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ORIGINAL ARTICLE

Combined use of 16S ribosomal DNA and automated ribosomal intergenic spacer analysis to study the bacterial community in catfish ponds

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Abstract

Aims: To apply culture-independent techniques to explore the bacterial community composition in catfish pond water.

Methods and Results: 16S rDNA libraries were constructed and sequenced from 15 pond water samples. Automated ribosomal intergenic spacer analysis (ARISA) was used to fingerprint each bacterial community. A broad diversity in bacterial species composition was found by 16S rDNA analysis. Alphaproteobacteria was the most represented class in all ponds, followed by Gammaproteobacteria and Gram-positive high G + C content bacteria. Uniqueness of bacterial communities from each individual pond was confirmed by ARISA. Catfish pathogens were detected sporadically.

Conclusions: Bacterial communities in a catfish aquaculture setting can vary from pond to pond at one given point. No correlation could be made between bacteria composition and fish strain or between bacterial profile and the presence of catfish pathogens in a particular pond.

Significance and Impact of the Study: This is the first report showing the composition of bacterial communities in catfish ponds. Fish health specialists and catfish aquaculture managers should be aware of the wide differences in bacterial communities between ponds and include this variable in fish husbandry practices.

Introduction

Channel catfish (*Ictalurus punctatus* Rafinesque) production is the largest aquaculture industry in the USA (Stickey 2000). More than 300 000 metric tons of channel catfish are processed annually accounting for half of the total USA aquaculture production. Because of production costs, nearly all commercially produced catfish are grown in earthen ponds filled either with groundwater or rainfall and run off waters (Hargreaves and Tucker 2004). These closed systems usually support high fish densities and constitute a very favourable environment for rapid spread of infectious bacteria and acute outbreaks of disease (Wagner *et al.* 2002).

Disease control is the main problem catfish growers must face accounting for more than 45% of total annual losses (USDA 2003a,b). Two main bacterial pathogens, *Edwardsiella ictaluri* and *Flavobacterium columnare*, causal agents of Enteric Septicemia of Catfish (ESC) and columnaris disease, respectively, are responsible for more than 80% of total losses associated with disease (USDA 2003a,b). While it is clear that negative environmental conditions, such as poor water quality and low dissolved oxygen, stress fish by making them more susceptible to disease, some ESC and columnaris outbreaks cannot be associated with stressful circumstances (Wagner *et al.* 2002). Even when catfish are raised under model conditions in experimental ponds, sporadic outbreaks caused by *Edw. ictaluri* and *Fl. columnare* are common during warmer months. When these outbreaks occur, typically only one or just a few ponds are affected, including replicate ponds from the same experiment where water,

stocked fish and rearing conditions are kept as similar as possible between replicates. Catfish ponds constitute a complex environment to study due to all the abiotic and biotic factors involved (Boyd and Queiroz 2001). One aspect that has been traditionally overlooked by fish pathologists is the role that autochthonous aquatic microbiota might play in relation to fish health.

Little is known about the ecology of bacterial catfish pathogens (*Edwardsiella* spp. and *Fl. columnare*) although some studies point out the capability of these bacteria to survive in water for days (Plumb and Quinlan 1986; Welker *et al.* 2005). However, isolation of bacterial catfish pathogens from asymptomatic fish or from the aquatic environment in the absence of an epizootic episode occurs very infrequently (*Fl. columnare*) or has not been reported at all (*Edw. ictaluri*). Nevertheless, conventional culture-dependent techniques favour predominant bacterial species able to rapidly grow under *in vitro* conditions. Pathogens, if present, are typically found in low numbers in the aquatic ecosystem and can be easily overcome by naturally occurring microbiota. Channel catfish ponds are eutrophic environments with high concentrations of micro-organisms. Analysis of the composition of bacterial communities in catfish pond water has never been attempted or has been a comparison between bacterial communities present in different ponds. The main aim of this study was to apply culture independent techniques to evaluate the composition of microbial environmental communities in experimental catfish ponds and to investigate a potential relationship between bacterial profiles and pathogen prevalence. In order to achieve these objectives we used two culture-independent techniques to explore bacterial diversity in catfish ponds: 16S ribosomal DNA analysis and automated ribosomal intergenic spacer analysis (ARISA).

Materials and methods

Catfish ponds

Five catfish groups were used in this study. Two strains of channel catfish (*I. punctatus*) USDA-103 and HS-5, one strain of blue catfish (*I. furcatus* Lesueur) D&B and two hybrid strains (♀USDA-103 channel × ♂D&B blue and ♀HS-5 channel × ♂D&B blue). A minimum of two replicas per strain was analysed. On 1 March 2004, 500 fingerling-sized fish from each group were randomly assigned to 15 earthen culture ponds. Experimental units, stocking rates and pond surface area are listed in Table 1. Aeration and feeding was provided according to standard practices (Tucker and Robinson 1991). Experimental ponds were located at North Auburn Upper Fisheries Research Station, Auburn, AL. Fish were reared under model conditions during the growing season (March until November). When abnormal fish behaviour or fish mortality was observed, moribund or fresh dead fish were taken to the Fish Disease Diagnostic Laboratory, Auburn University for diagnosis.

Table 1 Fish strains stocked at 12 500 ha^{-1} in 0·04 ha ponds

Fish strains	Ponds
USDA 103 channel catfish	E1, E6, E12, E15
HS-5 channel catfish	E19, E20, E23
D&B blue catfish	E4, E5, E11, E18
USDA 103 channel × D&B blue catfish	E14, E21
HS-5 channel × D&B blue catfish	E2, E17

Environmental samples

Water samples (500 ml) were collected between 5 and 10 cm below the surface and approximately 1 m off the edge of the pond. A total of 15 water samples (15 ponds) were collected on 15 November 2004. Average water temperature was 23·9°C and did not significantly differ from pond to pond. Dissolved oxygen, pH, hardness, alkalinity, ammonia and nitrite in the water were similar among ponds sampled (data not shown). Samples were centrifuged at 6000 ***g*** for 20 min. Pellets were subjected to total DNA extraction using the Pitcher *et al.* (1989) method.

Construction of the 16S rDNA libraries

16S rDNA gene was amplified from each environmental DNA using the universal primers: 63V (5′-CAGGCCTA ACACATGCAAGTC-3′) and 1387R [5′-GGGCGG(A/T) GTGTACAAGGC-3] according to Marchesi *et al.* (1998). Polymerase chain reaction (PCR) conditions were: initial denaturation at 95°C for 10 min, followed by 20 cycles of 95°C for 45 s, 55°C for 45 s and 72°C for 1 min. To minimize the PCR bias (Kanagawa 2003), the number of PCR cycles was decreased to 20. Amplified fragments (approximately 1300 bp) were electrophoresed, cleaned and cloned into a pGEMTeasy vector (Promega, Madison, WI, USA). Plasmid DNA from a total of 50 randomly selected clones per pond were extracted and sequenced at the Auburn University Sequencing Core. Clones were sequenced from both ends and a contiguous sequence of approximately 1300 bp was obtained from each clone. Sequences were compared with those in GenBank database using the BLAST program (Altschul *et al.* 1990). Clones were ascribed to bacterial classes when nucleotide identity were 96% or higher with sequences present in GenBank. When the taxonomic status of the best match sequence obtained

by BLAST was uncertain, clones were named as 'unidentified'.

ARISA

Automated ribosomal intergenic sequence analysis was generated by amplification of the sequence present between the 16S and the 23S rRNA genes using the universal primers: ITS-F (5′-GTCGTAACAAGGTAGCCGTA-3′) and ITS-R (5′-GCCAAGGCATCCACC-3′; Cardinale *et al.* 2004). Approximately 200 ng of environmental DNA were amplified in a reaction containing 1X PCR buffer, 1·5 U of *Taq* polymerase, 0·2 mmol l^{-1} (each) of deoxynucleoside triphosphate and 0·25 μmol l^{-1} (each) primer in a final volume of 25 μl. All reagents used for PCR were purchased from Promega. PCR conditions were as described above except for the number of cycles that was extended to a total of 35 cycles. Amplified products were electrophoresed on the NEN Global Edition IR2 DNA Analyzer (LI-COR, Lincoln, NE, USA) following manufacturer's instructions. ARISA images were processed with BIONUMERICS v. 4·0 (Applied Maths, Austin, TX, USA). Following conversion, normalization and background subtraction with mathematical algorithms, levels of similarity between fingerprints were calculated with the Pearson product-moment correlation coefficient (r). Cluster analysis was performed according to Arias et al. (2004) using the unweighted pair-group method with arithmetic mean (UPGMA).

Results

16S rDNA library

Analysis of the bacterial diversity by 16S ribosomal gene library revealed Alphaproteobacteria and Gammaproteobacteria along with Gram-positive high C + G content bacteria as equally dominant classes in pond water. Distribution of major eubacteria classes is shown in Table 2. All ponds presented Gammaproteobacteria although distribution ranged from 4% to 52%. *Plesiomonas shigelloides* accounted for 75% of all Gammaproteobacteria. Although it is not considered a primary pathogen, this bacterium has been associated with gastroenteritis in humans as well as with catfish infections. The most important group overall was the Alphaproteobacteria. This class was dominant in several ponds (E12, E15, E17, E18 and E19), being *Actinobacterium* and *Caulobacter* the predominant genera. Gram-positive high G + C content class represented 19% of total eubacteria with most clones showing high similarities with different species of *Mycobacterium*, *Clavibacter*, *Leifsonia* and *Streptomyces*.

Other represented classes were the Cytophaga-Flavobacterium-Bacteroides (CFB) group, Mycoplasmas and Chlamydias. More than 98% of all clones were found to have a similarity of 97% or higher with sequences present in GenBank. Only 11 clones presented high similarities with sequences of uncertain taxonomic statues and were considered unidentified.

Two species of bacterial catfish pathogens were identified during the analysis. *Edwardsiella tarda* was found in ponds E11, E14, E15, E17, E18 and E23 while *F. columnare* was detected in ponds E12 and E14.

ARISA

ARISA profiles were obtained from all ponds analysed (Fig. 1). Samples were amplified in duplicate to ensure ARISA reproducibility. Only peaks present in both gels were annotated. Amplified internal spacer region (ISR) fragments ranged from 100 bp up to over 750 bp. Because of settings used during electrophoresis only fragments below 750 bp could be analysed. The number of ISR-amplified fragments varied from 39 to 70. Pond E2

Table 2 Microbiota composition in channel catfish ponds determined by 16S rDNA library sequencing

Eubacteria class	Total (%)	E1	E2	E4	E5	E6	E11	E12	E14	E15	E17	E18	E19	E20	E21	E23
Alphaproteobacteria	22·8	4	11	4	7	4	0	29	2	14	24	23	21	12	3	13
Betaproteobacteria	3·6	0	0	4	4	2	2	0	3	2	5	0	0	3	2	0
Gammaproteobacteria	21·1	13	26	9	16	6	13	3	8	6	8	12	13	11	10	4
Deltaproteobacteria	2·5	0	0	0	2	0	2	0	4	2	2	0	0	2	5	0
CFB	6·7	2	0	3	1	0	4	5	7	7	0	0	0	15	0	6
Mycoplasma	5·6	14	0	0	0	2	2	6	4	0	0	0	0	0	12	2
Gram-positive high C + G	19·1	4	13	18	17	16	14	3	12	2	6	6	8	0	18	6
Bacillus	3·7	8	0	0	0	0	8	2	2	4	2	0	0	0	0	2
Chlamydias	3·3	4	0	0	0	15	0	0	0	4	0	0	0	2	0	0
Unidentified	1·5	0	0	3	0	0	0	0	0	0	0	3	4	1	0	0
Others	10·1	1	0	9	3	5	5	2	8	9	3	6	4	4	0	17

Numbers indicate sequenced clones corresponding to a specific eubacteria class. Fifty clones were sequenced from each pond.

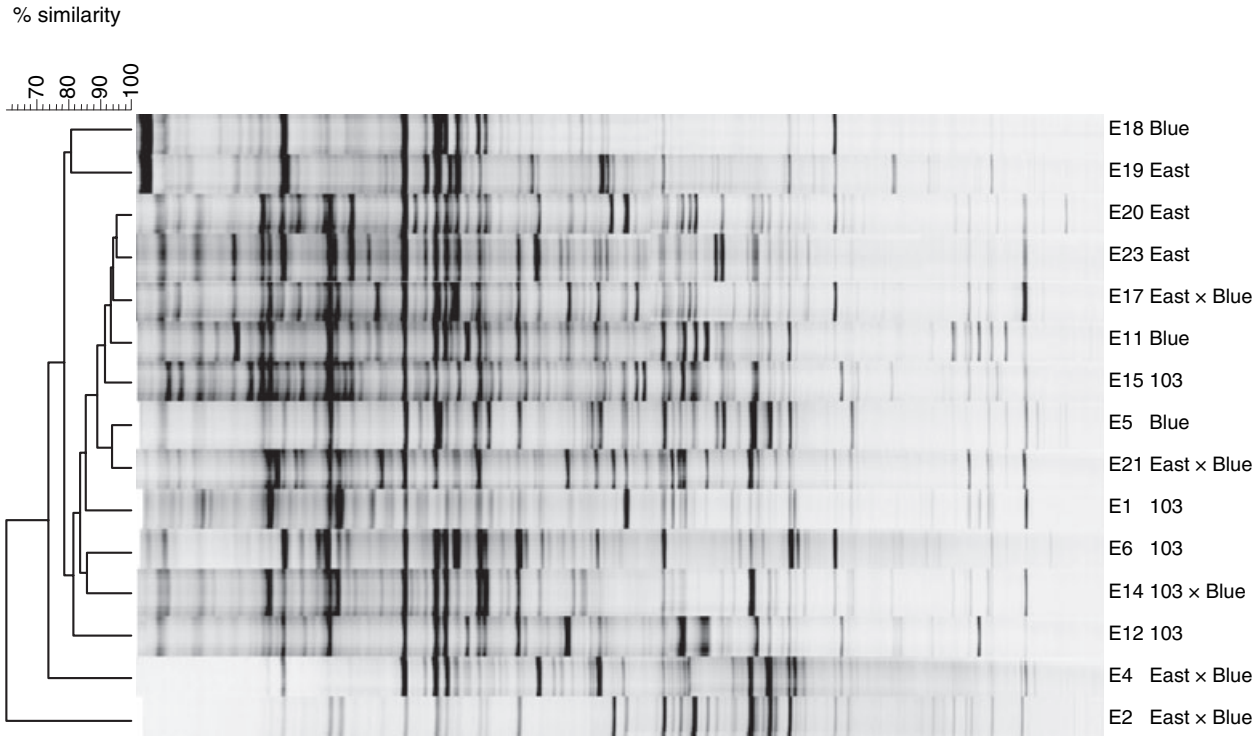

Figure 1 Fluorescent automated ribosomal intergenic spacer analysis (ARISA) patterns obtained from catfish pond water. The scale represents the percentage of similarity-based using the Dice coefficient. The dendrogram was constructed by using unweighted pair-group method with arithmetic mean (UPGMA).

showed the lowest diversity index with only 39 consistently amplified peaks. Regression analysis showed a weak positive correlation ($r^2 = 0{\cdot}66$) between number of bands displayed by ARISA and number of bacterial species identified in each pond by 16S rDNA library analysis.

Bacterial diversity in catfish ponds was revealed by ARISA as no identical profiles were obtained. All ponds showed different ARISA fingerprints although some common bands were found across all patterns. Ponds E5 and E21 shared a 93% pattern similarity while E11, E15, E17, E20 and E23 formed a cluster at 91% similarity. Rest of pond profiles presented more than 90% dissimilarity. Ponds E18 and E19 presented distinct ARISA profiles and clustered together at 80% similarity. Both ponds displayed one unique high-molecular weight band (over 700 bp). Finally, ponds E4 and E2 displayed fewer ARISA bands than the rest and clustered apart. No correlation between stocked fish strain and ARISA profiles could be inferred from our data.

Outbreaks

Several fish mortality episodes occurred during the length of the study; however, none could be correlated with deficient water quality or rearing practice. In July 2004, several ponds (E1, E18, E19 and E20) presented mortality-associated problems and nine fish were lost but cause was not identified. In September 2004, pond E6 suffered a confirmed columnaris outbreak and 61 fish were reported dead. During the same month, in ponds E15 and E17, a total of eight fish died but no infectious agent could be isolated. At sampling time, November 2004, no health problems were observed in any of the ponds analysed.

Discussion

The 15 culture ponds selected for this study were about the same size, and they had been identically managed, in terms of stocking density, feeding type and schedule, aeration, etc., except for the fish strains used to stock the ponds. Because of their close physical proximity they were also subjected to the same climatic conditions. Nevertheless, the bacterial communities found in each pond at the end of the growing season resulted quite different. As could be expected from an intensive aquaculture setting (Moreno *et al.* 1999; Cytryn *et al.* 2005), Alphaproteobacteria and Gammaproteobacteria were the dominant groups. Regarding other main constituents of the pond microbiota, it was surprising to find an elevated percentage of high G + C content Gram-positive bacteria in

most ponds. Specific studies on bacterial populations associated with farm-raised fish species have been scarce until recently, when culture-independent techniques have been applied to the study of bacterial population in different aquaculture settings (Sandaa *et al.* 2003; Jensen *et al.* 2004; Tanaka *et al.* 2004; Kapetanovic *et al.* 2005). Kapetanovic *et al.* (2005) showed that Gram-positive bacteria were dominant in trout fry but were quickly overtaken by Gammaproteobacteria when fry were transferred to outside pools. However, most studies showed a prevalence of Alphaproteobacteria and Gammaproteobacteria in a fish or shellfish farm environment (Sandaa *et al.* 2003; Jensen *et al.* 2004; Tanaka *et al.* 2004). Our results highlight the vast differences in bacterial communities from pond to pond even at the class taxonomy level. Therefore, bacterial communities should be considered a variable factor in channel catfish aquaculture.

Regarding the natural presence of pathogens in catfish ponds, we were able to detect two main catfish pathogens, *Edw. tarda* and *Fl. columnare*, in several ponds. Isolation of these two pathogens from catfish pond water samples in the absence of disease has been difficult in the past. The fact that these two pathogens can represent up to 8% (*F. columnare* in pond E14) or 10% (*E. tarda* in pond E17) of total sequenced clones seems to indicate these pathogens are easily overcome by saprophytic bacteria or might be stressed and unable to grow *in vitro*. This finding supports the idea of these two bacteria acting as facultative pathogens as they can be detect in water in the absence of an infective episode. However, no correlation could be made between the presence of these pathogens and the composition of the bacterial community in those ponds because vast differences in bacterial profiles were found between ponds. The only pond reported to suffer a confirmed columnaris outbreak (E6) presented a bacterial profile dominated by *Chlamydia* and Gram-positive high G + C content bacteria at the time of sampling. However, *Fl. columnare* was not detected thus indicating this pathogen was not a main constituent of the pond microbiota 2 months after the outbreak.

As no information is available on what might be a 'healthy' bacterial community for a catfish pond, it is difficult to evaluate which of all the different bacterial profiles found could benefit catfish culture. However, it is generally accepted that aquatic communities with low species diversity are unstable and subject to spectacular collapse, resulting in a host of attendant water quality problems (Tucker and Hargreaves 2004). This risk of sudden fluctuations of microbial composition for an aquaculture system is better understood for phytoplankton communities than for bacteria. We now understand some of the factors involved in phytoplankton community structure and periodicity and we are closer to be able to regulate these communities to establish the most appropriate phytoplankton balance for catfish culture. A similar approach should be used to study how bacterial populations change and how catfish culture is affected by these changes. Our study shows considerable differences in species diversity from pond to pond. For example, ponds E12 through E15 harboured more bacterial diversity than pond E2. Would this mean these ponds are better buffered against a bacterial outbreak? Further studies should be carried out in order to evaluate the 'fitness' of high *vs* low bacterial diversity ecosystem.

Sung *et al.* (2001) showed how bacterial communities influenced shrimp culture. According to their study, a decrease in species diversity is suggestive of environmental stress thus management practices should be implemented to correct the situation and avoid disease risks. The pathogenic population within a certain microbial community tends to shift in response to environmental stress. Finding the causing factors for such situations is typically difficult. For instance, temperature might be sufficient to explain why epizootic episodes caused by mesophilic bacteria (i.e. *Edw. ictaluri* and *Fl. columnare*) happen when water temperatures are optimal for the pathogen. However, not all the ponds are affected by outbreaks even though climatic and rearing conditions are the same. In this study we have shown for the first time how bacterial communities in a catfish aquaculture setting can vary from pond to pond at one given point. The availability of culture-independent methods to analyse bacterial diversity constitutes an invaluable tool to study the aquatic ecosystem. Nevertheless, construction and analysis of 16S rDNA libraries are still expensive methods especially when numerous samples are being processed. More inexpensive alternatives, such as denaturing gradient gel electrophoresis (DGGE), t-restriction fragment length polymorphism (t-RFLP) and ARISA have been developed in recent years and are currently being used to explore bacterial diversity in a wide variety of ecosystems (Jensen *et al.* 2004; Jasti *et al.* 2005; Yannarell and Triplett 2005; Yu *et al.* 2005). ARISA offers a cost efficient method to quantitatively analyse numerous samples simultaneously. In our study, ARISA confirmed the uniqueness of each bacterial pond community showed by 16S rDNA analysis. ARISA could be used to monitor changes in bacterial population year through. Because of the high variability found between ponds, it will be necessary to conduct routine surveys of bacterial populations starting prior to stocking. Understanding bacterial population dynamics in catfish ponds will help us to evaluate the role bacteria play in catfish aquaculture and integrate our knowledge into routine bacterial community management and better husbandry practices.

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References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic local alignment search tool. *J Mol Biol* **215**, 403–410.

Arias, C.R., Welker, T.L., Shoemaker, C.A., Abernathy, J.K. and Klesius, P.H. (2004) Genetic fingerprinting of *Flavobacterium columnare* isolates from cultured fish. *J Appl Microbiol* **97**, 421–428.

Boyd, C.E. and Queiroz, J. (2001) Feasibility of retention structures, settling basins, and best management practices in effluent regulation for Alabama channel catfish farming. *Rev Fisheries Sci* **9**, 43–67.

Cardinale, M., Brusetti, L., Quatrini, P., Borin, S., Puglia, A.M., Rizzi, A., Zanardini, E., Sorlini, C. *et al.* (2004) Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities. *Appl Environ Microbiol* **70**, 6147–6156.

Cytryn, E., Minz, D., Gelfand, I., Neori, A., Gieseke, A., de Beer, D. and van Rijn, J. (2005) Sulfide-oxidizing activity and bacterial community structure in a fluidized bed reactor from a zero-discharge mariculture system. *Environ Sci Technol* **39**, 1802–1810.

Hargreaves, J.A. and Tucker, C.S. (2004) *Biology and Culture of Channel Catfish*. Amsterdam, the Netherlands: Elsevier.

Jasti, S., Sieracki, M.E., Poulton, N.J., Giewat, M.W. and Rooney-Varga, J.N. (2005) Phylogenetic diversity and specificity of bacteria closely associated with *Alexandrium* spp. and other phytoplankton. *Appl Environ Microbiol* **71**, 3483–3494.

Jensen, S., Ovreas, L. and Torsvik, V. (2004) Phylogenetic analysis of bacterial communities associated with larvae of the Atlantic halibut propose succession form a uniform normal flora. *Syst Appl Microbiol* **27**, 728–736.

Kanagawa, T. (2003) Bias and artifacts in multitemplate polymerase chain reactions (PCR). *J Biosci Bioeng* **96**, 317–323.

Kapetanovic, D., Kurtovic, B. and Teskeredzic, E. (2005) Differences in bacterial population in rainbow trout (*Oncorhynchus mykiss* Walbum) fry after transfer from incubators to pools. *Food Technol Biotechnol* **43**, 193–198.

Marchesi, J.R., Sato, T., Weightman, A.J., Martin, T.A., Fry, J.C., Hiom, S.J., Dymock, D. and Wade, W.G. (1998) Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl Environ Microbiol* **64**, 795–799.

Moreno, Y., Arias, C.R., Meier, H., Garay, E. and Aznar, R. (1999) In situ analysis of the bacterial communities associated to farmed eel by whole-cell hybridization. *Lett Appl Microbiol* **29**, 160–165.

Pitcher, D.G., Saunders, N.A. and Owen, R.J. (1989) Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.

Plumb, J.A. and Quinlan, E.E. (1986) Survival of *Edwardsiella ictaluri* in pond water and bottom mud. *Prog Fish-Culturist* **48**, 212–214.

Sandaa, R.-A., Magneses, T., Torkildsen, L. and Bergh, O. (2003) Characterisation of the bacterial community associated with early stages of great scallop (*Pecten maximus*), using denaturing gradient gel electrophoresis (DGGE). *Syst Appl Microbiol* **26**, 302–311.

Stickey, R.R. (2000) *Encyclopedia of Aquaculture*. New York, NY, USA: R. R. Stickey, John Wiley & Sons, Inc.

Sung, H.-H., Hsu, S.-F., Chen, C.-K., Ting, Y.-Y. and Chao, W.-L. (2001) Relationships between disease outbreak in cultured tiger shrimp (*Penaeus monodon)* and the composition of *Vibrio* communities in pond water and shrimp hepatopancreas during cultivation. *Aquaculture* **192**, 101–110.

Tanaka, R., Ootsubo, M., Sawabe, T., Ezura, Y. and Tajima, K. (2004) Biodiversity and in situ abundance of gut microflora of abalone (*Haliotis discus hannai*) determined by culture-independent techniques. *Aquaculture* **241**, 453–463.

Tucker, C. and Hargreaves, J.A. (2004) *Biology and Culture of Channel Catfish*. Amsterdam, the Netherlands: Elsevier.

Tucker, C. and Robinson, E. (1991) *Channel Catfish Farming Handbook*. New York, NY, USA: Van Nostrand Reinhold.

USDA (2003a) *Part I: Reference of Fingerling Catfish Health and Production Practices in the United States*. Fort Collins, CO, USA: USDA, National Animal Health Monitoring System.

USDA (2003b) *Part II: Reference of Foodsize Catfish Health and Production Practices in the United States*. Fort Collins, CO, USA: USDA, National Animal Health Monitoring System.

Wagner, B.A., Wise, D.J. and Terhune, J.S. (2002) The epidemiology of bacterial diseases in food-size channel catfish. *J Aquat Anim Health* **14**, 263–272.

Welker, T.L., Shoemaker, C.A., Arias, C.R. and Klesius, P.H. (2005) Transmission and detection of *Flavobacterium columnare* in channel catfish *Ictalurus punctatus*. *Dis Aquat Organ* **63**, 129–138.

Yannarell, A.C. and Triplett, E.W. (2005) Geographic and environmental sources of variation in lake bacterial community composition. *Appl Environ Microbiol* **71**, 227–239.

Yu, C., Ahuja, R., Sayler, G. and Chu, K. (2005) Quantitative molecular assay for fingerprinting microbial communities of wastewater and estrogen-degrading consortia. *Appl Environ Microbiol* **71**, 1433–1444.