72) Otu028	*Porphyromonas* (2.64) Otu003
5	Rhodobacteraceae (1.06) Otu078	*Brevinema* (2.22) Otu012	*Prevotella* (0.70) Otu005	*Brevinema* (1.11) Otu012
6	*Sediminibacterium* (0.94) Otu007	*Prevotella* (1.62) Otu005	*Sediminibacterium* (0.68) Otu007	Proteobacteria (1.14) Otu028
7	*Amaricoccus* (0.95) Otu287	*Caulobacter* (1.31) Otu014	Chitinophagaceae (0.65) Otu009	*Brochothrix* (0.83) Otu063
8	Chitinophagaceae (0.85) Otu009	*Sphingomonas* (1.24) Otu035	*Peptococcus* (0.57) Otu016	Chitinophagaceae (0.80) Otu009
9	*Nakamurella* (0.85) Otu011	*Staphylococcus* (0.64) Otu013	*Clostridium* (0.46) Otu043	*Clostridium* (0.67) Otu043
10	*Prevotella* (0.79) Otu005	Rhodobacteraceae (0.21) Otu078	Rhodobacteraceae (0.36) Otu078	*Prevotella* (0.61) Otu005



The number of species in group OM 311
The number of species in group WH 168
The number of species in group OMxWH 196
The number of species in group WHxOM 197
The number of species shared between groups OM and WH is 57
The number of species shared between groups OM and OMxWH is 83
The number of species shared between groups OM and WHxOM is 77
The number of species shared between groups WH and OMxWH is 54
The number of species shared between groups WH and WHxOM is 52
The number of species shared between groups OMxWH and WHxOM is 69
The number of species shared between groups OM, WH and OMxWH is 43
The number of species shared between groups OM, WH and WHxOM is 41
The number of species shared between groups OM, OMxWH and WHxOM is 53
The number of species shared between groups WH, OMxWH and WHxOM is 41
The total richness of all the groups is 624

Fig. 3. Venn diagram of the average number of shared and unique operational taxonomic units of Bacteria in omul (OM), whitefish (WH), F1 hybrid ♀ whitefish × ♂ omul (WH × OM), and F1 hybrid ♀ omul × ♂ whitefish (OM × WH) hindgut libraries.

Discussion

This study analyzed hindgut microbiome communities in omul, whitefish and their first generation hybrid crosses (♀ omul × ♂ whitefish and vice versa) reared in aquaria at the same conditions of feeding, light intensity and water temperature, in order to investigate differences in gut microbial communities and detect frequently co-occurring microorganisms. For the first time, next generation sequencing (NGS) techniques were used for the study of the gut microbiome of juvenile coregonid fish and their F1 hybrids that, as yet, had only been studied within the lipid composition in muscle and liver (Vasilieva et al. 2016), morpho-functional peculiarities of erythrocytes (Yakhnenko et al. 2016) and the morphological diversity of ultrastructure of sensor auditory saccular epithelium (Sapozhnikova et al. 2017).

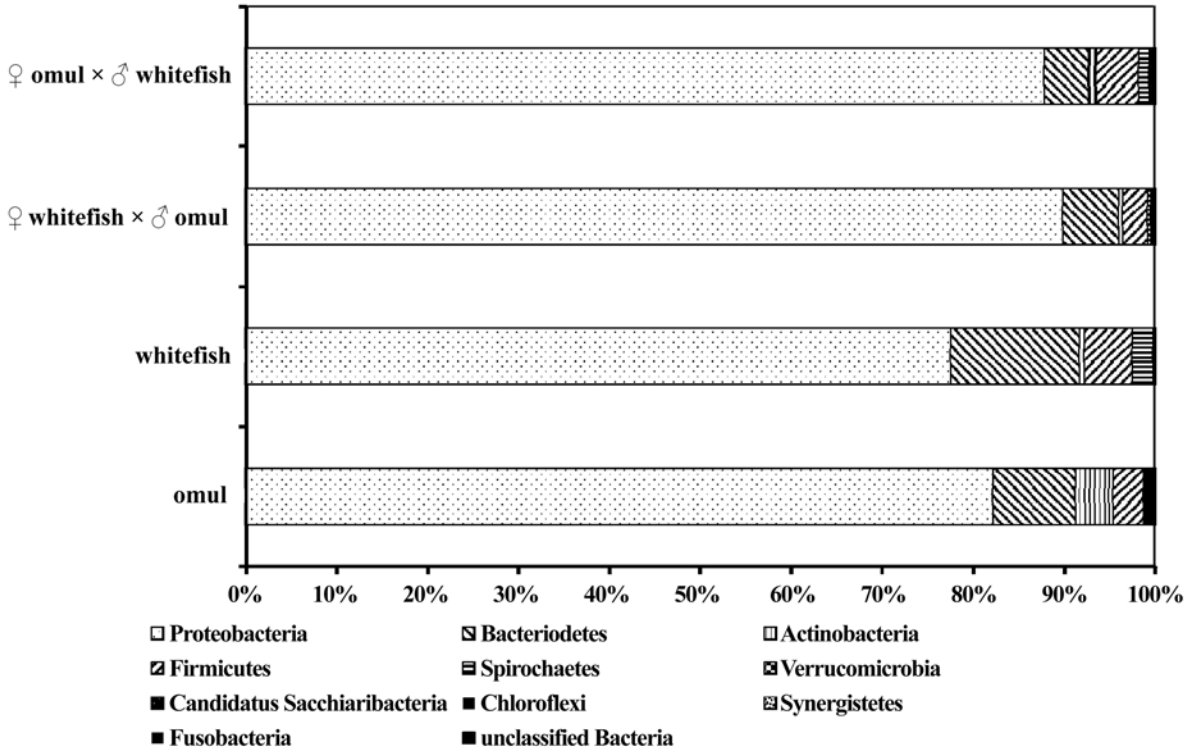


Fig. 4. Relative abundance of different bacterial phyla within the different microbiome communities. Phyla with total number of sequences less than 0.01 were not included in the analysis. Sequences that could not be classified into any known group were assigned as "unclassified Bacteria".

Comparative analysis of the microbiota associated with fishes inhabited or rared in the same environments allowed to find "core gut microbiome" as well as an individual phylotypes which might be essential for host organism and specify differences between fish species and microbiota (Skrodenyte-Arbaciauskiene et al. 2008; Smriga et al. 2010; Larsen et al. 2013; Carda-Diéguez et al. 2014; Kormas et al. 2014; Larsen et al. 2014; etc.). In order to get information on a microbiota diversity and its variety between different fish species, pooled samples from individual fish specimen were used for analysis of culturable microorganisms (Skrodenyte-Arbaciauskiene et al. 2008), clone libraries (Smriga et al. 2010) or high-thoughtput sequencing (Larsen et al. 2014). We used polled samples to evaluate "core gut microbiome" of the juvenile fishes and their F1 hybrids and to increase a diversity of minor phylotypes.

The bootstrap estimation of the clustering dichotomy revealed the significant differentiation of juvenile fishes and their F1 hybrids, the omul differed from ♀ omul × ♂ whitefish hybrid as well as whitefish differed from the others with 100 % probability (Fig. 2). The diversity index analysis between microbiome libraries showed the lowest OTU number in library of juvenile whitefish (Table 2). The differentiation between F1 hybrids ♀ whitefish × ♂ omul and ♀ omul × ♂ whitefish was detected suggesting that the inheritance of host fish influence microbiome communities at least in the pair of omul and hybrid ♀ omul × ♂ whitefish. The results obtained from estimation of most probable number of OTUs (Table 2) as well as from UPGMA dendrogramm (Fig. 2) revealed clustering dichotomy of microbiome communities, where omul and hybrid ♀ omul × ♂ whitefish formed distinctive cluster with a strong bootstrap support. Then, differenciation between WH × OM and WH is weakly supported with 75% bootstrap value.

The diversity indexes of Chao1 and Shannon showed lower values in comparison with other investigated wild and farmed fish species: Asian silver carp and gizzard shad (Ye et al. 2014), various fish species collected from Japan's coastal waters (Asakura et al. 2014), sea bass fed functional diets (Carda-Diéguez et al. 2014), in wild, organically-, and conventionally reared sea bream (Kormas et al. 2014), bighead carp (Li et al. 2014), but not with paddlefish (Li et al. 2014). The OTUs which occurred in only one microbiome had low relative abundance (0.6–6.4 %), suggesting their specific role at least in terms of dominance in

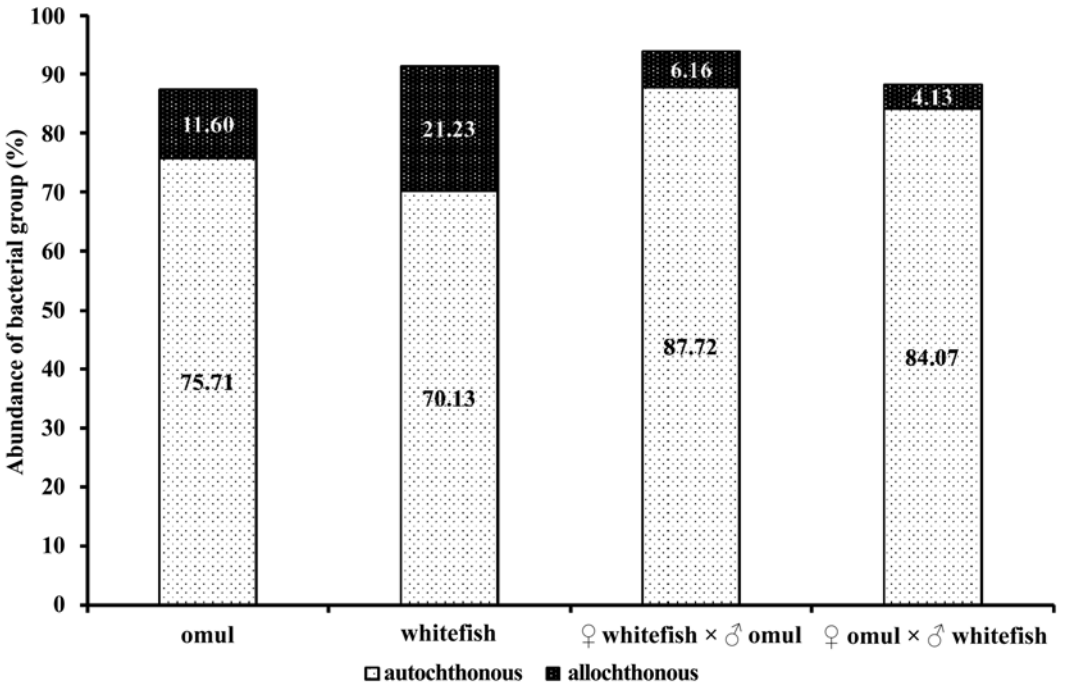


Fig. 5. Abundance of autochthonous and allochthonous bacterial groups among top 10 dominant OTUs in hindgut microbiome libraries of coregonid fishes and their F1 hybrids

the examined samples. The dominant phylum, in all individuals, was the Proteobacteria, appearing in 77 to 89 % of samples, but this was due to the dominance of a single OTU, related to *Serratia* sp. Representatives of the *Serratia* phylotype are Gram-negative, facultative anaerobic, rod-shaped bacteria of the Enterobacteriaceae family. The most common species in the genus, *S. marcescens*, is normally the only pathogen and usually causes nosocomial infections. However, a few strains were isolated from gut and associated microbiota of some protostome animals: *S. glossinae* (tsetse fly, Geiger et al. 2010), *S. ficaria* (fig wasp, Grimont et al. 1979), *S. nematodiphila* (nematode, Zhang et al. 2009), etc. Members of this genus produce the characteristic red pigment, prodigiosin and can be distinguished from other members of the family Enterobacteriaceae by their unique production of three enzymes: DNase, lipase and gelatinase (Garrity 2005).

A similar prevalence of Proteobacteria in the gut microbiome of fishes has been reported based on a meta-analysis of previously published data (Sullam et al. 2012), as well as the dominance of single OTU in bacterial communities for *Diaphorobacter* sp. (Kormas et al. 2014). In the report of Sullam et al. (2012), a strong distinction emerged among fish from saltwater vs. freshwater habitats, as well as the specific dominance of Aeromonadales and Enterobacteriales in the gut of freshwater fish, which agreed well with our data with *Serratia* sp. In our study, we have found only two OTUs which occurred across all investigated subjects at similar relative abundances: *Serratia* and *Achromobacter*. This is of particular interest because *Serratia* might be related to autochthonous microbiota, which representatives comprised a fraction from 70.13 to 87.72 % in total microbial community (Fig. 5). Whereas *Achromobacter* is strictly an aerobic bacterium found in a variety of freshwater environments and might be referred to allochthonous microorganisms. The presence of a common 'resident' or autochtonous OTU in all gut microbiomes, with high abundances exceeding 77 %, suggests the presence of a core microbiome in the coregonid juvenile gut bacterial community with a potential role in the nutrition or the immunity of the host. Other bacteria are varied between juvenile fishes, showing higher diversity in omul and hybrid hindgut, but not in whitefish. Additionally, whitefish gut microbiome contains a higher fraction of allochthonous microbiota in comparison with other juvenile fishes (Fig. 5), and does not include such bacteria as *Porphyromonas, Peptococcus* and *Clostridium*, whose fractions varied from 2.6–6.2, 0.3–0.6 and 0.3–0.7 %, respectively.

Taking into account that all 2-year-old fish were reared at the same conditions of feeding, light in-

tensity and water temperature, only heredity of host fishes could affect the microbiome communities. Omul showed an active behavior and a lesser food intake per feeding during rearing in comparison with more passive whitefish. The behavior of fish in the aquaria corresponded with that in nature. Omul is an active planctivorous migrant of pelagic waters of the lake. Whitefish is a bathypelagic benthophage, feeding near the bottom at relatively low speeds (Skryabin 1969; Smirnov & Shumilov 1974). Probably, the high fraction of allochthonous microbiota in whitefish gut and relatively simple diversity of the minor bacterial groups are consistent just with large amount of food intake, and, in turn, are associated with respective ecotype. To testify this, it whould be interesting to analyze the hindgut microbial community both after feeding and at least some time after emptying the intestine, regardless of whether the fish was cultivated artificially or caught in nature. The decreasing numbers of gut OTUs from the omul to hybrids and then to whitefish could be related to the more restricted diversity of food items of the whitefish compared with the omul. In order to clarify this, further research is required on the hindgut microbiome communities of wild whitefish and omul. Hybrids were in the intermediate position. However, each cross direction was closer to the species for which the female was used in the fertilization. Obviously, the composition of the microbial communities (Figs 1, 2) and peculiarities of feeding behavior of hybrids under the study agree with each other and also may be explained by heredity (intermediate position of F1 hybrids between parental species and the effect of maternal inheritance at least in the pair of omul and hybrid ♀ omul × ♂ whitefish).

In conclusion, this study revealed that the gut microbiome community structure in 2-year-old omul, whitefish and their F1 hybrids reflects the inheritance of host fish. However, a few OTUs were found to dominate in all individuals, implying the existence of a core bacterial community. The bacterial patterns of all hindgut microbiomes were very similar at the phylum level. All microbiome communities differed only in the composition of minor bacterial taxa. The reason for this, apparently, is a close phylogenetic relationship between the studied species, since their sympatric divergence into pelagic and benthic ecotypes has happened in a recent postglacial time (Sukhanova et al. 2012). Undoubtedly, associations of microbial community structures with the ecotypes of the studied species are needed to be further examined. Indeed, it would be plausible to trace these associations from the early to the late stages of the ontogenesis of the fish.

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