RESEARCH ARTICLE

Effects of poly-β-hydroxybutyrate (PHB) on Siberian sturgeon (*Acipenser baerii*) fingerlings performance and its gastrointestinal tract microbial community

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Abstract

Poly-β-hydroxybutyrate (PHB) is a natural polymer that can be depolymerized into water-soluble short-chain fatty acid monomers. These monomers can act as microbial control agents. In this study, the effects of partially replacing the diet of Siberian sturgeon fingerlings with 2% and 5% PHB were investigated. Replacing 2% of the diet with PHB improved weight gain, specific growth rate (SGR) and survival in the sturgeon fingerlings during the 10-week experimental period. Community-level physiological profiling and PCR-denaturing gradient gel electrophoresis (PCR-DGGE) were used to analyze the microbial community diversity and community organization in the sturgeon gastrointestinal tract. DGGE analysis revealed that PHB affected the intestinal microbial species richness and diversity. The highest species richness was observed with 2% PHB. DNA sequencing of the dominant bands in 2% and 5% PHB treatments revealed that PHB stimulated bacteria belonging to the genera *Bacillus* and *Ruminococcaceae*. Principal component analysis, Lorenz curves and the Shannon index of Biolog™ Ecoplate data revealed that aerobic metabolic potential of the bacterial community was different in the PHB-treated fishes as compared with the control situation. Overall, our results indicate that PHB act as microbial control agents and replacement of 2% of Siberian sturgeon fingerling diet with PHB has beneficial effects.

Introduction

The microbial community has an important role in the health and nutrition of the host (Burr *et al.*, 2005), and the surrounding environment, including the cultivation conditions, plays a key role in shaping the microbial community (Hansen & Olafsen, 1999). The possibility of steering the composition and functionality of a given microbial community through the incorporation of probiotics or prebiotics in the feed has been investigated. Prebiotics have been defined as non-digestible diet components that are metabolized by specific microorganisms beneficial to the health and growth of the host (Gibson & Roberfroid, 1995; Manning & Gibson, 2004). Positive effects of prebiotics on immuno-stimulation, production of beneficial metabolites in the gastrointestinal (GI) tract, mineral uptake and growth performance in human (Manning & Gibson, 2004), livestock (Patterson & Burkholder, 2003; Smiricky-Tjardes *et al.*, 2003) and fishes (Grisdale-Helland *et al.*, 2008; Akrami & Hajimoradloo, 2009; Yousefian & Amiri, 2009) have been reported. The production of short chain fatty acids (SCFA) due to the fermentation of prebiotic compounds in the GI tract resulting in the acidification of the colonic environment is of specific importance. For instance, SCFA are known to inhibit the growth of some potential pathogenic bacteria, such as *Salmonella* spp. and *Vibrio* spp. (Defoirdt *et al.*, 2007). In general, the growth inhibitory effect is believed to be caused by the undissociated form of the acid, which is able to penetrate the bacterial cell membrane. Once inside, the acid releases protons (H^+) in the neutral cytoplasm, which decreases the intracellular pH

(De Schryver *et al.*, 2010) and forces bacteria to redirect energy towards the efflux of the excess protons, thereby straining the cell metabolism and leading to lower cell growth and even cell death (Kato *et al.*, 1992).

Polyhydroxybutyric acid is an important member of family of polyhydroxyalkanoates (PHAs) and it can be degraded by bacteria (Kato *et al.*, 1992; Patnaik, 2005). It is a polymer belonging to the polyesters class that is produced under conditions of physiological stress and excess of carbon as a form of energy storage by bacteria such as *Alcaligenes eutrophus* and *Bacillus megaterium*. Poly-β-hydroxybutyrate (PHB) has been shown to be able to protect Artemia nauplii (*Artemia franciscana*) against vibriosis (Defoirdt *et al.*, 2007; Halet *et al.*, 2007; Van Cam *et al.*, 2009). Recent studies on PHB have been conducted in different aquaculture species such as European sea bass (*Dicentrarchus labrax*) juveniles and the larvae of the giant river prawn (*Macrobrachium rosenbergii*) (Defoirdt *et al.*, 2007; Nhan *et al.*, 2010). Results indicated that PHB increases weight gain (WG), survival and SCFA concentration in the GI tract. In PHB-treated giant river prawn, thiosulphate citrate bile salts sucrose agar counts (all *Vibrio* sp.) were found to be significantly lower when compared with control larvae, indicating that the addition of PHB inhibited the growth of these potentially pathogenic microorganisms.

In this study, the effects of PHB on Siberian sturgeon (*Acipenser baerii*) fingerling growth performance and its GI tract microbial community were investigated. The impacts of three commercial diets containing 0%, 2% and 5% PHB on the activity and structure of the microbial community in the GI tract were investigated with community-level physiological profile (CLPP) analyses (i.e. Biolog™ microplate) and by 16S rRNA denaturing gradient gel electrophoresis (DGGE) (Garland & Mills, 1991; Muyzer & Smalla, 1998).

Materials and methods

Experiment set-up

The 10-week experiments were conducted at the Laboratory of Aquaculture & Artemia Reference Centre (ARC) in 75-L tanks supplied with water flow-through (every 1 h, 100% water replaced), aeration and heaters to keep water temperature around 15 ± 1 °C. The light-dark cycle was controlled for 12 h light and 12 h dark. Fish were transferred to the tanks with initial weight 17.1 ± 0.8 g (Table 1) at the density of about 8 g L^{-1}. The fish were fed with commercial pellets (2.0 mm diameter) for sturgeon (Joosen-luyckx, Aqua Bio, Belgium). The amount of food was weighed every day to determine fish consumption ability and body weight. Fishes were fed three times daily (8:00, 13:00 and 18:00 h) with equal servings. The residual food were siphoned out of the tank and carefully recorded.

Table 1. Growth performance of Siberian sturgeon fingerlings fed the control diet and experimental diets in second trial (n = 3)

	Control	2% PHB	5% PHB	*P* value
Initial weight	17.5 ± 0.8	17.6 ± 0.7	18.07 ± 1.3	0.05
Final weight	69.1 ± 7.8	75.8 ± 2.7	67.5 ± 4.9	0.05
Weight gain	51.5 ± 8	58.1 ± 3	49.4 ± 3.8	0.05
SGR	2.2 ± 0.2	2.3 ± 0.1	2.1 ± 0.1	0.05
Survival	89.1 ± 9.3	96.6 ± 3.4	94.5 ± 5.4	0.05
FCR	0.75 ± 0.01	0.79 ± 0.03	0.84 ± 0.06	0.05

Values are average ± SD, n = 3.

Three diets, containing 0% (control), 2% and 5% (w/w) PHB, were evaluated in triplicate. PHB-containing diets were prepared 3 days prior to feeding to ensure they were completely dried. PHB was dissolved in chloroform: water solvent (80 : 20, v/v) and then mixed with food pellets at the above ratios. The diets were left exposed to air for chloroform evaporation, and kept at room temperature to dry. The experiment described was performed twice with similar results. Only the results of the second experiment are shown.

Measured parameters

The survival of the sturgeons over the 10 weeks was determined from daily observations. For fish weight and feeding rate in each tank, sturgeon fingerlings were weighed individually every 2 weeks following 24 h without feeding. The average WG/fish/treatment over the 10 weeks was calculated as follows: the average weight of the fish as measured at the beginning of the experiment was subtracted from the weight of fishes sampled on the final day of the experiment. The SGR was calculated as follow: SGR (%) = $\ln W - \ln W_0/t$, where W is the average weight after 10 weeks, W_0 is the average initial weight (measured at the beginning of the experiment), and t is the experiment period (10 weeks). The same approach was used to calculate the feed conversion ratio (FCR), expressed as the feed consumption (g) divided by the weight increase of the fish (g) per treatment. Three fish were randomly selected for measurement of pH in the GI tract, anaesthetized with 2 mL L^{-1} phenol ethanol (Sigma Company), dissected with scissors, and an incision was made in the gut for measurement of the midgut and hindgut pH using a biotrode pH electrode (Hamilton, Switzerland).

Bacterial community analysis – DGGE

Samples for the DNA extraction were collected at the end of the experiment. Three fish in each tank were sampled

at random and euthanized with 2 mL L^{-1} of 2-phenoxyethanol, with sodium bicarbonate to maintain the neutral pH. After dissection in sterile conditions, the spiral valve contents of three fish per replicate were gently removed. The three samples of the same tanks (replicate) were pooled and frozen at −20 °C. DNA extraction was performed using the CTAB buffer method (Boon *et al.*, 2003).

Amplification of the 16S rRNA gene by means of PCR was conducted as reported in Boon *et al.* (2002). Briefly, the V3 region was amplified using primer 338f (5′-ACTCCTACGGGAGGCAGCAG-3′) with a 40-base GC clamp attached to its 5′ end and primer 518r (5′-ATTACCGCGGCTGCTGG-3′). After 5 min denaturing at 94 °C, 35 cycles of the following procedure were carried out: 1 min of denaturation at 95 °C, 1 min of annealing at 55 °C, 2 min of primer extension at 72 °C; and a final extension at 72 °C for 10 min. DGGE was performed with a D-code universal mutation detection system (Bio-Rad). The denaturing gradient of the gel ranged between 45% and 55%. Electrophoresis was performed with a constant voltage of 38 V at 60 °C for 16 h. DGGE patterns were analyzed with BIONUMERICS software version 5.0 (Applied Maths, Sint-Martens-Latem, Belgium). UPGMA clustering analysis was carried out using Pearson correlation data.

Calculation of DGGE range weighted-richness (Rr) was performed as described by Marzorati *et al.* (2008), according to the formula: $R_r = (N^2 \times Dg)$, where N is the total number of bands in the pattern, and Dg the denaturing gradient between the first and the last band of the pattern. Community organization was evaluated using the Lorenz method (Lorenz, 1905; Marzorati *et al.*, 2008). A Lorenz curve was obtained as follows: for each DGGE lane, the respective bands were ranked from high to low based on their intensities. Consecutively, the cumulative proportions of bands were used as the x axis, and the y axis was represented by their respective cumulative proportions of intensities. Mathematically, this yields a convex curve. The more the Lorenz curve deviates from the theoretical perfect evenness line (i.e. the 45° diagonal); the less evenness can be observed in the structure of the studied community (Marzorati *et al.*, 2008).

Cloning and sequencing

The 16S rRNA genes were amplified using the primers P63f and R1378r, according to the protocol reported in Boon *et al.* (2002). The fragments were ligated in pCR2.1-TOPO plasmids and transformed into chemical competent TOP10 cells using the TOPO TA Cloning® kit (Invitrogen). The cells were grown in Luria–Bertani (medium) in the presence of ampicillin (50 mg L^{-1}) and 40 μL, 40 mg mL^{-1} X-gal in dimethylformamide at 37 °C for 16 h. The plasmid DNA was used to perform a PCR reaction with the primer couple 338f–518r, as described above. These fragments were run on a DGGE gel alongside PCR fragments generated with gut template DNA, identifying clones that generated the fragments boxed in Fig. 1. The plasmid DNA was used to perform M13 PCR (according to the instructions in the TOPO TA Cloning® kit). The fragments were purified using the QIAquick PCR Purification kit (QIAgen) and sent for sequencing to AGOWA (Germany).

CLPP – Biolog™ Microplates

CLPPs were determined with Biolog™ EcoPlates (Biolog Inc., Hayward, CA) including 30 different carbon sources and a negative control (water) (Insam, 1997). Three fish in each tank were sampled and euthanized using 2 mL L^{-1} of 2-phenoxyethanol. After dissection in sterile conditions, the hindgut (spiral valve) contents of three fish from the same replicate were gently removed and, after pooling, 1 g (wet weight equivalent) was suspended in 10 mL sterile saline solution (0.85% NaCl) with a Lab Blender (stomacher 400, model BA 7021, UK). Sample suspensions were filtered with a cellulose nitrate membrane filters (50-μm pore size) to remove food particles. The filtrate containing bacteria was further diluted 10 times with physiological solution to reach a final volume of 100 mL. Each well of the Biolog™ EcoPlates was filled with 130 μL of the final dilution (Garland & Mills, 1991).

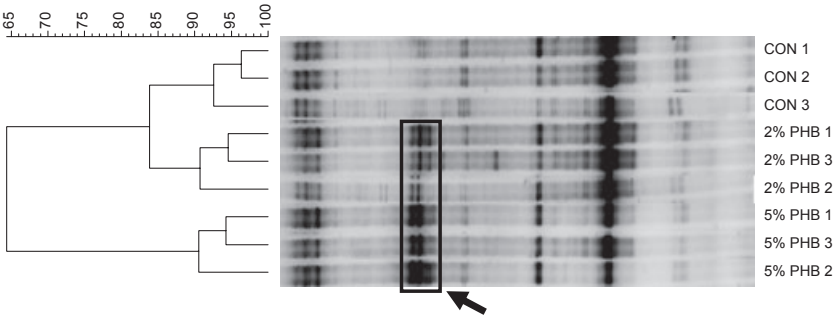

Fig. 1. DGGE band patterns based on the amplified bacterial DNA extracted from the intestinal matter of Siberian sturgeon fingerlings fed with different diets (control, 2% PHB, 5% PHB). The rectangle indicates bands that are more dominant in both PHB treatments than in the control treatment).

Plates were incubated at 25 °C in aerobic conditions and the absorption at 590 nm was read in a microtiter plate reader (TECAN, Model infinite M200, Austria) after 24, 48, 72 and 96 h of incubation. Biolog™ EcoPlate data were normalized by 'average well-colour development' (AWCD) as described by Garland & Mills (1991). In the following formula, C is the colour production within each well, R the absorption value in the control well, and n is the substrate number (31 in EcoPlates).

$$AWCD = \sum(\mathrm{C} - R)/n.$$

The Shannon diversity index (H') for Biolog™ EcoPlate data was calculated from the following equation: $H' = -\sum_{i=1}^{n}(P_i \ln P_i)$, where P_i is the relative absorption (Insam, 1997). Finally, the evenness based on Biolog™ Ecoplate data related to the functionality was calculated by means of the following equation: $E = H'/\ln S$, where H′ is the Shannon diversity index and S the number of wells with colour development.

Negative values were considered to be zero (Viti *et al.*, 2007). Principal Component Analysis (PCA) was used to detect differences between treatments in terms of both bacterial diversity and activity (on the readings from the Biolog™ EcoPlates) (Viti *et al.*, 2008). A Pareto–Lorenz curve was applied to investigate functional biodiversity in Biolog™ Ecoplate data.

Statistical analysis

The results are presented as mean values followed by the standard deviation (mean ± SD). One-way ANOVA was applied for growth performance parameters. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL). PCA scores were analyzed by multivariate analysis of variance (MANOVA). Pairwise comparisons were performed if a difference was detected among the treatments.

Results

Fish performances

The effect of the diets containing different amounts of PHB was examined with respect to WG, SGR, FCR and survival of sturgeon fingerlings. The treatment with 2% PHB resulted in the highest average values for WG and SGR, although the values were not statistically significant as compared to those obtained in the other treatments (Table 1). The FCR of sturgeons fed with PHB was also not significantly different from that of fishes fed the control diet. The survival of fishes fed with 2%, 5% and 0% PHB were 96.6%, 94.5% and 89.1%, respectively (Table 1). The GI tract pH was slightly alkaline for all treatments, with hindgut pH being higher than midgut pH. The midgut pH of the groups fed 2% PHB (7.2 ± 0.05) and 5% PHB (7.2 ± 0.09) diets was significantly ($P < 0.05$) lower than of those fed the control (7.4 ± 0.10) diets. The group fed a diet supplemented with 2% PHB had the lowest intestinal pH (7.8 ± 0.03). However, this was not significantly ($P > 0.05$) different from the pH of those fed a diet supplemented with 5% PHB (7.9 ± 0.10).

Bacterial community composition analysis – DGGE

DGGE analysis was used to assess the effect of the PHB treatment on the structure of the gut microbial community. This analysis was conducted on the total bacterial community at the end of the experiment. The intestinal microbial community patterns clustered according to the diet fed (Fig. 2). In addition, the patterns of fish from the control group and the 2% PHB group were more similar to each other than to the 5% PHB group. However, in the patterns obtained from fish fed PHB-containing diets, some specific bands became apparent (rectangle in Fig. 1), belonging to three groups of bacteria. They were phylogenetically similar to *Bacillus* sp. (99.5% similarity) and *Ruminococcaceae* sp. (either 66.8% or 66.3% similarity). To provide an ecological interpretation to the DGGE patterns, range-weighted richness and the community organization (Co) indexes were calculated, as suggested by Marzorati *et al.* (2008) (Table 2). Rr values for control, 2% PHB and 5% PHB were 32.7 ± 0.5, 44.2 ± 2.1, and 28.2 ± 1.6, respectively. The differences among treatments were all statistically significant ($P < 0.01$). The highest Rr value was obtained with the 2% PHB treatment, and the lowest one with the 5% PHB treatment. Finally, the DGGE patterns were used to calculate the Co index to describe the degree of evenness of a

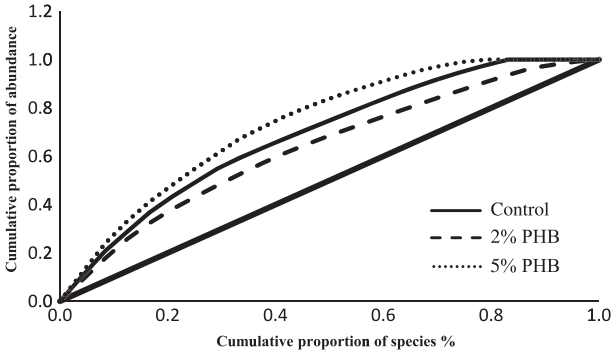

Fig. 2. Pareto–Lorenz distribution curves based on PCR-DGGE patterns.

Table 2. Range-weighted richness (Rr) and Co indexes calculated on the DGGE patterns of the intestinal microbial communities from sturgeon fingerlings fed with three different diets (control, 2% PHB and 5% PHB, respectively) (n = 3)

	Control	2% PHB	5% PHB
Rr	32.7 ± 0.5[a]	44.2 ± 2.1[b]	28.2 ± 1.6[c]
Co	37.5%	27.1%	47.1%

Values are average ± SD, n = 3.
Different letters within rows denote significant differences ($P < 0.05$).

given microbial community (Table 2). The Lorenz curves, a graphical representation of the Co parameter associated to the three treatments, showed that 2% PHB increased the evenness of the microbial community as compared to the control situation. In contrast, 5% PHB exerted a higher selective pressure, decreasing the degree of evenness of the microbial community (Fig. 1).

CLPP of intestinal microbiota

The AWCD of Biolog™ EcoPlates has been used as an index for aerobic metabolism of culturable bacteria under investigation. In the current experiment, this value is an indication of the catabolic diversity of the culturable bacteria under aerobic conditions. The positive AWCD activity was calculated by excluding the negative values, meaning that only the coloured wells were calculated. The results indicated that treatments supplemented with PHB enhanced the aerobic metabolic of culturable bacterial as shown by higher positive AWCD values (Fig. 3). The aerobic metabolism of the bacterial communities from the 2% and 5% PHB treatments were respectively 37% and 4.6% higher than the control after 96 h (Fig. 3).

The 31 carbon sources used in the Biolog™ EcoPlates assay belong to six categories: seven carboxylic acids, six amino acids, four polymers, two amines/amides, 10 carbohydrates, and two phenolic compounds. Supplementation with 5% PHB resulted in higher polymer metabolism, whereas addition of 2% PHB had almost no effect (Fig. 4a). In contrast, the relative utilization efficiency of other carbon sources including amino acids, carbohydrates and carboxylic acids was highest when fish were fed with 2% PHB (Fig. 4b–d). In particular, consumption of amino acids by the aerobic bacterial community in 2% PHB treatment after 96 h was approximately 50% and 63% higher than control and 5% PHB, respectively. A higher aerobic bacterial activity for carboxylic acid components was observed in 2% PHB treatment compared with the control (89%).

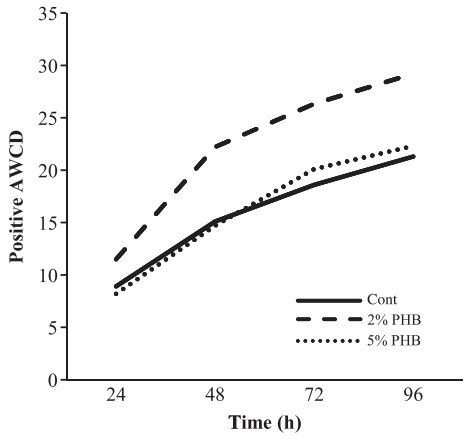

Fig. 3. Positive AWCD activity of intestinal bacteria from sturgeon fingerlings receiving three different diets (control, 2% PHB and 5% PHB).

The Shannon index has been used as a means to express the diversity level of aerobic bacterial metabolism. The aerobic bacterial community of sturgeon fingerlings fed with the 2% PHB diet showed the highest Shannon index (2.5 ± 0.2) indicating that these fish contained the most diverse community in terms of metabolic potential (Table 3).

A Lorenz curve was used to describe the evenness distribution of a microbial community in terms of the Biolog™ Ecoplate data. Our results showed an increase in evenness in the 2% PHB treatment (high aerobic metabolic potential) (Fig. 5). In contrast, the functional biodiversity with 5% PHB treatment was lower than that of the control.

PCA was performed on Biolog™ Ecoplate data. Analysis by MANOVA indicated that the aerobic bacterial activity in 2% PHB was significantly different with respect to the control and 5% PHB (no differences between control and 5% PHB) (Table 4, Fig. 6).

Discussion

In the previous two decades, antibiotics were widely used not only for treatment but also to promote the growth of fish, especially juvenile fish (Acar *et al.*, 2000). However, the banning of growth-promoting antibiotics usage in the EU and other countries has challenged researchers to find alternatives strategies (De Schryver *et al.*, 2010).

Recently, many alternative bio-control studies have been performed to find alternative ways to promote growth (Mahious *et al.*, 2006; Zhou *et al.*, 2007). In this study, the effects of PHB on the growth performance and the intestine bacterial community structure of Siberian sturgeon were investigated in 10-week trial. Our results indicated that there is no significant difference between fish fed on PHB-containing diets and control in terms of WG, survival, FCR or SGR, although treatment with 2% PHB performed the best in the trial. Analyzing the microbial community by PCR-DGGE and Biolog™ Ecoplates

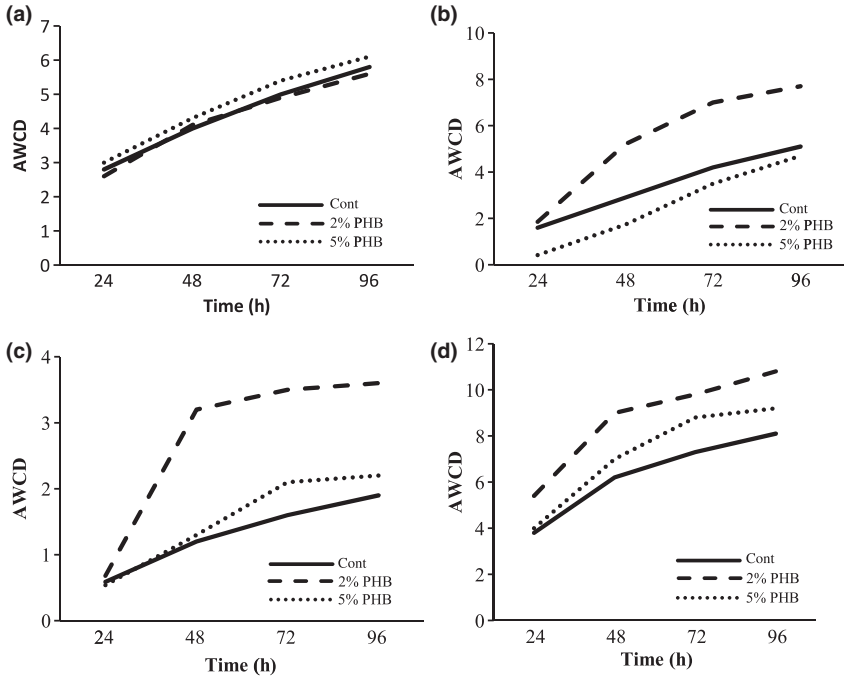

Fig. 4. The use of (a) polymers, (b) amino acids, (c) carboxylic acids, and (d) carbohydrates as a carbon source by intestinal bacteria from sturgeon fingerlings receiving three different diets (control, 2% PHB and 5% PHB).

Table 3. Shannon's index of diversity for the GI tract microbial communities from sturgeon fingerlings from Biolog™ EcoPlates data that received three different diets (control, 2% PHB and 5% PHB), after 24 h incubation

	Control	2% PHB	5%PHB
Shannon's index	2.2 ± 0.1[a]	2.5 ± 0.2[b]	2.1 ± 0.2[a]
Evenness	0.79 ± 0.1[a]	1.07 ± 0.1[b]	0.71 ± 0.1[a]

Values are average ± SD, $n = 3$.
Different letters within rows denote significant differences ($P < 0.05$).

Table 4. Comparisons of CLPP of the GI tract microbial community of the Siberian sturgeon fingerlings in dietary treatments by MANOVA

Treatments	Wilks' lambda test	*P* value
All three treatments	0.001	< 0.01
Control vs. 2% PHB	0.006	< 0.01
Control vs. 5% PHB	0.33	ns
2% PHB vs. 5% PHB	0.00	< 0.01

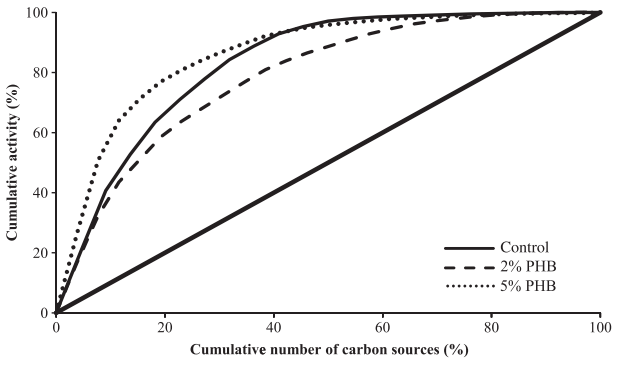

Fig. 5. Pareto–Lorenz distribution curves based on Biolog™ Ecoplate data.

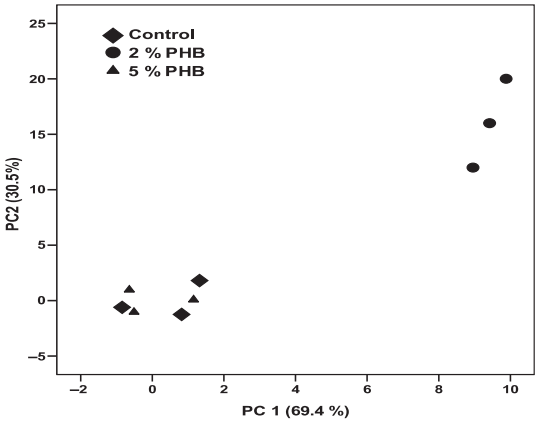

Fig. 6. Ordination diagram of CLPP from dietary treatment.

showed that there is a high aerobic metabolic potential in fish fed with 2% PHB. Sturgeon fingerlings fed on a diet containing 5% PHB were slightly retarded in their growth, which may be partly due to added PHB replacing part of the total feed. In a previous study by De Schryver *et al.* (2010) with sea bass larvae, the highest growth rate and the lowest FCR were observed with 5% PHB, indicating that the optimal PHB dose might be species-specific.

Growth-promoting effects achieved by including dietary compounds have been observed using inulin in turbot (*Psetta maxima*) larvae (Mahious *et al.*, 2006), xylooligosaccharides in crucian carp (*Carassius carassius*) juveniles (Baohua *et al.*, 2009) and Grobiotic™ AE in hybrid striped bass (Li & Gatlin, 2004, 2005). In contrast, no significant differences could be observed in Arctic charr (*Salvelinus alpinus*) fed with inulin (Olsen *et al.*, 2001), with salmon fed with galactooligosaccharide (Grisdale-Helland *et al.*, 2008), tilapia or Gulf sturgeon fed with mannanoligosaccharide (Pryor *et al.*, 2003; Genc & Yilmaz, 2007), Siberian sturgeon fed with arabinoxylooligosaccharides (Rurangwa *et al.*, 2008). These products have been classified as prebiotic, non-digestible diet components that are degraded by specific microorganisms beneficial to the health and growth of the host (Gibson & Roberfroid, 1995; Manning & Gibson, 2004). The prebiotic effect has been associated with the production of SCFA, which provides a beneficial environment for beneficial bacteria (De Schryver *et al.*, 2010).

PHB is an important member of the family of PHAs and it can be degraded by microbial extracellular hydrolytic enzymes to 3-hydroxybutyrate, becoming a carbon and energy source for the fish (Kato *et al.*, 1992; Patnaik, 2005; De Schryver *et al.*, 2010). High survival of sea bass larvae fed only on PHB particles (in comparison with starvation) was observed by De Schryver *et al.* (2010). Axenic *A. franciscana* could also degrade and metabolize PHB for energy purposes, as shown by Defoirdt *et al.* (2007). Both studies indicate that 3-hydroxybutyrate could be used as an energy source, although both organisms might not be able to grow on this substrate alone. Growth-promoting effects of PHB have been observed in sea bass (De Schryver *et al.*, 2010) and giant freshwater prawn (Nhan *et al.*, 2010) when it was incorporated in the feed. The released 3-hydroxybutyrate as a SCFA tends to decrease GI tract pH, as also observed by De Schryver *et al.* (2010) in sea bass juveniles.

The microbial community in the GI tract affects the host in many ways that modulate nutrition of the immune system (Blaut, 2002). In this study, we used two common methods to study the microbial community associated to the sturgeon GI tract: 16S rRNA gene fingerprinting by PCR-DGGE and CLPPs. Both methods indicated that the incorporation of 2% PHB in the diet improved the intestinal microbial community in terms of microbial diversity and activity.

Cluster analysis based on DGGE patterns demonstrated that the dietary treatments clustered into distinct groups. Range-weighted richness and Lorenz curves were applied to analyze the DGGE patterns. These methods revealed that treatment with 2% PHB increased the diversity of the intestinal microbial community. For instance, Lorenz curves describe the equality of distribution within a population and also indicate a relation between the structure of a microbial community and its functionality (Mertens *et al.*, 2005; Marzorati *et al.*, 2008; Wittebolle *et al.*, 2008). Lorenz curve analysis based on DGGE patterns showed that range-weighted richness and evenness in the 2% PHB treatment was higher than in the control and 5% PHB treatments. High growth rate and low mortality were observed in 2% PHB and hence it seems that there is a relationship between bacterial diversity in the GI tract and growth rate in Siberian sturgeon; however, probably because of the excellent conditions in the experimental tanks, even in the control (FCR < 0.85), significant differences were not observed. Our results are in agreement with a previous study by De Schryver *et al.* (2010) in sea bass which indicated that there is a relationship between fish growth rate, low mortality in the 5% PHB treatment and range-weighted richness. The DGGE patterns showed some bands apparently amplified from *Bacillus* sp. and *Ruminococcaceae* sp. in the PHB treatments that were absent (or less dominant) in the control. Previous studies have shown that these types of bacteria accumulate or degrade PHB according to environmental conditions (Liu *et al.*, 2010). Liu *et al.* (2010) isolated *Acidovorax* spp., *Acinetobacter* spp. and *Ochrobactrum* spp. from Siberian sturgeon, sea bass and prawn gut, respectively, that were able to degrade PHB by extracellular enzymes. Several studies have shown that *Bacillus* sp. and *Ruminococcaceae* sp. can be used as a probiotic, decreasing pathogenic bacteria and stimulating the non-specific immune system in fish (Sakai *et al.*, 1995; Rengpipat *et al.*, 1998; Daniels *et al.*, 2010). However, for further information these kinds of bacteria should be isolated from sturgeon gut, to investigate their effects as a probiotic.

Calculation of AWCD values for Biolog™ Ecoplates showed that the 2% PHB treatment resulted in a higher aerobic metabolism of culturable bacteria in the sturgeon GI tract. The sturgeon fingerlings fed a diet containing 2% PHB showed an increase in the consumption of amino acids, carboxylic acids and carbohydrates within different groups of carbon sources in the Biolog™ Ecoplates. The highest aerobic metabolism was observed with 2% PHB in the carboxylic acid group. Naturally organic acids such as carboxylic acids are a product of bacterial fatty acid catabolism and PHB is a fatty acid polymer that can be degraded and used by bacterial as an energy source (Azain, 2004). In Biolog™ Ecoplate substrates, carboxylic acids are the most diverse substrates in term of molecular weight and chemical configuration (Christian & Lind, 2007). Our results indicate that in the 2% PHB treatment, these kinds of carboxylic acids can be degraded quickly. The same trend in evenness and range-weighted richness was observed in the Biolog™ Ecoplate data and in the DGGE patterns.

There is a strong relation between species richness and productivity in ecosystems (Wilsey & Potvin, 2000; Wittebolle *et al.*, 2009). Results indicate that a well balanced diet with PHB increases bacterial species richness in the fish GI tract. PHB degradation in sturgeon gut alters GI tract pH, most probably because of β-hydroxybutyrate (SCFA) production, inducing changes in the composition of the bacterial community. However, in this case, PHB did not improve the sturgeon growth performance parameters when compared with studies of European sea bass and giant freshwater prawn.

In conclusion, our results and previous studies have shown that inclusion of PHB in the fish diet can be used as microbial control agent in aquaculture and that its effect on growth performances depends on both concentration and species.

References

Acar J, Casewell M, Freeman J, Friis C & Goossens H (2000) Avoparcin and virginiamycin as animal growth promoters: a plea for science in decision-making. *Clin Microbiol Infect* **6**: 477–482.

Akrami R & Hajimoradloo A (2009) Effect of dietary prebiotic inulin on growth performance, intestinal microflora, body composition and hematological parameters of juvenile Beluga, *Huso huso* (Linnaeus, 1758). *J World Aquacult Soc* **40**: 771–779.

Azain MJ (2004) Role of fatty acids in adipocyte growth and development. *J Anim Sci* **82**: 916–924.

Baohua X, Wang Y, Li J & Lin Q (2009) The effect of prebiotic xylooligosaccharides (XOS) on the growth performance and digestive enzyme activities of the allogynogenetic crucian carp, *Carassius auratus gibelio*. *Fish Physiol Biochem* **35**: 351–357.

Blaut M (2002) Relationship of prebiotics and food to intestinal microflora. *Eur J Nutr* **41**(suppl 1): 11–16.

Boon N, De Windt W, Verstraete W & Top EM (2002) Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. *FEMS Microbiol Ecol* **39**: 101–112.

Boon N, Top EM, Verstraete W & Siciliano SD (2003) Bioaugmentation as a tool to protect the structure and function of an activated-sludge microbial community against a 3-chloroaniline shock load. *Appl Environ Microbiol* **69**: 1511–1520.

Burr G, Gatlin D & Ricke S (2005) Microbial ecology of the GI tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture. *J World Aquacult Soc* **36**: 425–436.

Christian BW & Lind OW (2007) Multiple carbon substrate utilization by bacteria at the sediment–water interface: seasonal patterns in a stratified eutrophic reservoir. *Hydrobiologia* **586**: 43–56.

Daniels CL, Merrifield DL, Boothroyd DP, Davies SJ, Factor JR & Arnold KE (2010) Effect of dietary *Bacillus* spp. and mannan oligosaccharides (MOS) on European lobster (*Homarus gammarus* L.) larvae growth performance, gut morphology and gut microbiota. *Aquaculture* **304**: 49–57.

De Schryver P, Sinha AK, Kunwar PS, Baruah K, Boon N, Verstraete W, De Boeck G & Bossier P (2010) Poly-β-hydroxybutyrate (PHB) increases growth performance and intestinal bacterial range-weighted richness in juvenile European sea bass, *Dicentrarchus labrax*. *Appl Microbiol Biotechnol* **86**: 1535–1541.

Defoirdt T, Halet D, Vervaeren H, Boon N, Van de Wiele T, Sorgeloos P, Bossier P & Verstraete W (2007) The bacterial storage compound poly-beta-hydroxybutyrate protects *Artemia franciscana* from pathogenic *Vibrio campbellii*. *Environ Microbiol* **9**: 445–452.

Garland JL & Mills AL (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Appl Environ Microbiol* **57**: 2351–2359.

Genc MA & Yilmaz E (2007) Effects of dietary mannanoligosaccharides (MOS) on growth, body composition, and intestine and liver histology of the hybrid Tilapia (*Oreochromis niloticus* x *O-aureus*). *Isr J Aquacult* **59**: 10–16.

Gibson GR & Roberfroid MB (1995) Dietary modulation of the human colonic microbiota introducing the concept of prebiotics. *J Nutr* **125**: 1401–1412.

Grisdale-Helland B, Helland SJ & Gatlin DM (2008) The effects of dietary supplementation with mannanoligosaccharide, fructooligosaccharide or galactooligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). *Aquaculture* **283**: 163–167.

Halet D, Defoirdt T, Vervaeren H, Boon N, Van de Wiele T, Sorgeloos P, Bossier P & Verstraete W (2007) Poly-beta-hydroxybutyrate-accumulating bacteria protect gnotobiotic *Artemia franciscana* from pathogenic *Vibrio campbellii*. *FEMS Microbiol Ecol* **60**: 363–369.

Hansen GH & Olafsen JA (1999) Bacterial interactions in early life stages of marine cold water fish. *Microb Ecol* **38**: 1–26.

Insam H (1997) Substrate utilization test in microbial ecology. A preface to the special issue. *J Microbiol Methods* **30**: 1–2.

Kato N, Konishi H, Shimao M & Sakazawa C (1992) Production of 3 hydroxybutyric acid trimer by *Bacillus megaterium* B-124. *J Ferment Bioeng* **73**: 246–247.

Li P & Gatlin DM (2004) Dietary brewers yeast and the prebiotic Grobiotic™ AE influence growth performance, immune responses and resistance of hybrid striped bass (*Morone chrysops* * *M. saxatilis*) to *Streptococcus iniae* infection. *Aquaculture* **231**: 445–456.

Li P & Gatlin DM (2005) Evaluation of the prebiotic Grobiotic TMA and brewers yeast as dietary supplements for sub-adult hybrid striped bass (*Morone chrysops* * *M. saxatilis*)

challenged *in situ* with *Mycobacterium marinum*. *Aquaculture* **248**: 197–205.

Liu Y, De Schryver P, Van Delsen B, Maignien L, Boon N, Sorgeloos P, Verstraete W, Bossier P & Defoirdt T (2010) PHB-degrading bacteria isolated from the GI tract of aquatic animals as protective actors against *luminescent vibriosis*. *FEMS Microbiol Ecol* **74**: 196–204.

Lorenz MO (1905) Methods of measuring concentration of wealth. *J Am Stat Assoc* **9**: 209–219.

Mahious AS, Gatesoupe FJ, Hervi M, Metailler R & Ollevier F (2006) Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, *Psetta maxima* (Linnaeus, C. 1758). *Aquacult Int* **14**: 219–229.

Manning TS & Gibson GR (2004) Prebiotics. *Best Pract Res Clin Gastroenterol* **18**: 287–298.

Marzorati M, Wittebolle L, Boon N, Daffonchio D & Verstraete W (2008) How to get more out of molecular fingerprints: practical tools for microbial ecology. *Environ Microbiol* **10**: 1571–1581.

Mertens B, Boon N & Verstraete W (2005) Stereospecific effect of hexachlorocyclohexane on activity and structure of soil methanotrophic communities. *Environ Microbiol* **7**: 660–669.

Muyzer G & Smalla K (1998) Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Van Leeuwenhoek* **73**: 127–141.

Nhan DT, Wille M, De Schryver P, Defoirdt T, Bossier P & Sorgeloos P (2010) The effect of poly [beta]-hydroxybutyrate on larviculture of the giant freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture* **302**: 76–81.

Olsen RE, Myklebust R, Kryvi H, Mayhew TM & Ringø E (2001) Damaging effect of dietary inulin on intestinal enterocytes in Arctic char (*Salvelinus alpinus* L.). *Aquacult Res* **32**: 931–934.

Patnaik PR (2005) Perspectives in the modeling and optimization of PHB production by pure and mixed cultures. *Crit Rev Biotechnol* **25**: 153–171.

Patterson J & Burkholder K (2003) Application of prebiotics and probiotics in poultry production. *Poult Sci* **82**: 627.

Pryor GS, Royes JB, Chapman FA & Miles RD (2003) Mannanoligosaccharides in fish nutrition: effects of dietary supplementation on growth and GI villi structure in Gulf of Mexico sturgeon. *N Am J Aquacult* **65**: 106–111.

Rengpipat S, Phianphak W, Piyatiratitivorakul S & Menasveta P (1998) Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture* **167**: 301–313.

Rurangwa E, Delaedt Y, Geraylou Z, Van De Wiele T, Courtin CM, Delcour JA & Ollevier F (2008) *Dietary Effect of Arabinoxylan Oligosaccharides on Zootechnical Performance and Hindgut Microbial Fermentation in Siberian Sturgeon and African Catfish*. Aquaculture Europe, Krakow, September 15–18, pp. 569–570.

Sakai M, Yoshida T, Astuta S & Kobayashi M (1995) Enhancement of resistance to vibriosis in rainbow trout, *Oncorhynchus mykiss* (*Walbaum*) by oral administration of *Clostridium butyricum* bacteria. *J Fish Dis* **18**: 187–190.

Smiricky-Tjardes M, Grieshop C, Flickinger E, Bauer L & Fahey GJ (2003) Dietary galactooligosaccharides affect ileal and totaltract nutrient digestibility, ileal and fecal bacterial concentrations, and ileal fermentative characteristics of growing pigs. *J Anim Sci* **81**: 25–35.

Van Cam DT, Van Hao N, Dierckens K, Defoirdt T, Boon N, Sorgeloos P & Bossier P (2009) Novel approach of using homoserine lactone-degrading and poly-[beta]-hydroxybutyrate-accumulating bacteria to protect Artemia from the pathogenic effects of *Vibrio harveyi*. *Aquaculture* **291**: 23–30.

Viti C, Quaranta D, De Philippis Cort G, Agnelli A, Cuniglio R & Giovannetri L (2007) Characterizing cultivable soil microbial communities from copper fungicide-amended olive orchard and vineyard soils. *World J Microbiol Biotechnol* **24**: 309–318.

Viti C, Quaranta D, De Philippis R, Corti G, Agnelli A, Cuniglio R & Giovannetti L (2008) Characterizing cultivable soil microbial communities from copper fungicide-amended olive orchard and vineyard soils. *World J Microbiol Biotechnol* **24**: 309–318.

Wilsey BJ & Potvin C (2000) Biodiversity and ecosystem functioning: importance of species evenness in an old field. *Ecology* **81**: 887–892.

Wittebolle L, Vervaeren H, Verstraete W & Boon N (2008) Quantifying community dynamics of nitrifiers in functionally stable reactors. *Appl Environ Microbiol* **74**: 286–293.

Wittebolle L, Marzorati M, Clement L, Balloi A, Daffonchio D, Heylen K, De Vos P, Verstraete W & Boon N (2009) Initial community evenness favours functionality under selective stress. *Nature* **458**: 623–626.

Yousefian M& Amiri MS (2009) A review of the use of prebiotic in aquaculture for fish and shrimp. *Afr J Biotechnol* **25**: 7313–7318.

Zhou ZG, Ding ZK & Huiyuan LV (2007) Effects of dietary short-chain fructooligosaccharides on intestinal microflora, survival, and growth performance of juvenile white shrimp, *Litopenaeus vannamei*. *J World Aquacult Soc* **38**: 296–301.