Aquaculture xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Assessment of *Chlorogloeopsis* as a novel microbial dietary supplement for red tilapia (*Oreochromis niloticus*)

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ARTICLE INFO

Article history:
Received 25 October 2009
Received in revised form 1 December 2009
Accepted 3 December 2009
Available online xxxx

Key words:
Gut microbiota
DGGE
Cyanobacteria

ABSTRACT

A 9 week study was conducted to assess the effect of incorporating dietary *Chlorogloeopsis* on the growth performance, feed utilisation, body composition, haematology and gut microbiota of red tilapia (*Oreochromis niloticus*). *Chlorogloeopsis* cells were supplemented to the diet at 0.25%, 0.50% and 1.00%. Compared to the control group, no significant differences of growth parameters or body composition were observed in the *Chlorogloeopsis* fed fish. However, haematological and gut microbial load appeared to be more sensitive to dietary *Chlorogloeopsis*. Haematocrit levels increased from 31.0 ± 2.9% in the control group to 32.8 ± 4.2 and 33.3 ± 5.0 in the 0.50% and 1.00% *Chlorogloeopsis* groups, respectively. The total allochthonous microbial load and lactic acid bacteria levels were elevated but the microbial groups identified and microbial diversity did not differ between the dietary groups. PCR-DGGE fingerprints of the microbial profiles supported this finding but revealed clear differences between the allochthonous and autochthonous communities. Sequence analysis from DGGE fingerprints identified several bacterial clones closely related to bacteria previously isolated from the digestive tract of fish and revealed a *Lactobacillus* spp. found in all groups was present only as part of the allochthonous communities. Low level dietary applications of *Chlorogloeopsis* for tilapia aquaculture seem limited but further evaluation is required.

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1. Introduction

Over the past decade new initiatives towards the development and appraisal of novel functional dietary supplements in aquafeeds has been the focus of concerted research efforts. Such products have often been of microbial origin: immunostimulants (Gannam and Schrock 1999; Sakai 1999; Bricknell and Dalmo 2005), prebiotics (Burr et al., 2005; Gatlin et al., 2006) and probiotics (Balcázar et al., 2006; Kesarcodi-Watson et al., 2008; Wang et al., 2008a). Cyanobacteria are used in biotechnology, food, fertilizers, wastewater treatment and aquafeeds (Abed et al., 2009). Compared to bacteria, cyanobacteria are rich in proteins, carotenoids, biological cofactors and vitamins (Kobayashi and Kurata 1978). The application of photosynthetic bacteria and cyanobacterial cells (such as *Spirulina*) on growth parameters is well documented for several fish species (Matty and Smith 1978; Nandeesha et al., 1998; Mustafa et al., 1997; Palmegiano et al., 2008; Yanbo and Zirong 2006; Ramakrishnan et al., 2008) including tilapia *Oreochromis niloticus* (Takeuchi et al., 2002; Abdel-Tawwab and Ahmad 2009). Furthermore, Ramakrishnan et al. (2008) observed that dietary supplementation of *Spirulina* elevated the culturable gut microbial load of common carp (*Cyprinus carpio*). To the authors' knowledge *Chlorogloeopsis* spp., cyanobacteria belonging to the class *Cyanophyceae*, have never been used as an additive in aquafeeds.

Tilapiines, an omnivorous fish group, are one of the most important farmed fish groups; production of cultured tilapia has increased from 28,260 mt in 1970 to 2.5 million mt in 2007 (FAOSTAT, 2009). The aim of the present study was to provide an analysis of the dietary application of *Chlorogloeopsis* for red tilapia (*Oreochromis niloticus*).

2. Materials and methods

2.1. Diet formulations

Lyophilised *Chlorogloeopsis fritschii* was provided by Plymouth Marine Laboratory (PML Plymouth, UK). This robust fresh water species was selected due to its ease of sustained cultivation on the large scale, in addition to possessing low energy harvesting requirements. Four iso-nitrogenous and iso-energetic diets were formulated

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doi:10.1016/j.aquaculture.2009.12.004

Please cite this article as: Merrifield, D.L., et al., Assessment of *Chlorogloeopsis* as a novel microbial dietary supplement for red tilapia (*Oreochromis niloticus*), Aquaculture (2009), doi:10.1016/j.aquaculture.2009.12.004

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to produce a basal diet (control) and 3 experimental diets containing *Chlorogloeopsis* (basal diet containing 0.25%, 0.50% and 1.00%). Dietary ingredients were mixed in a Hobart food mixer (Hobart Food Equipment, Australia, model no: HL1400-10STDA) with warm water until a soft slightly moist consistency was achieved. This was then cold press extruded (PTM P6 extruder, Plymouth, UK) to produce a 2 mm pellet. Proximate composition was undertaken according to AOAC (1995) protocols and gross energy was analysed using a bomb calorimeter (Parr 1356, Parr Instrument Company, IL, USA). Dietary formulations and chemical composition is shown in Table 1.

2.2. Experimental design

Oreochromis niloticus fry were sourced from the Institute of Aquaculture (University of Stirling, UK) after a 3 week sex reversal treatment (alpha-methyl testosterone) to achieve all male phenotypes. Fry were transported to the Aquaculture and Fish Nutrition Research Aquarium, University of Plymouth, UK. After 6 weeks acclimation and on growing, 20 fish (7.3 g) were randomly distributed into 12×80-l fibreglass tanks, each provided with 99% recirculated aerated freshwater at a rate of 300 l h^{-1}. Each treatment was conducted in triplicate. Fish were fed 3–4% biomass day^{-1} provided in equal rations at 09.00, 13.00 and 17.00 h for 9 weeks. Daily feed was corrected every week by batch weighing following a 24 h starvation period. Water temperature was maintained at 27 ± 1 °C and dissolved oxygen maintained >80% saturation. A 12 h light/12 h dark photoperiod was maintained throughout the trial duration.

2.3. Growth performance and proximate analysis

Growth performance and feed utilisation was assessed by mean final weight, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER). Calculations were conducted using the following formulae: SGR = 100((lnFW − lnIW)/T), FCR = FI/WG and PER = WG/PI. Where FW = final weight (g), IW = initial weight (g), T = duration of feeding (days), WG = wet weight gain (g), PI = protein intake (g) and FI = feed intake (g). Fish were sampled at the end of the trial to determine carcass composition according to AOAC (1995) protocols. Gross energy was calculated using the conversion factors of 23.6, 39.5, and 17.0 kJ g^{-1} for protein, lipid, and nitrogen-free extract (NFE), respectively (Brett and Groves 1979).

Table 1
Dietary formulation and proximate composition of experimental diets.

Ingredient g kg^{-1}	Control	0.25%	0.50%	1.00%
Corn starch[a]	367.5	365.0	362.5	357.5
Fishmeal[b]	300.0	300.0	300.0	300.0
Lysamine pea protein[c]	164.7	164.7	164.7	164.7
Glutalys[c]	100.0	100.0	100.0	100.0
Fish oil[d]	30.0	30.0	30.0	30.0
Vitamin–mineral premix[e]	20.0	20.0	20.0	20.0
Vegetable oil[f]	17.8	17.8	17.8	17.8
Chlorogloeopsis[g]	0.0	2.5	5.0	10.0
Proximate composition (%)				
Dry matter	91.83	91.83	92.25	92.14
Crude protein	38.19	38.74	38.95	39.92
Lipid	8.04	8.49	8.16	8.07
Ash	5.52	5.49	5.81	5.32
NFE[h]	40.08	39.10	39.33	38.83
Gross energy (MJ kg^{-1})	19.37	19.85	20.04	19.96

[a] Sigma-Aldrich Ltd, UK.
[b] Scottish fishmeal 70, United Fish Products Ltd, UK.
[c] Roquette Frêres, France.
[d] Epanoil, Sevenseas Ltd, UK.
[e] Premier nutrition vitamin/mineral premix: 121 g kg^{-1} calcium, Vit A 1.0 µg kg^{-1}, Vit D3 0.1 µg kg^{-1}, Vit E (as alpha tocopherol acetate) 7.0 g kg^{-1}, Copper (as cupric sulphate) 250 mg kg^{-1}, Magnesium 15.6 g kg^{-1}, Phosphorous 5.2 g kg^{-1}.
[f] Sunflower oil.
[g] *Chlorogoeoplsis fritschii*; PML Plymouth, UK.
[h] Nitrogen-free extracts (NFE) = dry matter − (crude protein + crude lipid + ash).

2.4. Haematology

Blood was sampled from 4 fish per tank at the end of the trial. Samples were taken from the caudal vein using a 25 gauge needle and 1 ml syringe. Haematocrit levels were determined by drawing fresh blood into microhaematocrit tubes and centrifugation at 3600 *g* for 5 min in a microhaematocrit centrifuge. Haematocrit values were measured and read as % packed cell volume (%PCV). Blood smears were prepared for the determination of total leukocyte levels and differential leukocyte counts as described by Merrifield et al. (2009a). Thrombocytes, lymphocytes, monocytes and granulocytes were identified following the descriptions of Hrubec et al. (2000). Additional blood was left to clot for 3 h before serum was removed. Serum samples were stored at −80 °C until analysis of lysozyme activity. Lysozyme activity was assessed as described elsewhere (Merrifield et al., 2009a) and one unit of lysozyme activity was defined as a decrease in absorbance of 0.001 unit min^{-1}.

2.5. Microbial analysis

At the end of the trial 3 fish per tank were euthanized by overdose (200 mg l^{-1} water for 10 min) of tricaine methane sulphonate (MS222; Pharmaq, Fordingbridge, UK) followed by destruction of the brain. The intestine in its entirety was aseptically excised and the digesta from the posterior section was removed; the intestinal mucosal tissue was excised, washed thoroughly with phosphate-buffered saline 3 times (PBS; Oxoid, Cambridge, UK) before homogenization in a macerator (MSE, London, UK) for 30 s. As inter-fish variation has been reported previously (Spanggaard et al., 2000; Liu et al., 2008) the resulting material from 3 fish per tank was pooled into 1 sample (after Hovda et al., 2007) to assess differences between the dietary groups.

2.5.1. Culture-dependent analysis

Samples were serially diluted with PBS and 100 µl was spread onto duplicate agar plates. Aerobic heterotrophic bacteria were determined by plate counts after 7 days aerobic incubation at 28 °C on tryptone soya agar (TSA; Oxoid) after Rawling et al. (2009). A total of 349 isolates were identified by morphology/standard biochemical tests (colony morphology, cell morphology, Gram stain, production of catalase, oxidase, glucose fermentation, motility and endospore formation) and Microbact™ 24E test kits (Oxoid) after Merrifield et al. (2009b). Additionally, de Man, Rogosa, Sharpe plates (MRS; Oxoid) were used for the enumeration of lactic acid bacterial (LAB) using the pour plate method.

2.5.2. Culture-independent analysis

For PCR-DGGE analysis the replicate samples from the control, 0.50% and 1.00% groups were pooled into single samples, each representing the microbial community of 9 fish. DNA extraction and PCR amplification of the 16 S rRNA V3 region was conducted as described by Merrifield et al. (2009c). DGGE was performed using a Dcode Universal Mutation Detection System (Bio-Rad laboratories, Italy). Twelve µl of PCR products were run on an 8% polyacrylamide gel (16 cm×16 cm×1 mm) with a denaturing gradient of 40–60% (where 100% denaturant is 7 M urea and 40% formamide). The gel was run at 65 V for 16 h at 60 °C in 1×TAE buffer (66 mM Tris, 5 mM Na acetate, 1 mM EDTA). Visualization of the DGGE bands was achieved with SYBR Green staining (Molecular Probes, Eugene, OR, USA). The Gel was scanned in a Bio-Rad universal hood II (Bio-Rad laboratories, Italy) and optimized for analyses by enhancing contrast and greyscale. Bands of interest were excised for sequence analysis. DNA was eluted from excised bands in TE buffer at 4 °C overnight before re-PCR as

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described previously except the forward primer lacked the GC clamp. The PCR products were cleaned (QIAquick PCR Purification Kit; Qiagen) and sequenced by GATC laboratories (GATC-biotech laboratories, Germany). Nucleotide sequences (GenBank accession numbers GU117869–GU117874) were then submitted to a BLAST search in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to retrieve the closest known alignment identities for the partial 16 S rRNA sequences.

2.6. Statistical analysis

Data was transformed where necessary and statistical analysis was carried out using a One-Way ANOVA (SPSS 15.0 Chicago, IL, USA). *Post hoc* LSD test was used to determine significant differences between means and significance was accepted at the $P<0.05$ level. Primer V6 (Clarke and Gorley 2006) was used to calculate species richness, evenness and diversity of the PCR-DGGE fingerprints according to the following formulae: Margalef's species richness: $d=(S-1)/\log(N)$; Pielou's evenness: $J'=H'/\log(S)$ and Shannons diversity index: $H'=-\Sigma(pi(\ln pi))$. Where N = total number of individuals (total intensity units), S = number of observed taxonomical units (presumed species) and pi = the proportion of the total number of individuals in the ith species.

3. Results

3.1. Growth parameters and feed utilisation

No differences of growth performance, feed utilisation, K-factor and HSI were observed in any of the *Chlorogloeopsis* fed groups compared to the control group (Table 2). Body composition also remained unaffected by dietary incorporation of *Chlorogloeopsis* (data not shown).

3.2. Haematology

Haematological data is presented in Table 3. Compared to the control group haematocrit levels, measured as %PCV, significantly increased from 31.01 ± 2.89% in the control group to 32.75 ± 4.24% ($P=0.037$) and 33.30 ± 4.97% ($P=0.020$) in the 0.50% and 1.00% groups, respectively. Leukocytes remained unaffected. The highest lysozyme activity was observed in the 1.00% group (464 ± 91 U ml^{-1}) but this was not significantly different from the control group (382 ± 175 U ml^{-1}).

3.3. Intestinal microbiology

3.3.1. Culture-dependent

Culturable microbial populations associated with the tilapia posterior intestine are presented in Table 4. Autochthonous LAB levels in all groups were statistically too low to enumerate (i.e. less than 20 colonies at the lowest dilution). Autochthonous TVC were higher in the 1.00% *Chlorogloeopsis* group. Additionally, compared to the control the allochthonous TVC and LAB levels were also elevated in the 0.50% and 1.00% *Chlorogloeopsis* groups. Dominant bacterial genera from all feeding groups were identified as *Pseudomonas* spp. and *Micrococcus* spp. Minor differences between microbial groups were present but no clear differences were evident. The Shannon–Weaver diversity indices also revealed no clear patterns in the intestinal microbiota.

3.3.2. Culture-independent

PCR-DGGE fingerprints revealed that microbial profiles within the intestine of the control, 0.50% and 1.00% groups were all highly similar within the respective communities (i.e. autochthonous and allochthonous) (Fig. 1). However, it is clear that the autochthonous and allochthonous populations are distinctly different from one another as populations from these regions were clustered together into two distinct groups (Fig. 1 and Table 5). Furthermore, the species richness (d) of the autochthonous microbial communities (1.99) was lower than that of the allochthonous populations (2.6–2.8). Similarly, the autochthonous microbial communities were less diverse (H') than the allochthonous populations, $H'=\sim 3.0$ and 3.4 respectively. Sequence

Table 2
Growth performance, feed utilisation and biological indices of tilapia after 9 weeks feeding on experimental diets. Dietary codes: control (C) and diets supplemented with *Chlorogloeopsis* (0.25%, 0.50% and 1.00%). $n=3$.

	C	0.25%	0.50%	1.00%
Initial weight (g)	7.30 ± 0.05	7.33 ± 0.10	7.32 ± 0.08	7.33 ± 0.03
Final weight (g)	42.41 ± 1.51	44.77 ± 4.09	41.43 ± 0.34	42.34 ± 2.06
SGR (% d^{-1})	2.79 ± 0.05	2.87 ± 0.12	2.75 ± 0.02	2.78 ± 0.08
FCR	0.90 ± 0.01	0.95 ± 0.06	0.91 ± 0.02	0.91 ± 0.03
K[a]	1.79 ± 0.04	1.83 ± 0.03	1.83 ± 0.03	1.79 ± 0.04
HSI[b]	1.51 ± 0.48	1.68 ± 0.51	1.59 ± 0.37	1.57 ± 0.42

There were no significant differences between groups.
[a] Condition factor (K): $K=$ (final weight × 100)/final length^3.
[b] Hepatosomatic index (HSI): HSI = (liver weight/final weight) × 100.

Table 3
Haematological parameters of tilapia after 9 weeks feeding on experimental diets. Dietary codes: control (C) and diets supplemented with *Chlorogloeopsis* (0.25%, 0.50% and 1.00%).

	C	0.25%	0.50%	1.00%
PCV (%)[1]	31.0 ± 2.9[a]	30.8 ± 2.6[a]	32.8 ± 4.2[b]	33.3 ± 5.0[b]
Lysozyme (U ml^{-1})[1]	382 ± 175	366 ± 11	323 ± 101[a]	464 ± 91[b]
Leukocyte count[2]	24.8 ± 10.1	25.5 ± 8.7	24.4 ± 11.7	25.1 ± 9.5
Thrombocytes (%)[3]	59.2 ± 9.4	59.6 ± 7.6	60.9 ± 5.8	62.6 ± 9.7
Lymphocytes (%)[3]	31.8 ± 6.1	32.0 ± 6.0	29.8 ± 4.0	29.4 ± 6.1
Monocytes (%)[3]	5.2 ± 2.1	5.2 ± 2.2	6.1 ± 2.0	5.0 ± 2.7
Granulocytes (%)[3]	3.8 ± 1.9	3.2 ± 2.0	3.2 ± 1.9	3.0 ± 1.4

[ab] Values in the same row with the different superscripts are significantly different ($P<0.05$).
[1] $n=12$.
[2] Expressed as leukocytes per 1000 blood cells. $n=8$.
[3] $n=6$.

Table 4
Levels (log CFU g^{-1}) of LAB, TVC and composition of aerobic heterotrophic autochthonous and allochthonous microbiota of tilapia fed experimental diets. Dietary codes: control (C) and diets supplemented with *Chlorogloeopsis* (0.25%, 0.50% and 1.00%). $n=3$ (pooled from 9 fish).

	Autochthonous				Allochthonous			
	C	0.25%	0.50%	1.00%	C	0.25%	0.50%	1.00%
LAB	–[a]	–[a]	–[a]	–[a]	4.97	4.36	6.13	7.50
TVC	5.81	6.47	6.78	8.04	7.62	7.50	7.32	8.32
TVC composition (%)								
Pseudomonas spp.[b]	43.1	47.7	31.8	33.3	29.3	37.2	32.4	65.9
Enterobacteriaceae[c]	2.0	–	–	2.2	–	–	–	2.3
Acinetobacter spp.	2.0	–	–	–	–	–	–	–
Aeromonas spp.[d]	15.7	2.3	–	13.3	9.8	–	–	9.1
Other Gram–negative rods	2.0	–	–	–	–	–	10.8	–
Micrococcus spp.	21.6	25.0	15.9	15.6	26.8	2.3	24.3	4.5
Staphylococcus spp.	–	–	–	2.2	–	–	–	4.5
Bacillus spp.	–	11.4	–	–	–	7.0	2.7	–
Other Gram-positive rods	–	4.5	25.0	2.2	4.9	23.3	8.1	9.1
Irregular stained rods	3.9	–	–	–	–	2.3	–	4.5
Not identified[e]	9.8	9.1	27.3	31.1	29.3	27.9	21.6	–
Diversity[f]	1.573	1.392	1.385	1.540	1.447	1.423	1.582	1.217

– not detected.
[a] Adherent LAB levels were statistically too low to enumerate (i.e. less than 20 colonies at the lowest dilution).
[b] Identified by Microbact™ 24E as *Pseudomonas* spp.
[c] Identified by Microbact™ 24E as *Serratia marcescens*-like.
[d] Identified by Microbact™ 24E as *Aeromonas* spp., *Aeromonas hydrophila* and *Aeromonas veronii*.
[e] Did not grow after re-streaking or died prior to positive identification.
[f] Shannons diversity index: $H'=-\Sigma(pi(\ln pi))$.

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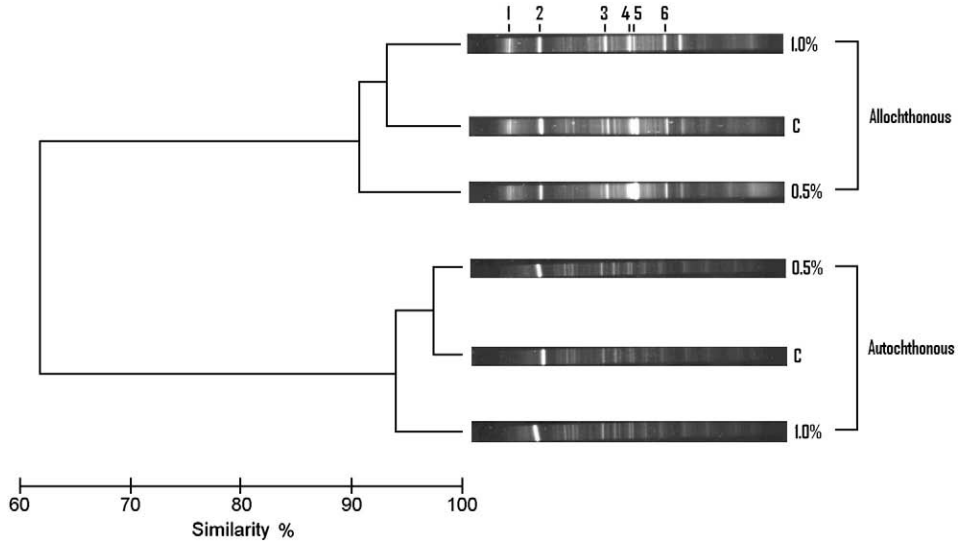


Fig. 1. Dendrogram and PCR-DGGE fingerprints of the posterior intestinal microbiota of red tilapia after feeding on experimental diets for 9 weeks. Dietary codes: control (C) and diets supplemented with *Chlorogloeopsis* (0.5% and 1.0%). Numbers (1–6) indicate bands excised for sequence analysis.

analysis from the DGGE bands revealed that most bacteria were uncultured and not identified to species level (Table 6). LAB (*Weissella* and *Lactobacillus* spp.) were identified which supports the culture-based data that LAB were among the main microbial groups. However a band identified as *Lactobacillus* spp., which was present in all dietary groups, was found uniquely only as part of the allochthonous microbiota. Other clones were closely related to uncultured bacteria previously isolated from the gastrointestinal tract of fish (i.e. Atlantic salmon *Salmo salar*, yellow catfish *Pelteobagrus fulvidraco*, grass carp *Ctenopharyngodon idellus*) or insects (i.e. *Apriona germari*, *Spheno-phorus levis*).

4. Discussion

Dietary supplementation of microbial cells such as yeast and bacteria has proved effective at enhancing growth performance, digestive function and carcass composition of several fish species (Bogut et al., 2000; Bagheri et al., 2008; Burr et al., 2008) including tilapia (El-Haroun et al., 2006; Wang et al., 2008b). Furthermore, previous studies have reported that low level dietary inclusion (0.1–5%) of photosynthetic bacteria can affect fish growth parameters, feed utilisation, body composition and product quality (Yanbo and Zirong 2006; Zhao et al., 2006; Ramakrishnan et al., 2008; Abdel-Tawwab and Ahmad 2009). However, contrary to these findings the application of the cyanobacteria *Chlorogloeopsis* in the present study had no effect on growth, feed utilisation, carcass analysis or biological indices. Future studies will demonstrate whether higher levels of *Chloro-gloeopsis fritschii* affect these parameters in tilapia.

In the present study, dietary supplementation of *Chlorogloeopsis* influenced haematocrit levels and serum lysozyme activity but the reason for this is not clear and it is recommended that future dietary cyanobacterial studies assess blood chemistry and haematological parameters. However, dietary supplementation with bacterial cells has been shown to influence tilapia haematocrit levels in previous studies; yet the results are somewhat contradictory: compared to control fish, levels have been reported to be elevated (Aly et al., 2008a), reduced (El-Rhman et al., 2009) and unaffected (Aly et al., 2008b) with dietary administration of potential probiotic bacterial species. The reason for these contradictory outcomes is not clear.

Ramakrishnan et al. (2008) reported that dietary inclusion of the cyanobacteria *Spirulina* increased microbial load within the gut of common carp. Similarly in the present study, considerably higher levels of total culturable bacterial levels and LAB levels were observed in fish fed 1.0% *Chlorogloeopsis*. However, only minor differences between the microbial types within the total viable load were evident. This is in contrast to the findings reported for other species, such as mice, where dietary cyanobacteria (*Nostoc commune*, *Spirulina platensis* and *Afanizominon flos-aquae*) had a more pronounced effect on the gastric microbial profiles and species identified (Rasmussen et al., 2009). Such differences may be due to the different dietary patterns, resident endogenous microbiota and gastric morphology of the host species. Indeed, cyanobacteria form part of the natural diet for many fish species, including tilapia (Dempster et al., 1993; Figuerdo and Giani 2005), which is not the case for mice.

Dominant bacterial genera from the culture-dependent analysis in all feeding groups within the present study were identified as *Pseu-domonas* spp. and *Micrococcus* spp.. *Pseudomonas* spp. and *Micrococ-cus* spp. have been identified from the intestine of cultured tilapia but generally constitute only minor components of the microbial community (Sugita et al., 1996; Al-Harbi and Uddin 2003; Hu et al., 2007).

In the present study, PCR-DGGE revealed that *Lactobacillus* spp. and *Weissella* spp. were components of the gut microbial populations. However, culture-based analysis revealed that LAB levels were only statistically quantifiable as allochthonous populations and not adherent autochthonous populations. Furthermore, a band, identified as *Lactobacillus* spp. was present uniquely as part of the allochthonous

Table 5
Ecological parameters of PCR-DGGE fingerprints of the posterior intestinal microbiota of red tilapia after feeding on experimental diets for 9 weeks. Dietary codes: control (C) and diets supplemented with *Chlorogloeopsis* (0.50% and 1.00%). $n = 3$ (pooled from 9 fish).

	S[a]	Richness[b]	Evenness[c]	Diversity[d]
Autochthonous				
C	22	1.999	0.9822	3.036
0.50%	22	1.996	0.9854	3.046
1.00%	22	1.992	0.9828	3.038
Allochthonous				
C	33	2.822	0.9854	3.446
0.50%	30	2.589	0.9828	3.343
1.00%	31	2.663	0.9829	3.375

[a] Number of bands, presumed species.
[b] Margalef's species richness: $d = (S-1)/\log(N)$.
[c] Pielou's evenness: $J' = H'/\log(S)$.
[d] Shannons diversity index: $H' = -\Sigma(pi(\ln pi))$.

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Table 6
Isolated bacterial bands and their closest relatives (BLAST) from PCR-DGGE of the posterior intestinal communities of tilapia.

Band	Accession number	Groups/regions present	Nearest neighbour(s)	Similarity to nearest neighbour(s)	Accession number of nearest neighbour(s)
1	GU117869	All groups — allochthonous	Uncultured bacterium from the hindgut of Atlantic salmon	94%	EU697166
2	GU117870	All groups/regions	Uncultured *Weissella* sp.	97%	FJ195789
3	GU117871	All groups/regions	Uncultured bacterium from the hindgut of Atlantic salmon	97%	EU697167
			Uncultured bacterium from the hindgut of Atlantic salmon	97%	EU009390
4	GU117872	All groups/regions	Uncultured bacterium from the intestine of mulberry longicorn beetle (*Apriona germari*)	97%	EU560749
			Uncultured bacterium from the intestine of sugarcane weevil (*Sphenophorus levis*)	97%	FJ626273
5	GU117873	All groups — allochthonous	Uncultured bacterium from the intestine of yellow catfish (*Pelteobagrus fulvidraco*)	94%	GQ360029
			Uncultured bacterium from the intestine of grass carp (*Ctenopharyngodon idellus*)	94%	EU585894
6	GU117874	All groups — allochthonous	Uncultured *Lactobacillus* sp.	100%	GQ422710.1

microbiota. These results suggest that LAB populations within the intestinal tract of tilapia are predominantly allochthonous transient visitors, which seems to confirm our previous findings (Rawling et al., in press) where LAB levels were only statistically quantifiable as allochthonous populations. Additionally, an isolate identified as closest relation to an uncultured bacterium from the hindgut of Atlantic salmon was found uniquely present as part of the allochthonous microbial community. Allochthonous communities were higher in complexity and richness than that of the autochthonous microbiota. Rarely have culture-independent techniques been utilised to compare the autochthonous and allochthonous microbial communities of the fish gut. Despite observing a similar number of PCR-DGGE bands (presumed species) present in each group, Merrifield et al. (2009c) observed clear differences between pairwise comparisons of microbial fingerprints of the autochthonous and allochthonous populations of the rainbow trout (*Onchorhynchus mykiss*) intestine. We suggest that the autochthonous communities of fish require further investigation, and might well be sub-categorised further to microbes present only within the mucus layer and those directly adhered to the epithelial mucosa. The majority of bacterial sequences isolated from the DGGE within the present study could not be identified to the species level as the V3 sequences were highly similar to unidentified clones previously isolated from gastrointestinal tract of fish or insects. Culture-independent approaches of identifying gastric microbiota often lead to the isolation of novel or unidentified bacteria likely because such organisms are fastidious and difficult to culture and are thus not well documented. It is not surprising that bacteria thrive in rich organic intestinal contents and that closely related bacterial species are found within the gut of different host species.

5. Conclusion

The endogenous gut microbiota has been implicated as an important factor in the mucosal development and maturation of the fish gut and immune system (Rawls et al., 2004; Bates et al., 2006; Gómez and Balcázar 2008). Furthermore, it has been suggested that gastric populations aid digestive function and provide competition against pathogenic visitors to the gut (Birkbeck and Ringø 2005; Gómez and Balcázar 2008; Ringø et al., 2009). In this context, as cyanobacteria are a natural component of the diet for many fish species (Dempster et al., 1993; Figuerdo and Giani 2005) and are often included in aquafeeds (FAO 2008), it is important that future studies assess the effect of cyanobacteria on the gut microbiota of fish. From the present study, and our previous study (Rawling et al., in press), we conclude that LAB are primarily allochthonous populations within the gut of cultured tilapia. The present study suggests that low level dietary applications of *Chlorogloeopsis* for tilapia aquaculture are limited but further evaluation is required.

Acknowledgements

This work was funded by the TSB and the BBSRC Renewable Materials LINK Programme. The authors kindly thank Dr Michele Kiernan, Ben Eynon, Liz Preston and Nikki Blatchford for their technical assistance.

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Please cite this article as: Merrifield, D.L., et al., Assessment of *Chlorogloeopsis* as a novel microbial dietary supplement for red tilapia (*Oreochromis niloticus*), Aquaculture (2009), doi:10.1016/j.aquaculture.2009.12.004

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Please cite this article as: Merrifield, D.L., et al., Assessment of *Chlorogloeopsis* as a novel microbial dietary supplement for red tilapia (*Oreochromis niloticus*), Aquaculture (2009), doi:10.1016/j.aquaculture.2009.12.004