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Effects of microbe- and mussel-based diets on the gut microbiota in Arctic charr (*Salvelinus alpinus*)

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ABSTRACT

A major challenge in aquaculture is finding nutrient resources that do not compete with human demand and do not threaten ecological sustainability. Single cell proteins, such as filamentous fungi and yeasts, have similar nutrient profiles to fish meal, grow fast under optimal conditions and contain high amounts of protein, making them attractive candidates as alternative nutrient sources for farmed fish. Moreover, the cell walls of yeasts and filamentous fungi have bioactive properties, potentially mediated via the intestinal microbiota, that positively affect the intestinal health of fish. The microbiota in fish is not well explored and it is uncertain how different dietary components influence its composition.

Five experimental diets were fed to Arctic charr (*Salvelinus alpinus*) to investigate their effects on gut microbiota. The fish meal in a reference diet was replaced with either intact or extracted yeast cells of the species *Saccharomyces cerevisiae*, the filamentous fungi *Rhizopus oryzae* or meal from blue mussel (*Mytilus edulis*). The microbiota was characterised in samples collected from the proximal and distal intestine using 16S rRNA gene amplicon sequencing with Illumina MiSeq.

Sequence data showed that the gut microbiota was dominated by *Firmicutes* and *Proteobacteria*, which represented 85% of total community abundance, with lactic acid bacteria representing 36.2%. Principal component analysis (PCA) of the data revealed that the microbiota in proximal and distal regions of the intestine had similar composition and that replacement of fish meal with yeast and filamentous fungi affected microbiota composition, primarily with higher relative proportions of *Photobacterium* and *Lactobacillus*.

Lactic acid bacteria were a dominant fraction of the intestinal microbiota in Arctic charr. Microbial based feeds were associated with similar changes in microbiota composition, but contrasting to the fish-meal based reference diet. Microbiota composition was similar in the proximal and distal gut, but dietary responses were specific to gut segment.

1. Introduction

Aquaculture is a growing industry world-wide, thanks in part to rising demand for seafood that cannot be met by supply from fisheries. However, the need for fish meal and fish oil as protein and lipid sources for fish feeds has led to a situation that is both ecologically and economically unsustainable (FAO, 2014). Many studies have investigated alternative sources of protein that meet the nutritional requirements of farmed fish species (Gatlin et al., 2007). Among plant feedstuffs, soybean meal is considered to be a high quality protein source and costs about half as much as fish meal (FAO, 2014).

Inclusion of plant feedstuffs in fish feeds has increased, but plant-based materials contain anti-nutritive factors that can negatively affect the intestinal health of fish (Knudsen et al., 2008). These compounds are novel to most farmed fish species, particularly carnivorous species (Krogdahl et al., 2010). Soybean meal is problematic as a feedstuff for Atlantic salmon due to its high content of saponins, which can increase intestinal permeability and induce enteritis (Knudsen et al., 2008). Other plant feedstuffs, such as oilseeds, legumes and cereal grains, have been used as a protein source in fish feeds (Gatlin et al., 2007), but as the aquaculture sector grows it will increase competition for these agricultural feed resources (Tacon et al., 2011).

As an alternative to plant feedstuffs, single cell proteins (SCP), such as microalgae, bacteria and yeast, are increasingly being researched as alternative protein sources for inclusion in fish feeds (Langeland et al., 2016; Vidakovic et al., 2015; Øverland et al., 2013).

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The nutritional value of SCP derives from their high content of crude proteins and additional components such as B-vitamins, pigments and β-glucans, increasing their potential to replace fish meal protein (Sanderson and Jolly, 1994; Tacon, 1995). Studies have shown that fish meal protein can be partly replaced with the yeast *Saccharomyces cerevisiae* with no negative effects on fish performance in fact replacement of up to 30% of fish meal with SC in the diet can even improve feed efficiency (Oliva-Teles and Gonçalves, 2001). It has also been shown that diets with most of the protein from fish meal or filamentous fungi (*Rhizopus oryzae*) biomass have comparable metabolic effects when fed to Arctic charr (Abro et al., 2014a).

Among SCP, yeasts are currently the most widely used in aquafeeds (Rumsey et al., 1991; Tacon, 1995; Vidakovic et al., 2015). Aside from being a protein source, studies have shown that β-glucans and mannan-oligosaccharides derived from yeast cell walls can increase resistance to sea lice infection, improve feed uptake and counteract soybean meal-induced enteritis in Atlantic salmon (*Salmo salar*) (Refstie et al., 2010). In addition, yeast SCP can improve the intestinal barrier function (Ohland and MacNaughton, 2010) and yeast β-glucans can stimulate intestinal maturation of rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*) (Gatesoupe, 2007). However, a study on the intestinal barrier in Arctic charr (*Salvelinus alpinus*) showed decreased function in fish fed intact SC compared with fish feed a reference diet based on fish meal (Vidakovic et al., 2015).

Rhizopus oryzae is a protein-rich filamentous fungus with an amino acid profile similar to fish meal and has been used in a few studies to replace fish meal protein (Abro et al., 2014a; Bankefors et al., 2011). Abro et al. (2014b) found that *trans*-epithelial resistance was higher in the distal intestine of Arctic charr fed a diet of *R. oryzae* than a fish meal-based diet. In addition, the Arctic charr fed the *R. oryzae* diet had reversed mannitol transport, indicating that dietary inclusion of filamentous fungi or digested metabolites from micro-fungal biomass affected gut integrity.

It has been shown in numerous studies that the gastro-intestinal (GI) microbiota is very important for fish health by promoting nutrient supply, preventing colonisation by pathogens and maintaining mucosal immunity (Greiner and Bäckhed, 2011; Nayak, 2010). There are multiple examples of how dietary habits and feed ingredients affect the microbial composition in the GI tract of many animal species (Kim et al., 2011; Ley et al., 2008; Ringø et al., 2006; Zhang et al., 2014).

The gut microbiota of aquatic animals is involved in digestion of algal cells, amino acid production and secretion of inhibitory compounds that protect against bacterial pathogens in the gut (Austin, 2006; Nayak, 2010). It also plays a role as an immuno-stimulant (Ringø and Gatesoupe, 1998). The distal part of the GI tract is a favourable ecological niche for microorganisms, since it can be colonised by a wide range of microbes (Skrodenytė-Arbačiauskienė, 2007). Earlier studies have shown that *Firmicutes* and *Proteobacteria* are phyla that predominates in the intestine of carnivorous fish species (Schmidt et al., 2016; Wong et al., 2013). However, several factors, such as environment, fish species and feed, influence the composition at a higher taxonomic resolution and it has for example been shown that the intestinal microbiota of freshwater and marine fish species differ in microbial composition (Cahill, 1990; Sakata et al., 1980).

There is a growing interest in controlling diseases within aquaculture using alternative dietary components, such as probiotics and prebiotics, to reduce the use of antibiotics within the aquaculture industry (Pérez et al., 2010). It has been shown that administration of certain lactic acid bacteria can counteract the effects of pathogenic microorganisms in rainbow trout (*Oncorhynchus mykiss*), possibly by competition for nutrients and adhesion sites (Balcázar et al., 2007; Vendrell et al., 2008). In aquaculture, previous studies have examined the link between diet and microbial composition, especially focusing on soybean meal as a replacement for fish meal in Atlantic cod (*Gadus morhua*) (Ringø et al., 2006), rainbow trout (Heikkinen et al., 2006) and Atlantic salmon (Bakke-McKellep et al., 2007). The microbiota of Arctic charr is rather unexplored and it is uncertain how different dietary components influence its composition.

The aim of the present study was to investigate how alternate protein sources, primarily of microbial origin, influence the composition of the intestinal microbiota of Arctic charr. The impact of different diets on the microbiota in the proximal and distal region of the intestine was examined using 16S rRNA gene amplicon sequencing.

Table 1
Formulation (g kg^{-1} feed), proximal chemical composition (g/kg^{-1} DM) and energy content (MJ kg^{-1} DM) of the five experimental diets[2].

Ingredient	Experimental diet[1]				
	REF	MYE	ISC	ESC	RHO
Fish meal	467.9	280.4	280.9	281.7	279.0
Fish oil	89.4	89.3	91.7	97.0	81.6
Soy protein concentrate	36.4	36.4	28.1	31.3	36.2
Soybean meal	114.3	103.9	83.2	114.7	113.7
Rapeseed oil	34.6	32.0	33.9	34.7	27.1
Wheat gluten	33.8	38.9	60.3	38.6	36.2
Wheat	124.8	124.6	102.0	130.1	100.2
Titanium oxide	5.2	5.2	5.2	5.2	5.2
Min-vit premix	15.6	15.6	15.6	15.6	15.5
Cellulose	78.0	53.5	10.0	77.9	44.7
Mytilus edulis	–	220.0	–	–	–
Intact *Saccharomyces cerevisiae*	–	–	289.0	–	–
Extracted *S. cerevisiae*	–	–	–	172.6	–
Rhizopus oryzae	–	–	–	–	260.1
Proximate chemical composition					
Dry matter (g/kg^{-1})	912	917	913	929	908
Crude protein (Nx6.25)	493	498	492	494	480
Crude lipid	201	201	190	174	186
Ash	201	74	67	75	73
Gross energy	24.1	24.4	23.9	23.2	23.9

[1] REF = reference diet, MYE = diet with blue mussel (*M. edulis*), ISC = diet with intact yeast (*S. cerevisiae*), ESC = diet with extracted yeast (ESC), RHO = diet with filamentous fungi *R. oryzae*.
[2] From Vidakovic et al. (2015).

2. Material and methods

2.1. Study design

The experiment was carried out at Kälarne Research Station (Vattenbrukscentrum Norr AB, Sweden). The experiment was performed in compliance with laws and regulations concerning experiments on live animals overseen by the Swedish Board of Agriculture and was approved by the ethics committee for animal experiments in Umeå, Sweden. The experimental diets were formulated based on a reference diet with fish meal as protein source (REF), with 40% of the fish meal replaced with test ingredients on a dry matter (DM) and crude protein (CP) basis (Table 1). The test ingredients were: extracted *S. cerevisiae* (ESC), intact *S. cerevisiae* (ISC), filamentous fungus *R. oryzae* (RHO) and mussel meal from *M. edulis* (MYE). All diets were isonitrogenous (CP = 45%) and isoenergetic (gross energy = 20 MJ kg^{-1}), for further information on diet composition see Vidakovic et al. (2015). All fish were fed a restricted ratio of 1% of the total fish biomass in each tank using automatic feeders (Arvo-Tec T 2000, Huutokoski, Finland) delivering feed continuously during the light period (12:12 h). Fish with a mean start weight of 47.8 ± 8.6 g were fed the experimental diets for 99 days and were then euthanised with an overdose of 300 mg L^{-1} tricaine methane sulphonate (MS-222; Western Chemical Inc., Ferndale, Washington, USA). Since the fish used in this experiment were

also used in a growth study, for more details regarding feeding and rearing conditions see Vidakovic et al. (2015).

2.2. Collection and preparation of intestinal samples

Intestinal samples were collected from 20 individual adult Arctic charr (*Salvelinus alpinus*) (ESC; n = 5, ISC; n = 3, RHO; n = 3, MYE; n = 3, REF; n = 6). The fish were dissected and the intestines were sectioned with strings below pyloric caecum, ileorectal valve (between proximal and distal intestine) and anus (distal intestine), in order to avoid flow of digesta between the distal and the proximal intestine. After sectioning, the whole intestine of each fish was wrapped in aluminium foil and flash-frozen in liquid nitrogen. Thereafter, the samples were stored at −80 °C until DNA extraction was performed.

The intestinal samples were thawed, dissected and the mucosa and faeces from the proximal (foregut) and distal (hindgut) intestines were scraped separately using a scalpel under sterile conditions. DNA was isolated from 200 mg of intestinal content by bead beating in cycles of 2 × 60 s in a Mini Bead-Beater 8 (Biospec Products INC, Bartlesville, Oklahoma, USA) in tubes containing 0.1 mm Zirconia/silica beads. The DNA was extracted using QIAamp DNA Mini Kit (Qiagen Gmbh, Hilden, Germany) according to the manufacturer's protocol. The isolated DNA was stored at −20 °C until further analysis.

2.3. Generation of 16S amplicon libraries

The V4 region of the 16S rRNA genes were amplified from the isolated DNA using the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′ (Hugerth et al., 2014) and 805R (5′-GACTACHVGGGTATCTAATCC-3′ (Herlemann et al., 2011). Phusion High-Fidelity Master Mix with HF Buffer was used in all of the 25 μL PCR reactions (ThermoFisher Scientific, Osterode, Germany). The PCR started with an initial denaturation step at 98 °C for 30 s, followed by 35 cycles of 98 °C for 10 s, 60 °C for 30 s and 72 °C for 4 s and finally an elongation step at 72 °C for 2 min. The presence of PCR products was confirmed through gel electrophoresis before further analyses were conducted.

To target the specific PCR product, gel purification was conducted using the GeneJet Gel Extraction Kit (Thermo Fisher Scientific, Osterode, Germany) according to the manufacturer. The purified PCR product was used as template in a second PCR step using a set of barcoded primer combinations giving every sample an individual tag for identification (Hugerth et al., 2014). The second PCR step was conducted using the same master mix under the following conditions: initial denaturation step at 98 °C for 30 s followed by 10 cycles of 98 °C for 10 s, 62 °C for 30 s and 72 °C for 5 s and a final elongation step at 72 °C for 2 min.

The size and presence of the PCR product was confirmed by gel electrophoresis and the product was subsequently purified with magnetic beads from Agencourt AMPure XP (Beckman Coulter Inc, Brea, California, USA) according to the manufacturer. The purified PCR products were quantified using a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, California, USA) and then mixed and pooled in equimolar proportions in the final mixture. The amplicon library was sequenced using the Illumina MiSeq platform at the National Genomics Infrastructure (NGI) hosted by Science for Life Laboratory (Solna, Sweden).

2.4. Sequence analysis

The sequences generated were trimmed for quality according to the following criteria: the adaptor and primer sequences were removed from the sequences. In addition, any base from the 3′ end with quality below 10 was trimmed. Reads that contained N bases longer than 300 bp or did not contain primer sequences were discarded. The paired end reads obtained were trimmed with Cutadapt. The trimmed pair end reads were further processed using the QIIME software package, version 1.8 (Caporaso et al., 2010). Sequence data were assigned to operational taxonomic units (OTUs) sharing 97% or higher homology using an open reference OTU picking strategy. Chimeric sequences were removed by ChimeraSlayer (Haas et al., 2011). The final OTU table was filtered based on the criterion that the OTU was observed in at least three samples. In addition, reads matched with chloroplasts and mitochondria were filtered from the OTU table. The remaining sequences were subsampled (according to the sample containing the smallest set of sequences N = 5524) to equalise number of sequences between different samples. The 16S rRNA gene sequences were deposited in the NCBI Sequence Read Archive (SRA) under the accession number PRJNA351976.

2.5. Statistical analysis

Principal component analysis (PCA) was used to explore how samples clustered to find correlations between microbiota composition and diet or gut segment. The variables used in the PCA was relative abundance of the different OTUs found in the samples. Differences in overall microbiota composition related to diet or gut segment were determined with permutational multivariate analysis of variance (PERMANOVA). The diversity of the intestinal microbiota was assessed using the Chao-1 richness index and Shannon diversity index. Moreover, to investigate how the treatment influenced specific OTUs, a univariate approach was used to identify the treatment effects on all OTUs present with an average abundance of >1%. The effects of diet were determined by a Kruskal-Wallis test for equal medians and when differences were significant a Mann-Whitney test was used to identify the diets that differed. All statistical analyses were performed using Paleontological Statistics Software Package for Education (PAST) software version 3.0 (Hammer et al., 2001) and differences were considered significant at $P < 0.05$.

3. Results

3.1. Fish growth

The fish used in this study were part of a previous growth trial (Vidakovic et al., 2015) and revealed that growth rate was influenced by the SCP diets, while feed conversion ratio was not. Fish fed the ESC and RHO diets had lower specific growth ratethan fish fed the REF diet, 0.95, 0.97 and 1.08 respectively. More detailed information about growth performances, relative weight and nutrient retention is given in (Vidakovic et al., 2015). No significant differences in growth rate were found for fish fed the MYE and ISC diets compared with the REF diet.

3.2. Analysis of community composition

Taxonomic mapping of the OTUs showed that two phyla covered 85% of the total relative abundance in the dataset: *Firmicutes* (65.7%) and *Proteobacteria* (19.3%) (Fig. 1). Among the *Firmicutes*, the largest proportion was classified as lactic acid bacteria (LAB), which had a mean relative abundance of 36.2% in the dataset. The most dominant OTU was *Leuconostocaceae*, which had a mean relative abundance of 14.2%. The major representative of the *Proteobacteria* phylum was *Photobacterium* with an abundance of 13.6%

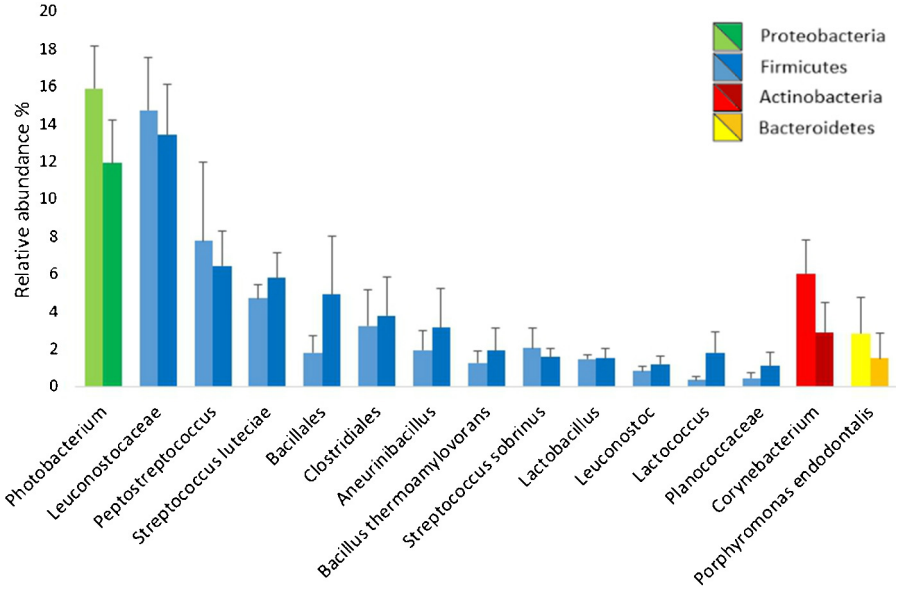

Fig. 1. The top 15 OTUs (with average relative abundance >1%) in the intestine of Arctic charr, grouped into phyla and shown in descending order within a given phylum. Lighter shades of each colour represent the proximal intestine and darker shades the distal intestine.

Table 2
Dietary effects on diversity indices for the microbiota in the proximal and distal intestine of Arctic charr.

	Experimental diet					
	REF	MYE	ISC	ESC	RHO	P-value
Proximal:						
Chao-1	59.5 ± 7.7	39.0 ± 5.0	90.3 ± 5.7*	76.6 ± 3.4	86.0 ± 4.5*	0.032
Shannon	2.66 ± 0.14	2.12 ± 0.24	2.84 ± 0.06	2.89 ± 0.03	2.77 ± 0.08	0.092
Distal:						
Chao-1	55.2 ± 6.9	35.7 ± 3.9	89.0 ± 3.5*	85.3 ± 0.7*	82.3 ± 2.3*	0.004
Shannon	2.60 ± 0.16	2.39 ± 0.17	2.92 ± 0.03	2.91 ± 0.08	2.90 ± 0.13	0.093

REF = fish meal-based reference diet, MYE = mussel (*Mytilus edulis*) meal-based diet, ISC = intact *S. cerevisiae* diet, ESC = extracted *S. cerevisiae* diet and RHO = *Rhizopus oryzae* diet. All values are expressed as mean ± SE.

* indicate experimental diets where the diversity differ significantly compared with the reference diet ($P < 0.05$).

3.3. Microbial diversity

Shannon diversity and Chao-1 richness indices for the proximal and distal gut differed depending on diet (Table 2). However, there were no significant differences in diversity between the regions. In the proximal region, the highest Chao-1 richness was found in fish fed the ISC and RHO diets, both significantly higher than the REF diet ($P = 0.028$ for both). Shannon diversity showed a similar pattern to Chao-1, but with no significant differences between the diets (Table 2). In the distal region, the highest values of Chao-1 richness were found again in fish fed microbe-based diets and the lowest in those fed the MYE and REF diets ($P = 0.004$). Similar to the proximal region, Shannon diversity showed a similar pattern to Chao-1, but with no significant differences between the diets (Table 2).

3.4. Feed impact on gut microbiota

Principal component analysis (PCA) was performed to examine correlations between the diet type, gut segment and the composition of the intestinal microbiota (Fig. 2). The first and second principal components together explained 64% of the variation in the dataset and showed a clear separation between samples according to diet indicating a strong correlation between the relative abundance of dominant OTUs and different diets. The microbial diets clustered separately from the REF diet (ESC $P = 0.0033$, ISC $P = 0.0062$, RHO $P = 0.01$; PERMANOVA). In addition, the composition of the microbiota in fish fed the MYE diet was different from that in fish fed the microbe-based diets when samples from the proximal and distal regions were pooled (ESC $P = 0.0009$, ISC $P = 0.0028$, RHO $P = 0.0028$). However, there was no separate clustering of samples from the proximal and distal gut, indicating that there was no apparent difference in microbiota composition between the regions.

3.5. Dietary effects on specific OTUs

The OTUs with mean relative abundance >1% in the dataset were also analysed with univariate analysis in order to study the impact of diet on specific OTUs. The OTU that varied most between diets was taxonomically classified as *Photobacterium* and had significantly higher abundance in fish fed microbe-based feeds than in fish fed REF (Fig. 3). This difference was evident for both the proximal ($P = 0.007$) and distal ($P = 0.005$) regions of the intestine. Diet also had an impact on an OTU classified as *Porphyromonas endodontalis*, which was present in lower abundance in fish fed the ESC or ISC diets than in fish fed REF diet (proximal $P = 0.021$, distal $P = 0.289$). Moreover, *Lactobacillus* spp. were significantly more abundant in the proximal intestine of fish fed the microbe-based diets than the REF diet ($p = 0.015$). However, this effect was not observed for the distal region. In addition, diet type affected abundance of *Peptostreptococcus*, for which there was a clear difference between fish

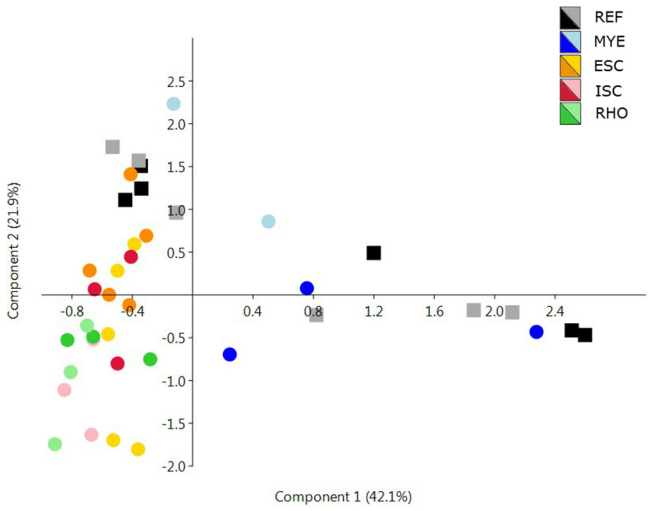

Fig. 2. Principal component analysis (PCA) of the bacterial community structure in the proximal and distal intestine of Arctic charr. In total 39 samples were analysed and each sample is represented by a symbol. Grey/black squares = samples from fish fed a fish meal-based reference diet (REF); light/dark blue dots = samples from fish fed mussel (*Mytilus edulis*) meal (MYE); light/dark orange spots = samples from fish fed extracted *Saccharomyces cerevisiae* (ESC); pink/red spots = samples from fish fed intact *S. cerevisiae* (ISC); light/dark green spots: samples from fish fed *Rhizopus oryzae* (RHO). Lighter shades of each colour represent the proximal intestine and darker shades the distal intestine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fed the ESC and MYE diets as regards abundance in the distal region ($P = 0.028$), but not in the proximal region. However, the overall abundance of this OTU did not differ between regions in fish fed the REF diet.

4. Discussion

To our knowledge, this is the first study to characterise the proximal and distal intestine of Arctic charr using high-throughput sequencing techniques (i.e. Illumina MiSeq platform). Characterisation of the microbiota from the proximal and distal regions of the intestine revealed a community dominated by the phylum *Firmicutes*, which was more than 3-fold more abundant than the second most abundant phylum, *Proteobacteria*. Within the *Firmicutes* phylum, the LAB group comprised the largest fraction, with *Leuconostocaceae* being the most abundant OTU (Fig. 1). This result is consistent with previous studies on rainbow trout that reported a high presence of LAB, especially *Weissella*, *Streptococcus* (Desai et al., 2012) and *Leuconostoc* (Desai et al., 2012; Ingerslev et al., 2014). Ringø and Strom (1994) found that *Lactobacillus* spp. constituted a large percentage (~20%) of the intestinal microbiota of Arctic charr. The beneficial properties some LAB might have on fish have been investigated in many studies and they are reported to be involved in fermentation processes within the distal intestine of Atlantic salmon (Bakke-McKellep et al., 2007), while some strains have probiotic properties that can improve the health and quality of farmed fish (Ringø and Gatesoupe, 1998).

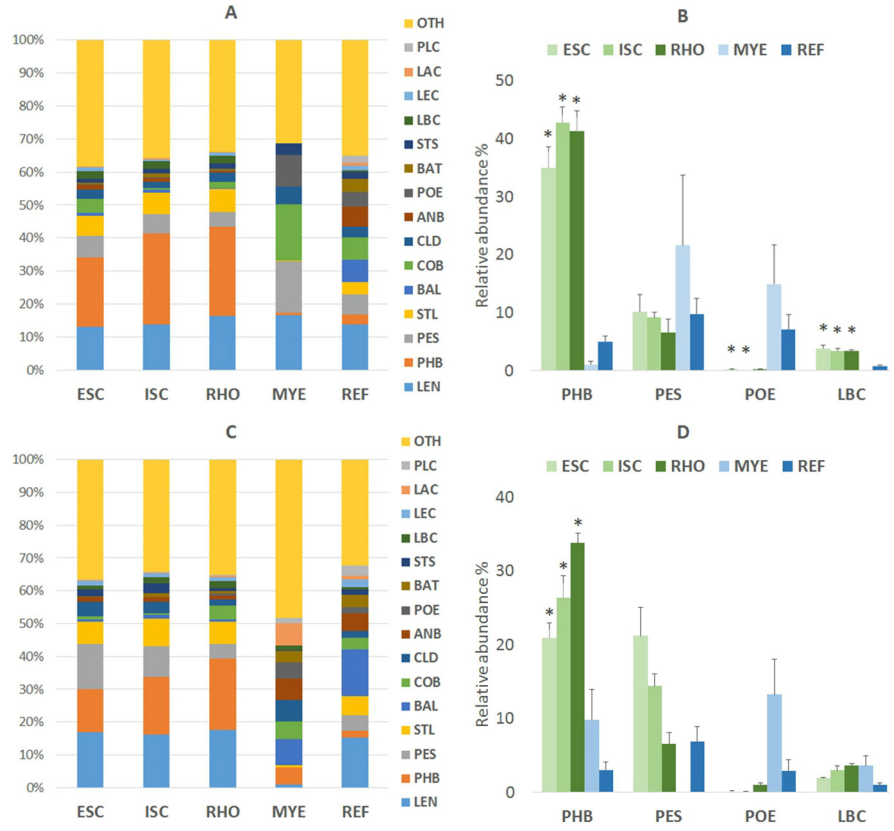

Fig. 3. Microbial composition and dietary effects on specific OTUs in the proximal and distal intestine of Arctic charr. Distribution of the top 15 OTUs (with average relative abundance >1%) in the proximal intestine (Panel A) and distal intestine (Panel C). Panels B and D show relative abundance (% ± SE) of OTUs for which there was a significant diet effect in the proximal intestine and distal intestine. Significant difference ($p < 0.05$) from the REF diet is indicated by *. Abbreviations used in the figure: ESC = diet with extracted yeast (*Saccharomyces cerevisiae*), ISC = diet with intact yeast (S. cerevisiae), RHO = diet with filamentous fungi *Rhizopus oryzae*, MYE; diet with blue mussel (*Mytilus edulis*), REF = reference fish meal-based diet. LEN = *Leuconostocaceae*, PHB = *Photobacterium*, PES = *Peptostreptococcus*, STL = *Streptococcus luteciae*, BAL = *Bacillales*, COB = *Corynebacterium*, CLD = *Clostridiales*, ANB = *Aneurinibacillus*, POE = *Porphyromonas endodontalis*, BAT = *Bacillus thermoamylovorans*, STS = *Streptococcus sobrinus*, LBC = *Lactobacillus*, LEC = *Leuconostoc,* LAC = *Lactococcus*, PLC = *Planococcaceae*, OTH = Other. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The proximal and distal region of the intestine differ in physiological function and have a different repertoire of digestive enzymes, and thus the substrates available for microbes also differ between the proximal and distal gut. We did not find any differences in microbiota composition or microbial diversity related to intestinal region. Analysis of the microbiota composition using PCA revealed that samples from the proximal and distal intestine from the same fish clustered together, indicating similar composition. It has previously been shown that the distal region of Arctic charr intestine has a higher number of bacteria than the proximal region (Ringø et al., 1995), while the reverse has been reported for rainbow trout (Austin and Al-Zahrani, 1988). Vidakovic et al. (2015) showed that feeding Arctic charr the ISC and RHO diets resulted in increased paracellular permeability in the distal region. It has previously been shown that dietary *R. oryzae* can result in disturbed integrity and a leakier distal intestine in Arctic charr (Abro et al., 2014b). The decrease in intestinal barrier function of fish fed fungal diets could be an effect of cell wall components from yeast or the filamentous fungi, such as chitin/chitosan (Abro et al., 2014c), These bioactive compounds could influence the microbiota composition in the intestine. However, more research is needed on the link between gut microbiota and intestinal barrier function.

The gut microbiota in Arctic charr was dependent on the diet and we found higher diversity in fish fed the microbe-based diets compared with the REF diet (Table 2). Multivariate analysis of the compositional data showed that clustering of OTUs from fish fed the microbe-based diets differed from the REF diet. Similar dietary effects on the microbiota have been observed in previous feeding studies. Ingerslev et al. (2014) found that diets of plant origin correlated with a higher abundance of *Firmicutes*, while diets of marine origin increased the abundance of *Proteobacteria* in the intestine of rainbow trout. A study by Lyons et al. (2015) also found *Proteobacteria* to be the dominant phylum in rainbow trout fed a fish meal-based diet. However, the results from the present study contradict previous findings, since we observed an association between the microbe-based diets and the relative abundance of *Photobacterium*, which belong to the phylum *Proteobacteria*. *Photobacterium* is common in marine environments, with some luminous species occurring as symbionts in the light-generating organs of several fish species (Dalgaard et al., 1997). To our knowledge, *Photobacterium* has not been found in freshwater environments, only in the GI of migrating fish (Budsberg et al., 2003). The high abundance of *Photobacterium* in Arctic charr fed the microbe-based diets was unexpected, since this taxa is primarily of marine origin (Baumann and Baumann, 1977), although our microbe-based diets did contain some fish meal. The higher abundance of *Photobacterium* in fish fed lower rates of marine ingredients requires further studies.

We found significantly higher abundance of *Lactobacillus* in the proximal intestine of Arctic charr fed the microbe-based diets (Fig. 3). We also found numerically higher abundance of *Lactobacillus* in the distal intestine of fish fed the microbe-based feeds, but the difference was not significant. The elevated abundance of *Lactobacillus* in fish fed the microbe-based diets could be explained by the cell wall components in the microbe-based feeds having prebiotic properties. The yeast cell wall is made up of beta-glucans, mannan-oligosaccharides (MOS) and chitin. Beta-glucans have been shown in vitro to have a beneficial effect on the stress tolerance of intestinal *Lactobacillus* (Stack et al., 2010). It has also been shown that whole yeasts have similar effects as refined MOS when fed to pigs, resulting in elevated *Lactobacillus* levels (White et al., 2002). In addition, MOS can function as prebiotics by providing favourable conditions for growth of *Lactobacillus* in monogastric animals (Flickinger and Fahey, 2002). However, we obtained similar results for the diets with intact yeast (ISC) and extracted yeast (ESC), where the cell wall had been removed, and the effect could possibly be due to a symbiotic relationship between yeast and *Lactobacillus*.

We observed an effect of diet on *Porphyromonas endodontalis* and *Peptostreptococcus*. *Porphyromonas* spp. has previously been found in the intestinal microbiota of Atlantic salmon (Zarkasi et al., 2016) and rainbow trout (Kim et al., 2007) while *Peptostreptococcus* has been found in the GI tract of freshwater fish (Romero et al., 2014). We could not find any evidence linking these two bacteria with microbe-based diets and further studies are needed to determine their importance.

5. Conclusions

The microbe-based test feeds were associated with similar changes in microbiota composition, primarily characterized by an elevated abundance of *Photobacterium* and *Lactobacillus* when compared to reference fish meal-based diet. Bacterial composition was also similar in the proximal and distal intestine of Arctic charr, but dietary responses were specific to gut segment. Bacterial diversity was similar in distal and proximal intestine but the microbe-based diets was associated with higher Chao-1 richness than the mussel and fish meal-based diets.

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