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A preliminary *in vitro* assessment of GroBiotic®-A, brewer's yeast and fructooligosaccharide as prebiotics for the red drum *Sciaenops ocellatus*

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This study examined the effects of brewers yeast, fructooligosaccharide (FOS), and GroBiotic®-A, a mixture of partially autolyzed brewers yeast, dairy components and dried fermentation products, on the intestinal microbial community of red drum, *Sciaenops ocellatus*. Gastrointestinal (GI) tracts were aseptically removed from three sub-adult red drum previously maintained on a commercial diet and placed in an anaerobic chamber. Intestinal contents were removed, diluted and incubated *in vitro* in one of four liquid media: normal diet alone, diet + 2% (w/w) GroBiotic®-A, diet + 2% brewers yeast, and diet + 2% FOS. After 24 and 48 h of incubation at 25°C, supernatants were removed for volatile fatty acid (VFA) analysis and DNA was extracted for denaturing gradient gel electrophoresis (DGGE) analysis. Polymerase chain reaction (PCR) was performed on a highly conserved region of M 16S rDNA and the amplicons were subjected to DGGE. The microbial community (MC) fingerprint was used to distinguish microbial populations. The intestinal contents incubated with GroBiotic®-A had significantly ($P < 0.05$) higher acetate and total VFA concentrations at 48 h compared to the other treatments. DGGE analysis demonstrated that the microbial community was significantly altered by Grobiotic®-A and brewers yeast.

Keywords: Prebiotics; gastrointestinal tract microbes; anaerobic bacteria; denaturing gradient gel electrophoresis (DGGE).

Introduction

Recently there has been increased interest in altering the intestinal microbiota of animals by introducing beneficial bacteria to the gastrointestinal (GI) tract or adding supplements to the diet. There are two general approaches used to modify the GI tract bacteria. The first approach is the use of probiotics, which are viable microorganisms that benefit the host.[1] Probiotics have been studied in swine[2,3], chickens[4], and humans[1] as well as fishes.[5–8] The second commonly used approach to modify the GI tract microbial community is the addition of prebiotics to the diet. Prebiotics are defined as "nondigestable food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon."[9] Prebiotics have been found to have beneficial effects in humans,[10,11] poultry,[4,12] and swine.[13,14] Reports from these studies reveal that prebiotics can modify the GI tract microbial community to enhance non-specific immune responses[15], increase fermentation products,[13] as well as improve mineral uptake,[16] and livestock performance indices such as protein efficiency ratio and feed conversion ratio.[16] Smiricky-Tjardes et al.[13] demonstrated that dietary transglacto-oligosaccharide increased the concentrations of the volatile fatty acids (VFAs) propionate and butyrate in the small intestine of swine. Prebiotics such as oligofructose have been reported to increase bioavailability of glucose and trace elements in the diet.[17,18] Oligofructose has been shown to increase feed efficiency and weight gain in broiler chicks, while mixed results have been seen in pigs.[19] These potential benefits of prebiotics have been poorly investigated in fishes.

The GI microbial community, especially the anaerobic microbiota, of fishes have been poorly studied and understood. The majority of studies characterizing the microbial community of fish have been aerobic studies,[20–23] which can be useful for determining the dominant facultative

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anaerobic bacteria, but are not appropriate for isolating strict anaerobic bacteria. Using aerobic methods to culture bacteria have led some investigators to conclude that anaerobic bacteria in fish play a minor role in the GI tract microbial community.[23] Anaerobic studies of the GI tract of fishes are essential to evaluate the effects of the entire microbial community on the host.[24]

Prebiotics have received considerable attention from the terrestrial livestock industry as a way to improve the disease resistance and to increase growth performance of the host organism. However, little attention has been given to prebiotics in aquaculture. In fishes, the limited work done with prebiotics has focused on *in vivo* studies. Previous studies have examined different dietary components on the culturable facultative intestinal microbial community. Linoleic acid and other dietary fatty acids, as well as dietary carbohydrate components, have been shown, to alter the aerobic/facultative intestinal microbiota of Artic char using classical microbiological techniques.[21,25,26] When linoleic acid was supplemented in the diet of Artic char, the total viable counts from the GI tract aerobic/facultative microbial community were increased 10-fold as compared with fish fed a diet without linoleic acid.[25]

The purpose of the current study was to determine if Grobiotic®-A, brewers yeast and the known terrestrial prebiotic fructooligosaccharide (FOS) could be effective prebiotics for red drum (*Sciaenops ocellatus*). The supplements were evaluated *in vitro* with GI tract inoculum from red drum and the anaerobic microbial community was assessed using denaturing gradient gel electrophoresis (DGGE) and volatile fatty acid (VFA) production.

Methods and materials

Preparation of samples

Red drum were fed a commercial diet (EXTR 400, Rangen, Angleton, TX) twice daily and maintained in a recirculating culture system 8 ppt salinity with optimal water quality. The temperature was maintained at 25°C by conditioning the ambient environment, and a 12:12 light:dark was maintained with artificial lights and timers. The fish appeared healthy at the start of the experiment.

The GI tracts of three healthy sub-adult red drum from the recirculating system were aseptically harvested 4 h after the fish were fed the commercial diet containing 40% protein and 10% lipid. The GI tracts were placed into 50-ml conical tubes and transported to an anaerobic chamber (Coy Laboratory Products, Detroit, MI) with an atmosphere of 10% CO_2, 5% H_2, and 85% N_2 gas. The intestinal contents from each fish were removed independently by squeezing and diluted separately 1:3000 with anaerobic dilution solution (ADS) (K_2HPO_4, 0.45 g/L; KH_2PO_4, 0.45 g/L; $(NH_4)_2SO_4$, 0.45 g/L; NaCl, 0.90 g/L; $MgSO_4 \times 7H_2O$, 0.225 g/L; $CaCl_2 \times 2\ H_2O$, 0.12 g/L; cysteine, 0.6 g/L; resazurin, 0.02 g/L; and sodium bicarbonate, 1.59 g/L).[27,28] After removal of the contents, the intestines were subsequently discarded. The ADS had been placed into the anaerobic hood the previous day to remove any oxygen. Five milliliters of the diluted intestinal contents or sterile ADS were added to 15-ml tubes containing 0.3 g of diet. This created non-inoculated tubes (without the diluted GI tract microbiota) and inoculated tubes, one tube containing the diluted intestinal contents from a single fish (with the GI tract microbiota). All inoculated and non-inoculated treatments were tested in triplicate, independent tubes. The same commercial diet that was fed to the fish was used as the medium to which the three prebiotics were added to a concentration of 2% on a dry-weight basis. The prebiotics evaluated included GroBiotic®-A, a mixture of partially autolyzed brewers yeast, dairy ingredient components and dried fermentation products[29] (International Ingredient Corporation, St. Louis, MO); partially-autolyzed brewers yeast (International Ingredient Corporation, St Louis, MO); and FOS (Encore Technologies, Plymouth, MN). Each treatment was evaluated in triplicate. The tubes were allowed to incubate at 25°C for 0, 24 and 48 hours. A portion (1 mL) was removed for DNA isolation and PCR at each time interval. The remaining portions of the cultures were centrifuged at 20,000 × *g*, and 1 mL of the supernatant was used for VFA analysis.

Volatile fatty acid analysis

Volatile fatty acid analysis was done according to the methods of Hinton et al.[30] as follows: 1 ml of culture was centrifuged at 20,000 × *g* for 10 min and supernatants were stored at −20°C until analysis was preformed by gas chromatography using a Shimadzu Gas Chromatograph GC-14A (Shimadzu, Columbia, MD) equipped with a flame ionization detector, an 80/120 Carobpack™ B-DA/4% Carbowax® 20M (2 m × 2 mm ID) glass column, with an oven temperature of 175°C and detector temperature of 175°C. The flow rate was 24 ml/min. The peak profiles were obtained with a CR501 autointegrator. All samples had 20 mM 2-methylbutyric acid added as an internal standard. The concentrations of the VFA at each incubation interval were subjected to analysis of variance and Duncan's multiple range test for comparison using the Statistical Analysis System.[31]

DNA isolation and PCR

Genomic DNA was isolated from the initial intestinal content sample and from 1 ml of each subsequent culture with a QIAamp DNA Mini Kit (Qiagen, Valencia, CA) using the method supplied by the manufacturer. The bacteria in each sample were pelleted by centrifuging at 5,000 x *g* for 10 min. Each pellet was suspended in 180 μL of enzyme solution (20 mg/ml lysozyme, 20 mM Tris-HCl (pH 8.0), 2 mM EDTA, 1.2% Triton®) for 30 min at 37°C.

PCR was conducted using the method of Hume et al.[32]. The use of bacteria-specific PCR primers to conserved regions flanking the variable V3 region of 16S rDNA was used. The primers (50 pmol of each primer; primer 2, 5′-ATTACC GCGGCTGCTGG-3′; primer 3 with a 40 base pair GC clamp (33) 5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGG CACGGGGGGCCTACGGGAGGCAGCAG-3′) were mixed with Jump Start Red-Taq Ready Mix (Sigma Chemical Company, St. Louis, MO) according to the manufacturer's instructions, 250 ng of pooled (83 ng/replicate) template DNA from each of the three replicates was added along with 10 μg of bovine serum albumin (BSA) to help stabilize the reaction. The PCR amplifications were performed on a PTC-200 Peltier Thermal Cycler (MJ Research, Waltham, MA). A touchdown PCR program was used to minimize artificial by-products. The program used was as follows: 1) denaturation at 94.9°C for 2 min; 2) denaturation at 94.0°C for 1 min; 3) annealing at 67°C for 45s, −0.5°C per cycle; (to minimize formation of artificial products) (Hume et al. 2003); 4) extension at 72°C for 2 min; 5) repeat steps 2 to 4 for 17 cycles; 6) denaturation at 94°C for 1 min; 7) annealing at 58°C for 45 sec; 8) repeat steps 6 to 7 for 12 cycles; 9) extension at 72°C for 30 min; 10) 4°C final.

Denaturing gradient gel electrophoresis

Denaturing gradient gel electrophoresis (DGGE) was run following the method of Hume et al.[32] as modified from Muyzer et al.[33] The amplicons were separated on 8% polyacrylamide gels [(vol/vol) acrylamide-bisacrylamide ratio of 37.5:1 (Bio-Rad, Richmond, CA)] with a 30% to 60% urea-formamide gradient (100% denaturing 7M urea and 40% formamide) using a Dcode System (Bio-Rad, Hercules, CA). The amplicons were mixed with an equal volume of 2X loading buffer [0.05% (wt/vol) bormophenol blue; 0.05% (wt/vol) xylene cyanol; and 70% (vol/vol) glycerol] and 7 μL was loaded into each sample well (16-well comb). The gels were run at 60 volts for 17 hours in 0.5X Tris-Acetate-EDTA buffer (TAE) (20 mM Tris (pH 7.4); 10 mM sodium acetate; 0.5 M ethylenediaminetetraacetic acid (EDTA); Bio-Rad, Hercules, CA) at 59°C. Gels were stained for 30 min with SYBR® Green I (USA Amersham Life Sciences, Cleveland, OH) diluted 1:10,000. The fragment analysis pattern relatedness was determined with Molecular Analysis Fingerprinting software (v 1.6; Bio-Rad, Hercules, CA). This analysis is based on the Dice similarity coefficient and the unweighted pair group method using arithmetic averages (UPGMA) for clustering. Comparisons between sample band patterns were expressed as a percentage similarity coefficient (%SC).

Sequencing

Six bands from the common bands in the non-inoculated, GroBiotic-A®, and the brewer's yeast treatments were targeted for sequencing (designated by the arrows in Figure 1). Plugs from these six bands were removed using sterile 200 μl tips. The plugs were then incubated overnight in Jump Start Red-Taq Ready Mix (Sigma Chemical Company, St. Louis, MO) according to the manufacturer's instructions. The samples were then amplified with the same primers as before except primer 3 did not have the 40 base pair GC clamp. The samples were then reamplified using a blunt end polymerase. The blunt end products were then used in Zero Blunt® TOPO® polymerase chain reaction (PCR) Cloning Kit for Sequencing (Invitrogen, Carlsbad, California) according to the methods provided in the kit. Three clones were sequenced and then analyzed using at the National Center for Biotechnology Information (NCBI) to identify the genus and/or species.

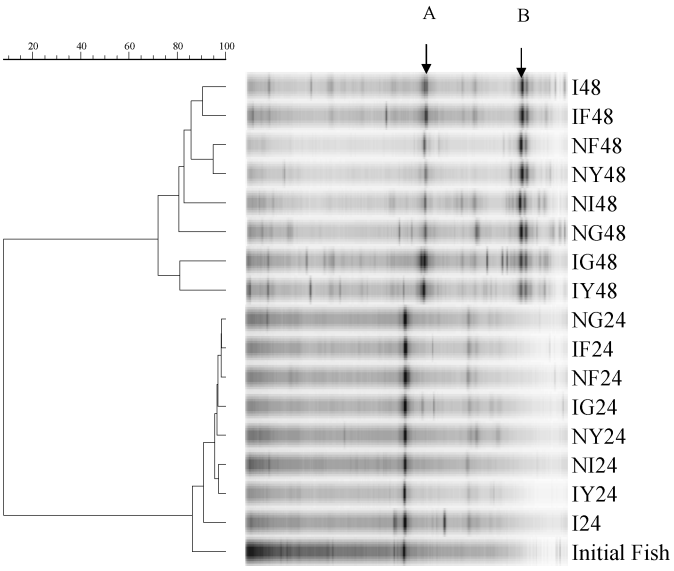

Fig. 1. Denaturing gradient gel electrophoresis of bacterial 16S rDNA amplicons from anaerobic cultured red drum intestinal contents. The bar above figure indicates percentage similarity coefficients. NI = non-inoculated + basal diet; NG = non-inoculated + basal diet + 2% GroBiotic®-A; NF = non-inoculated + basal diet + 2% fructoliogosaccharide (FOS); NY = non-inoculated + basal diet + 2% brewers yeast; I = inoculated + basal diet; IG = inoculated + basal diet + 2% GroBiotic®-A; IF = inoculated + basal diet + 2% FOS; IY = inoculated + basal diet + 2% brewers yeast. The number indicates incubation time in hours. Initial fish = DNA isolation directly from the intestinal contents.

Results

Volatile fatty acids

The microbial community cultured from the GI tract of red drum was acetogenic (Table 1). After 24-h incubation in an anaerobic environment, acetate production was significantly ($P < 0.05$) increased with the addition of

Table 1. *In vitro* acetate and propionate production by the intestinal bacteria from red drum cultured under anaerobic conditions at 27°C[1].

	Incubation time (h)			*Incubation time (h)*		
	0	*24*	*48*	*0*	*24*	*48*
	Acetate			*Propionate*		
No inoculum (NI)	0.77 ± 0.6	12.44 ± 11.6^{B}	33.17 ± 16.5	0.83 ± 0.6^{AB}	0.00 ± 0.0	0.00 ± 0.0^{B}
NI + FOS[2]	0.76 ± 0.4	10.31 ± 4.9^{B}	44.68 ± 26.2	0.58 ± 0.4^{AB}	0.00 ± 0.0	0.02 ± 0.0^{B}
NI + GroBiotic®-A	0.48 ± 0.4	10.06 ± 2.9^{B}	50.41 ± 25.5	0.47 ± 0.1^{B}	0.00 ± 0.0	0.42 ± 0.7^{AB}
NI + Yeast	0.92 ± 2.2	21.45 ± 2.3^{B}	53.26 ± 15.5	2.23 ± 2.2^{A}	0.00 ± 0.0	1.26 ± 1.6^{A}
Inoculum (I)	0.75 ± 0.7	16.18 ± 12.9^{B}	42.58 ± 15.1	072 ± 0.5^{AB}	0.00 ± 0.0	0.00 ± 0.0^{B}
I + FOS	1.19 ± 0.8	26.99 ± 12.5^{B}	51.93 ± 27.9	0.77 ± 0.3^{AB}	0.00 ± 0.0	0.00 ± 0.0^{B}
I + GroBiotic®-A	1.00 ± 0.9	88.73 ± 86.9^{A}	67.85 ± 19.2	0.90 ± 0.4^{AB}	0.53 ± 0.9	0.00 ± 0.0^{B}
I + Yeast	1.00 ± 0.9	42.88 ± 17.0^{AB}	49.51 ± 9.3	0.87 ± 0.6^{AB}	0.22 ± 0.4	0.16 ± 0.3^{AB}
Anova P[3]	0.8527	0.1122	0.6600	0.3700	0.5310	0.2638

[1]Within column means ± SD (μmole/ml; n = 3) without a common superscript letter differ significantly ($P < 0.05$).
[2]fructooligosaccharide.
[3]means compared within incubation time.

GroBiotic®-A compared to diet alone or the addition of FOS in the inoculated samples and all of the non-inoculated samples. After 48 h the differences were not significant ($P \geq 0.05$). Propionate production did not vary at each incubation time (Table 1). Butyrate production was significantly ($P < 0.05$) increased after 24 h for the sample containing GroBiotic®-A compared to the other samples (Table 2). However, after 48 h no differences were apparent. Total volatile fatty production was increased ($P < 0.05$) after 24 h for the samples containing GroBiotic®-A compared to the other samples (Table 2), differences were not significant after 48 h although samples containing GroBiotic®-A had the highest VFA production.

DGGE analysis

The DNA isolated from the 0-h samples was low in concentration and did not amplify and thus was omitted from the DGGE analysis. The cluster analysis separated the samples into two groups. One group was composed of the 24-h samples that differed significantly from the group composed of the 48-h samples (Fig. 1; 8%SC). The banding patterns for

Table 2. *In vitro* butyrate and total volatile fatty acid (VFA) production (μmole/ml) by the intestinal bacteria from red drum cultured under anaerobic conditions at 27°C.

	Incubation time (h)			*Incubation time (h)*		
	0	*24*	*48*	*0*	*24*	*48*
	Butyrate			*Total VFA*		
No inoculum (NI)	0.00 ± 0.0	0.00 ± 0.0^{B}	9.02 ± 8.6	1.61 ± 1.2	12.44 ± 11.7^{B}	47.36 ± 23.1
NI + FOS[1]	0.00 ± 0.0	0.00 ± 0.0^{B}	6.43 ± 7.1	1.34 ± 0.8	1.031 ± 4.9^{B}	55.96 ± 35.5
NI + GroBiotic®-A	0.00 ± 0.0	0.00 ± 0.0^{B}	25.45 ± 35.6	0.95 ± 0.4	10.06 ± 2.9^{B}	86.16 ± 54.1
NI + Yeast	0.04 ± 0.1	0.00 ± 0.0^{B}	15.55 ± 9.3	3.19 ± 2.4	21.45 ± 2.3^{B}	76.78 ± 32.7
Inoculum (I)	0.00 ± 0.0	0.00 ± 0.0^{B}	10.34 ± 17.7	1.48 ± 1.3	16.18 ± 12.9^{B}	59.10 ± 32.0
I + FOS	0.00 ± 0.0	0.22 ± 0.4^{B}	8.37 ± 8.0	1.96 ± 0.8	27.21 ± 12.8^{B}	62.34 ± 30.4
I + GroBiotic®-A	0.00 ± 0.0	2.73 ± 3.4^{A}	9.40 ± 14.9	1.90 ± 0.8	92.47 ± 88.3^{A}	78.13 ± 16.1
I + Yeast	0.00 ± 0.0	0.28 ± 0.5^{B}	4.78 ± 2.7	1.87 ± 1.19	48.80 ± 26.8^{AB}	55.67 ± 12.1
	$P = 0.4663$[2]	$P = 0.1568$[2]	$P = 0.8219$[2]	$P = 0.5785$[2]	$P = 0.0989$[2]	$P = 0.7924$[2]

[1]Within column means ± SD (μmole/ml; n = 3) without a common superscript letter differ significantly ($P < 0.05$).
[2] fructooligosaccharide.
[3]means compared within incubation time.

the 24-h samples indicated that microbial populations were not altered extensively by the addition of the prebiotics. Bacterial populations after 24 h of anaerobic incubation were either highly related, greater than 90%SC, or could be considered identical with greater than 95%SC. The samples that did not have any diluted digesta added to the tubes (non-inoculated) were similar to the other 24-h samples. The banding pattern of the initial sample, that is, DNA isolated from the original digesta, also was most similar to the 24-h samples (86%SC). The 48-h incubation samples had a greater number of bands than found in the 24-h cultures, possibly indicating a greater proliferation of diverse species. The banding patterns from inoculated samples treated with GroBiotic®-A and brewers yeast were very different from the rest of the 48-h samples with less than 80%SC (Fig. 1). Samples with GroBiotic®-A and brewers yeast had close to 80% SC, exhibiting little similarity with each other. The samples with GroBiotic-A®and only sterile ADS also were significantly different from the other samples (80% similarity). This indicated that GroBiotic®-A was possibly stimulating the growth of different bacteria when compared to the other prebiotics. This analysis only examined the culturable anaerobic bacterial population.

Sequencing

The three clones from each band did not all return the same species when run through the BLAST database (nr database); however, usually two out of three were the same. The upper common band was most likely *Lactococcus lactis* (arrow A) and the lower common band was *Aeromonas* sp. (arrow B) (Fig. 1).

Discussion

This study is the first to examine the production of VFAs *in vitro* in cultured red drum. Acetate was produced in the highest concentrations and comprised 76–89% of VFA production in inoculated samples and 61–82% in the non-inoculated samples. Previous *in vitro* studies have examined effects of various oligosaccharides on the culturable intestinal microbiota of red seabream (*Pagus major*), common carp (*Cyprinus carpio*), and rainbow trout (*Oncorhynchus mykiss*).[34–37] Unlike the VFA profiles in the current study, butyric and propionic were produced by the intestinal microbiota of rainbow trout and common carp in much higher proportions and the cultures were not mainly acetogenic.[36,37] *In vivo* studies have examined VFA production in the cool temperate species *Cebidichthys violaceus* reporting that acetate accounted for 100% of the VFAs produced. But in the warm temperate species *Medialuna californiensis* and two subtropical species, *Kyphosus bibibbus* and *K. vaigeinsis,* acetate production accounted for less than 20% of the VFAs produced.[38] *In vitro* acetate production after 48 h was higher than in previous *in vivo* measurements[39,40] indicating that the culturable acetogenic species of microbes from red drum were more abundant and had more substrate available than *in vivo* communities. Smith et al.[39] found that largemouth bass (*Micropterus salmoides*) in summer had acetate concentrations of 33.5 mM in the GI tract. Mountfort et al.[40] examined temperate herbivorous fishes and found acetate concentrations ranging from 8.3 to 37.5 mM in the GI tracts of three temperate herbivorous species (*K. sydneyanus*, *Odax pullus*, and *Aplodactylus arctidens*). Thus, the concentrations from *in vitro* experiments were approximately 2 to 3 times higher than *in vivo* measurements. Tilapia has been shown to transport VFAs across the intestinal walls[41,42] and thus the increased VFA production could be used as an energy source for the host fish. The increase in VFA production also has been shown to have a beneficial effect on the host immune response by modulating leukocyte activity in both mice and humans.[43]

Based on the variation in VFA production in the individual samples from this study, it appears that the intestinal community varies from fish to fish. This VFA production variability also could be due to bacterial species not being uniformly distributed among samples or unique members of the intestinal community occurring in individual fish. The VFA profiles did not seem to indicate a change in the microbial community after 48 hours.

In the current study, GroBiotic®-A and brewers yeast altered the culturable anaerobic microbial community *in vitro* while the FOS did not when compared to the microbial community resulting from incubation of the diet alone. The microbial community in the non-inoculated samples containing 2% Grobiotic®-A also was altered indicating that the anaerobic/facultative microbial community already present in the feed can be modified using this prebiotic after 48 h incubation in anaerobic conditions. GroBiotic®-A is an autolyzed yeast product that contains a high level of lactose that can be fermented thus facilitating the change in the anaerobic community. The lack of detectable change in the FOS samples could be due to the lack of culturable microbes in the red drum GI tract that are adapted to use β-linked carbohydrates or changes in the microbiota were below the detection level of DGGE. The present experiment only examined the culturable community associated with the GI tract of tank-reared red drum. It has been estimated that only 5 to 20% of the species in the GI tract of mammals can be cultured using current media and methods.[44,45]

Results from the current study demonstrated that a single species of bacteria dominated the microbial community after being cultured for 24 h in an anaerobic environment. The difference in culturable microbiota between the initial, 24-h samples and 48-h samples could be due to various reasons such as competition from species that are more suitable for culture, different substrates being present in the medium because the fish may not have extracted nutrients

consistently from the diet or some nutrients may have been exhausted in the tube and not replaced as would happen if the fish was consuming food. Another possibility is that *in vivo* microbial interactions are disrupted *in vitro*.

The two major bands present in all 48-h samples (Figure 1) are most likely *Lactococcus lactis* for the upper band and *Aeromonas* sp. for the lower band. However, it is possible that other species having DNA that is chemically equivalent may have been present. The GI tract samples incubated with brewers yeast and GroBiotic®-A showed a higher complexity when compared to the other samples indicating that more species were present in these samples. However, after 48 h, many bands were detected indicating that community bacteria previously undetectable were multiplying in numbers sufficient to reveal DGGE bands. In the inoculated GroBiotic®-A and brewers yeast samples, there appeared to be more bands above the *Lactococcus lactis* band and more bands in between the *Lactococcus lactis* and the *Areomonas* sp. bands when compared to the inoculated sample without prebiotics. These bands most likely were less numerous species that were able to use the diet additives as a carbon source and able to compete with the more abundant species, thus increasing the complexity of the DGGE profiles. Larval coho salmon (*Onchorhynchus kisutch*) were reported to have simple DGGE profiles consisting of only four bands with *Pseudomonas* sp. and *Aeromonas* sp. being the dominant species detected.[46] The uptake of a potential probiotic organism for haddock (*Melanogrammus aeglefinus*) larva was confirmed using DGGE.[47] However, the changes in the microbial community related to diet and age were not analyzed and only certain microbial species were determined from each gel. The banding patterns from the Plante et al.[47] *in vivo* study and the current *in vitro* experiment showed differences with the banding patterns from the current study showing a dominant species while the *in vivo* samples showed a more complex pattern with more than one species being dominant at different life stages. This difference is probably due to numerous species that cannot be cultured, but reside in the GI tract of a living host.

In the current study, *Aeromonas* sp. and *Lactococcus lactis* were found to be the dominant species in culture. Using molecular techniques (DNA sequencing and Restriction Fragment Length Polymorphism), Pond et al.[48] demonstrated that the dominant bacteria of rainbow trout (*Oncorhynchus mykiss*) was *Clostridium gasigenes*, an anaerobe. Pond et al.[48] also reported that the intestinal tract bacteria varied from fish to fish, thus complicating potential comparisons among treatments. Recently, the effects of inulin on the culturable facultative intestinal microbiota have been assessed in Atlantic salmon (*Salmo salar*), Arctic char (*Salvelinus alpinus*), and turbot (*Psetta maxima*).[49–51] In all three studies the attached culturable microbial community from the fish fed inulin-supplemented diet was less diverse and numerous. Ringø et al.[50] investigated the effects of inulin, containing fructooligasaccharides, on aerobic bacteria associated with the GI tract of Arctic char using DNA sequences of 16S rDNA from 18 culturable species. They determined that inulin changed the community by decreasing the number of bacteria adhering to the GI tract wall. However, in the present *in vitro* study we did not detect any changes in the microbial community of red drum inoculum incubated with 2% FOS. The lack of detectable change could be due to the intestinal contents being removed and the resulting samples obtained likely did not include bacteria that adhere to the intestinal wall.

Conclusions

The addition of brewers yeast and GroBiotic®-A to the GI tract contents of red drum altered the microbial community *in vitro*. These *in vitro* results combined with the previous *in vivo* studies conducted with GroBiotic®-A and hybrid striped bass indicate that the detected change in the microbial community may be beneficial to red drum. Beneficial effects such as increased growth performance and disease resistance were conferred to hybrid striped bass fed diets containing GroBiotic®-A.[29,52] Since the intestinal microbial communities of fish have been poorly studied, *in vitro* experiments might not be adequate at this time and need further refinement to predict the fate of potential prebiotics in fish intestinal tracts. Future studies need to be conducted to identify the microbial species that benefit the host and determine if this inherently artificial system can be used as a predictor of changes that occur *in vivo*.

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