Environmental Research 160 (2018) 385–390

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Analysis of potential risks from the bacterial communities associated with air-contact surfaces from tilapia (*Oreochromis niloticus*) fish farming☆

Maria Jose Grande Burgos, Jose Luis Romero, Rubén Pérez Pulido, Antonio Cobo Molinos, Antonio Gálvez*, Rosario Lucas

Área de Microbiología, Departamento de Ciencias de la Salud, Facultad de Ciencias Experimentales, Universidad de Jaén, 23071 Jaén, Spain

ARTICLE INFO

Keywords:
Tilapia
Aquaculture
Bacterial diversity
Microbiological risks

ABSTRACT

Tilapia farming is a promising growing sector in aquaculture. Yet, there are limited studies on microbiological risks associated to tilapia farms. The aim of the present study was to analyse the bacterial communities from solid surfaces in contact with air in a tilapia farm in order to evaluate the presence of bacteria potentially toxinogenic or pathogenic to humans or animals. Samples from a local tilapia farm (tank wall, aerator, water outlets, sink and floor) were analyzed by high throughput sequencing technology. Sequences were assigned to operational taxonomic units (OTUs). *Proteobacteria* was the main phylum represented in most samples (except for one). *Cyanobacteria* were a relevant phylum in the inner wall from the fattening tank and the wet floor by the pre-fattening tank. *Bacteroidetes* were the second phylum in relative abundance for samples from the larval rearing tank and the pre-fattening tank and one sample from the fattening tank. *Fusobacteria* showed highest relative abundances in samples from the larval rearing tank and pre-fattening tank. Other phyla (*Verrucomicrobia*, *Actinobacteria*, *Firmicutes*, *Planktomycetes*, *Acidobacteria*, *Chloroflexi*, *Chlorobi*, *Gemmatiomonadetes* or *Fibrobacters*) had lower relative abundances. A large fraction of the reads (ranging from 43.67% to 72.25%) were assigned to uncultured bacteria. Genus *Acinetobacter* (mainly *A. calcoaceticus/baumanni*) was the predominant OTU in the aerator of the fattening tank and also in the nearby sink on the floor. The genera *Cetobacterium* and *Bacteroides* showed highest relative abundances in the samples from the larval rearing tank and the pre-fattening tank. Genera including fish pathogens (*Fusobacterium*, *Aeromonas*) were only detected at low relative abundances. Potential human pathogens other than *Acinetobacter* were either not detected or had very low relative abundances (< 0.01%). The results of the study suggest that the main risk factors to be monitored in tilapia farm are putative human pathogenic *Acinetobacter* and potential cyanotoxin-producing cyanobacteria.

1. Introduction

Tilapia is an aquaculture food commodity of economic and global importance (Rafael, 2008). In 2014, the world aquacultue production of tilapia and other cichilids amounted 5308020 t (FAO, 2014). In Spain, tilapia farming is still very limited, but the sector is expected to rise in the near future (FAO, 2017). Tilapia farming generates and anthropogenic environment where different microbial communities develop. Deciphering the composition of bacterial communities in aquaculture ecosystems can be relevant for safety assessment of the food, evaluation of the risk of exposure to human pathogens, and adopting control measures intended to decrease the spread of possible pathogenic bacteria.

One study on bacteria associated with tilapia farming (pond water, pond sediment, fish gill and intestine) based on culture-dependent methods (Pakingking et al., 2015) revealed that *Aeromonas hydrophila*, *Bacillus* spp., *Plesiomonas shigelloides*, *Shewanella putrefaciens*, *Pseudomonas fluorescens*, *Staphylococcus* spp. and *Vibrio cholerae* were the dominant bacteria identified in the gills and intestine of tilapia. These bacteria also dominated in the pond sediment and rearing water, except for the nil isolation of *S. putrefaciens* and *V. cholerae* in the water samples examined, indicating that resident bacteria in the pond water and sediment congruently typify the composition of bacterial microbiota in the gills and intestine of tilapia which under stressful conditions may propel the ascendance of disease epizootics (Pakingking et al., 2015).

☆ This work was supported by the University of Jaén (Research Structure AGR230).

* Corresponding author. Present address: Área de Microbiología, Departamento de Ciencias de la Salud, Facultad de Ciencias Experimentales. Edif. B3, Universidad de Jaén, Campus Las Lagunillas s/n., 23071 Jaén, Spain.
E-mail address: agalvez@ujaen.es (A. Gálvez).

http://dx.doi.org/10.1016/j.envres.2017.10.021
Received 27 August 2017; Received in revised form 10 October 2017; Accepted 12 October 2017
Available online 20 October 2017
0013-9351/

Studies based on high-throughput sequencing (HTS) technologies are providing new insight into the microbiota from different environments, including fish and fish farms. By studying the bacterioplancton communities of tilapia ponds, Fan et al. (2016) concluded that the dominant phylum in all water samples were similar, and they included *Proteobacteria*, *Cyanobacteria*, *Bacteroidetes*, *Actinobacteria*, *Planctomycetes* and *Chlorobi*, distributed in different proportions in the different months and ponds. One study on the composition of water, feed and gut bacteria communities of Nile tilapia larvae revealed the presence of representatives of *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, *Nitrospirae* and *Planktomycetes* with different relative abundances depending on the sampling environment investigated (Giatsis et al., 2015). Another study analyzed the intestinal microbiota of tilapia (*Oreochromis niloticus*) after the application of a multi-species probiotic. *Firmicutes* were the dominant phyla in the control group, while reads for *Proteobacteria*, *Cyanobacteria*, *Actinobacteria*, *Bacteroidetes*, *Fusobacteria*, *Nitrospirae*, *Spirochaetes* and the phylum TM6 were detected at lower relative abundances (Standen et al., 2015).

While the microbiota of water, ponds and tilapia gut has been studied to a large extent, there are no previous studies on the bacterial communities from wet surfaces in contact with air, where bacterial biofilms can develop. Such environments could be a source for pathogenic or toxinogenic bacteria. Biofilms are complex structures where bacteria are embedded within a self-produced extracellular matrix (Costerton et al., 1999; Donlan and Costerton, 2002). Biofilm formation confers an increased tolerance to disinfection processes, facilitating persistence of bacteria in the environment (Donlan and Costerton, 2002; Steenackers et al., 2012). Biofilms can be important as reservoirs of bacteria that can further colonize other environments such as the water, food, or animal tissues. Since wet surfaces in contact with air may act as reservoirs of unwanted bacteria that may be protected from disinfection in biofilms, the aim of the present work was to provide insights on the bacterial communities from different wet surfaces in contact with air in a tilapia farm as possible sources of bacteria pathogenic to humans or fish, or relevant for their toxin production capacity.

2. Materials and methods

2.1. Sample preparation

Samples were taken from a tilapia (*Oreochromis niloticus*) fish farm in Andalucia in the month of April 2015. The tanks (larval rearing, pre-fattening, fattening) had concrete walls. Water was recirculated and held at a constant temperature of 29 °C. Samples (in triplicate) were taken from wet surfaces in contact with air (Table 1) by rubbing the surfaces (ca. 2 cm^2 each) with sterile swabs. Samples were kept on ice for not longer than 24 h before analysis. The content of each swab was recovered in 1 ml sterile saline solution inside a sterile Eppendorf test tube by manual agitation. The process was repeated once with fresh saline solution. The resulting suspensions were centrifuged (13.500 × *g*, 5 min) and the sediments recovered for each sample in triplicate were resuspended into 0.5 ml sterile solution and pooled as a single sample for DNA extraction and further analysis.

2.2. DNA extraction, sequencing and analysis

DNA was extracted by using a GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich) following instructions provided by the manufacturer. DNA concentration and quality were measured with a NanoDrop spectrophotometer (Thermo Scientific, United Kingdom).

The sequence of the V3-V4 region of 16 S rRNA gene was used as the taxonomic basis to estimate bacterial populations present in the samples (Caporaso et al., 2011) using Illumina technology. Library preparation and sequencing was done at the facilities of Fundación Parque Científico de Madrid (Madrid, Spain). The quality of the DNA was determined by agarose gel electrophoresis. Accurate concentration of DNA in the samples was determined using a fluorimetric method with Quant-IT PicoGreen reagent (Thermo Fischer, Madrid, Spain) in a Quantifluor ST fluorometer (Promega, Alcobendas, Madrid). The oligonucleotide primers used for the first PCR reaction were 16 SV3-V4-CS1 ACACTGACGACATGGTTCTACACCTACGGGNGGCWGCAG (forward) and 16SV3-V4-CS2 5´ TACGGTAGCAGAGACTTGGTCTGACTACHVGGGTATCTAATCC (reverse), where the underlined regions are the CS1 and CS2 Fluidigm adapter nucleotide sequences, while the non-underline sequences are locus-specific sequences targeting conserved regions within the V3 and V4 domains of prokaryotic 16S rRNA genes (Klindworth et al., 2013). Each PCR reaction contained DNA template (~10–12 ng), 5 µl forward primer (1 µM), 5 µl reverse primer (1 µM), 12.5 µl Q5® High-Fidelity 2X Master Mix (New England Biolabs, Ipswich, MA, USA), and PCR grade water to a final volume of 25 µl. PCR amplification was carried out as follows: 98 °C × 30 s, 20 cycles of 98 °C × 10 s, 50 °C × 20 s, 72 °C × 20 s, then 72 °C × 2 min and held at 4 °C. PCR products were visualized using agarose gel electrophoresis. Successful PCR products were cleaned using AMPure XP magnetic bead based purification (Beckman Coulter, Brea, CA, USA). Afterwards, a second PCR was applied under the same primers and conditions as above (except that only 8 cycles were completed) to add the individual barcode to each of the samples, as well as to incorporate Illumina-specific sequences in the amplicon libraries. Individual libraries were analyzed using a Bioanalyzer 2100 (Agilent, Madrid) to estimate the concentration of the specific PCR products and a pool of samples was made in equimolar amounts. The pool was further cleaned with AMPure XP magnetic beads, and the exact concentration of the library was measured by real time PCR using Illumina specific primers (Kapa Biosystems, Wilmington, MA, USA). Paired-end sequencing of the library was performed on an Illumina MiSeq sequencer (San Diego, CA, USA) using the MiSeq Reagent Kit (v3) with the longest read length set to 2 × 300 base pairs (bp). After demultiplexing, paired end reads were joined together with the fastq-join program (https://expressionanalysis.github.io/ea-utils/). Only reads that that had quality value (QV) scores of ≥ 20 for more than 99% of the sequence were extracted for

Table 1
Description of samples, number of sequences (reads) and observed diversity for 16 S rRNA amplicons analyzed in this study.

Sample name	Origin	No. of reads	Shannon index	Simpson index	Chao1 index
UJA-T3–1	Larval rearing tank aerator	73,925	3.62	0.94	473.50
UJA-T3–2	Larval rearing tank water outlet (to filter)	86,366	3.43	0.93	394.80
UJA-T3–3	Fattening tank inner wall	91,877	3.65	0.95	518.31
UJA-T3–4	Pre-fattening tank water outlet (to filter)	76,598	3.44	0.94	391.00
UJA-T3–5	Pre-fattening tank water overflow (to floor)	76,650	3.46	0.93	456.02
UJA-T3–6	Floor by pre-fattening tank	83,870	3.74	0.94	684.63
UJA-T3–7	Floor by pre-fattening tank	80,165	3.83	0.94	610.00
UJA-T3–8	Fattening tank inner wall	70,542	2.83	0.78	585.01
UJA-T3–9	Fattening tank aerator	76,011	3.13	0.89	397.91
UJA-T3–10	Floor drain by fattening tank and aerator	100,564	2.95	0.83	540.32

further analysis. All sequences with ambiguous base calls were discarded. After filtering, sequence reads were assigned to operational taxonomic units (OTUs) based on sequence similarity for each read to 16 S rRNA genes from the Era7 Bioinformatics DB7 database which combines the major databases Silva, GreenGenes, RDP, Ena and Refseq (Era7 Bioinformatics, Granada, Spain). Each read was assigned to the taxon corresponding to the Best Blast Hit over a threshold of similarity (e < 1E-15). Cluster analysis was done with Statistica (V. 10.0. Statsoft, Palo Alto, CA). Biodiversity indexes were calculated with Microsoft Excel programme.

3. Results

3.1. Bacterial communities from the tilapia farm samples

The numbers of assigned reads after filtering ranged from 70542 for the fattening tank wall to 100564 for the floor drain (Table 1). Reads were assigned to 29 phyla including 227 families and 965 genera in total. Biodiversity indexes (Table 1) indicated a high diversity for all samples, with marked differences between samples for Shannon´s diversity index and Chao 1 index.

The relative abundances of the main phyla detected changed according to the sample origin. *Proteobacteria* was the main phylum represented, with highest relative abundances exceeding 65% in samples from the fattening tank aerator and the nearby floor drain (Fig. 1). This phylum had lowest relative abundance (18.66%) in sample UJA-T3-8 from the fattening tank inner wall, where *Cyanobacteria* were the predominant group (55.28%). *Cyanobacteria* were also a relevant phylum in samples from the wet floor by the pre-fattening tank (UJA-T3-6, 12.53%; UJA-T3-7, 19.13%) and less relevant in the fattening tank aerator (UJA-T3-9, 8.12%) and sink (UJA-T3-10, 6.40%). *Bacteroidetes* were the second phylum in relative abundance for many of the samples (ranging from 23.88% to 33.31% for samples from the larval rearing tank and the pre-fattening tank and one sample from the fattening tank). *Fusobacteria* showed highest relative abundances in samples from the larval rearing tank (UJA-T3-2, 18.39%) and pre-fattening tank (UJA-T3-4, 17.88%; UJA-T3-5, 12.67%). Other phyla such as *Verrucomicrobia*, *Actinobacteria*, *Firmicutes*, *Planktomycetes*, *Acidobacteria*, *Chloroflexi*, *Chlorobi*, *Gemmatiomonadetes* or *Fibrobacteres* had lower relative abundances in general or only had a higher relative abundance in a limited number of samples.

A large fraction of the reads (ranging from 43.67% to 72.25%) were assigned to uncultured bacteria (Fig. 2). Among them, uncultured Bacteroidetes and uncultured cyanobacteria showed highest relative abundances. The first was detected in all samples at relative abundances ranging from 2.85% (UJA-T3-9) to 16.65% (UJA-T3-5). Uncultured cyanobacteria showed highest relative abundances in samples UJA-T3-6 (10.76%), UJA-T3-7 (15.15%), UJA-T3-8 (45.16%), UJA-T3-9 (6.98%) and UJA-T3-10 (3.15%).

Genus *Acinetobacter* (mainly *A. calcoaceticus/baumanni*) was the predominant OTU in the aerator of the fattening tank (UJA-T3-9; 27.80% relative abundance) and also in the nearby sink on the floor (UJA-T-10; 39.16%). *Cetobacterium* (mainly *C. somerae*) had high relative abundances in samples from the water outlet of the larval rearing tank (UJA-T3-2; 18.37%) and the pre-fattening tank water outlet (UJA-T3-4; 17.87%) and water overflow (UJA-T3-5; 12.66%). Genus *Bacteroides* also showed highest relative abundances in the samples from the larval rearing tank (UJA-T3-2; 7.81%) and the pre-fattening tank (UJA-T3-4, 5.66%; UJA-T3-5, 4.40%).

The following genera (Fig. 2) had relative abundances > 2.0% only in a few samples (*Pseudomonas*, *Flectobacillus*, *Plesiomonas*, *Mycobacterium*) or only in one sample (*Flavobacterium*, *Dyella*, *Sphingomonas*, *Paenibacillus*, *Porphyromonas*, *Ideonella*, *Haliscomenobacter*, *Planktothrix*, *Phormidium*, *Newskia*, *Silanimonas*, *Rhodanobacter*).

Other genera (not listed in Fig. 2) had relative abundances comprised between 1% and 2% for at least one of the samples (*Nordella, Pseudoxanthomonas, Thermomonas, Lysobacter, Shewanella, Aeromonas, Pedobacter, Undibacterium, Rubriviax, Nitrobacter, Actinomyces, Prosthecobacter, Exiguobacterium, Chryseobacterium, Anabaena* and *Novosphingobium*).

With respect to specific fish pathogens, OTUs assigned to genus *Flavobacterium* were detected in all samples, at relative abundances from 0.60% to 1.90% except for samples from sink on the floor (UJA-T3-10; 6.13%). Nevertheless, the main species detected were *F. cucumis* and *F. indicum* while the fish pathogen *F. columnare* was only detected in two samples and only at relative abundances < 0.01%. *Aeromonas* was also detected in all samples, at relative abundances < 1.7%, but the fish pathogen *A. hydrophila* had a highest relative abundance of only

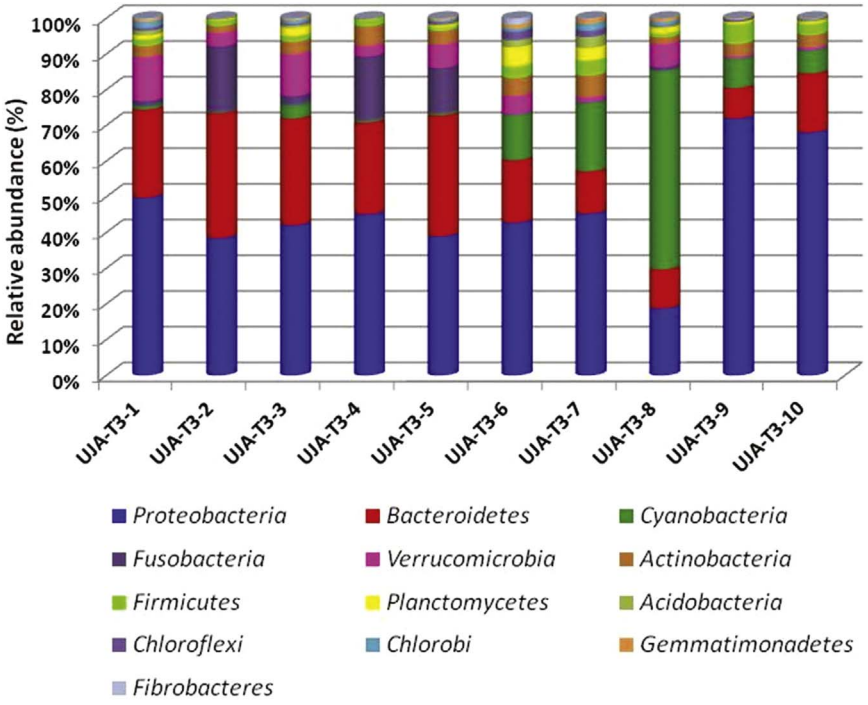

Fig. 1. Relative abundance of OTUs sorted by *Phylum* based on paired-end 16 S rRNA gene sequencing analysis of DNA from the sampled tilapia farm. Sample codes are described in Table 1.

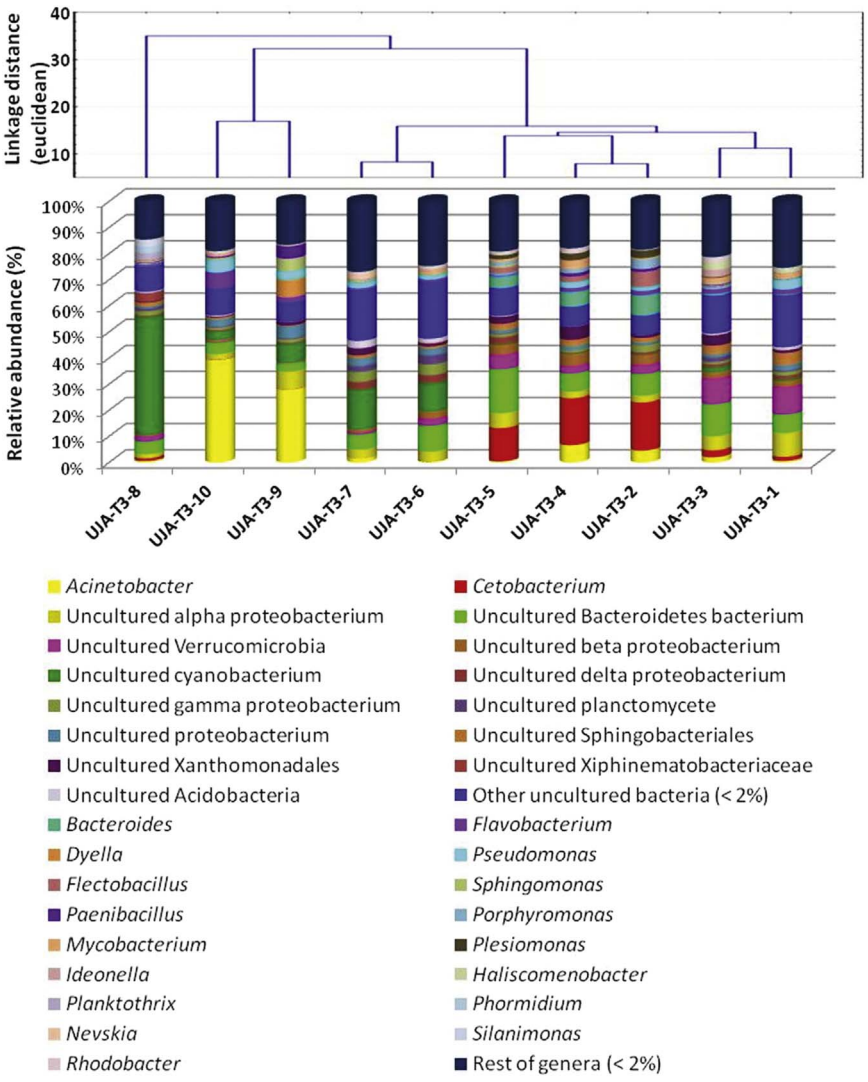

Fig. 2. Cluster analysis of samples. OTUs assigned to species level were sorted by *Genus*. OTUs assigned to uncultured bacteria were sorted by the corresponding group. Sample codes are described in Table 1.

0.5% (UJA-T3-10), and *A. salmonicida* was not detected. OTUs corresponding to other fish pathogens (*Edwardsiella tarda*, *Francisella* sp., *P aeruginosa* group, *P. plecoglossicida Streptococcus iniae*, *Streptococcus agalactiae*, *Vibrio anguillarum*, *Vibrio harveyi* or *Photobacterium* sp.) were not detected or had very low relative abundances (< 0.01%).

OTUs corresponding to genera *Escherichia, Salmonella* and *Listeria* had relative abundances below 0.01%. Furthermore, most of the reads assigned to genus *Listeria* belonged to *Listeria welshimeri*, and none was assigned to *Listeria monocytogenes. Salmonella enterica* accumulated less than 10 counts, and *Escherichia coli* was not detected. OTUs assigned to genus *Legionella* showed highest relative abundances in samples UJA-T3 (0.19%) and UJA-T7 (0.15%). However, the numbers of reads assigned to *Legionella pneumophila* were < 20.

Cluster analysis (Fig. 2) revealed that samples UJA-T3-1 to UJA-T3-7 formed a major cluster clearly separated from the rest of the samples. Within this cluster, highest similarities were observed between samples UJA-T3-6 and UJA-T3-7 (with uncultured cyanobacteria and uncultured gammaproteobacteria as most abundant OTUs) as well as between samples UJA-T3-2 and UJA-T3-4 (sharing high relative abundances of *Cetobacterium* and *Bacteroidetes*). Furthermore, samples from the fattening tank aerator and the nearby sink on the floor also had similar compositions whose main feature was the high relative abundance of *Acinetobacter*-assigned OTUs.

4. Discussion

Fish farming may generate environments where toxinogenic or pathogenic bacteria may proliferate. Wet surfaces in contact with air deserve special attention, because they can behave as bacterial reservoirs and facilitate the spread of microorganisms. Results from the present study revealed that *Acinetobacter*-affiliated OTUs (mainly *A. calcoaceticus/baumanni*) were dominant in at least two samples from the tilapia farm (the aerator by the fattening tank and the close sink on the floor). The majority of *Acinetobacter* species are nonpathogenic, environmental microorganisms, however those species adapted to clinical environments are now causing serious health problems in the nosocomial environment (Wong et al., 2017). Particularly, *Acinetobacter baumannii* has been listed as one of the most important nosocomial pathogens (Joly-Guillouet al, 2005; Peleg et al., 2008; Antunes et al., 2014). This bacterium attaches to and forms biofilm structures on abiotic surfaces, and particularly at the liquid–air interfaces (Tomaras et al., 2003). Liquid-air interfaces are important for dissemination of bacteria through aerosols. In view of the obtained results, further studies should be carried out in order to evaluate the presence of *Acinetobacter* in aerosols and the possible risks of exposure for workers in the tilapia farms. Another reason to consider acinetobacters as bacteria of concern in the seafood industry is that environmental isolates may carry antimicrobial resistance traits. Yoon et al. (2014) showed that the amikacin resistance gene *aphA6* coding for an enzyme that confers

resistance to amikacin, the most active aminoglycoside for the treatment of nosocomial infections due to *Acinetobacter* spp. originated from the environmental species *Acinetobacter guillouiae*. A recent study from our group reported that *Acinetobacter calcoaceticus* and *Acinetobacter oleivorans* strains isolated from salmon slices and prawns sold at supermarkets were multiply resistant to antibiotics (Romero et al., 2017). Furthermore, the *A. calcoaceticus* isolates carried the quaternary compounds resistance gene *qacEΔ1* and the sulphonamide resistance gene *sul1*. Therefore, antibiotic resistance in *Acinetobacter* from fish farming deserves to be investigated.

Regarding other potential human pathogenic bacteria, OTUs assigned to genus *Legionella* were comprised between 0.15% and 0.19% in two samples, but the numbers of reads assigned to *L. pneumophila* were too low to reach a conclusion about the risk of exposure to this bacterium. A recent study (Pereira et al., 2017) reported that the minimum concentration of *L. pneumophila* detected with Illumina MiSeq using universal primers for *Bacteria* was 10^2 genome copies per assay, and that this method underestimated the load of *Legionella* in water samples. Therefore, more specific methods should be used in order to evaluate the real concentrations of pathogenic legionellae in the tilapia farm. Other human pathogenic bacteria did not represent a relevant fraction of the microbial community in the studied samples. Therefore, they do not seem to represent a risk for humans through food contamination. Similarly, the main pathogenic bacteria described for tilapia (Newaj-Fyzul et al., 2008; Pech et al., 2017) also had very low relative abundances or were not detected, suggesting that the studied environments are not relevant reservoirs of fish pathogens.

In the present study, the predominant OTUs from the larval rearing tank and pre-fattening tank samples belonged to *Cetobacterium* (mainly *C. somerae*), presumably originated from the fish feces. *C. somerae* is as vancomycin-resistant, microaerotolerant anaerobe that produces acetate as main metabolic end-product (Finegold et al., 2003). *C. somerae* was first isolated from stools of children with late-onset autism undergoing treatment with vancomycin and also from the feces of a 47-month old male child during the first course of oral treatment with vancomycin (Finegold et al., 2003). However, it has not yet been clarified whether *C. somerae* is indigenous to the intestinal tract and feces of human children, particularly patients being treated with vancomycin (Finegold et al., 2003; Tsuchiya et al., 2008). Its vancomycin resistance seems to be intrinsic, and no mobile genetic elements associated with this trait have been described to date in the bacterium. *C. somerae* has also been isolated as a vitamin B_{12} producing bacterium from the intestine of freshwater fish such as common carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*) and ayu (*Plecoglossus altivelis*) (Tsuchiya et al., 2008). One study carried out by high-throughput sequencing reported that *Ceobacterium* was part of the microbiota of freshwater fish, mainly from carnivorous, omnivorous and filter-feeding species (Liu et al., 2016). The fact that other bacteria also described as relevant in tilapia feces were not detected in the present study, or had a very low relative abundance would suggest a low capacity for binding to and surviving on wet surface-air interfaces. There are no previous studies on the biofilm-forming capacity of *Cetobacterium*, but this train may be important for survival of the bacterium in microaerophylic environments outside of the host.

In the present study, uncultured cyanobacteria were the predominant group in one sample from the tank wall (where a green patch of biomass could be seen by the naked eye) and also had high relative abundances in samples from the floor by the fattening tank and in the fattening tank aerator. Cyanobacteria have been detected in the bacterioplancton communities of tilapia ponds (Fan et al., 2016) and also from the intestinal microbiota of tilapia (Standen et al., 2015). Cyanobacteria may produce an array of cyanotoxins (reviewed by Buratti et al., 2017). Ingestion of cyanotoxins may cause severe poisoning of humans, animals and livestock (Buratti et al., 2017). High concentrations of cyanotoxins in freshwater primarily result from surface scum formation. However, cyanotoxins may accumulate in fish via direct feeding on phytoplankton, through uptake of dissolved toxins after lysis of blooms via epithelial absorption, or from exposure through the food web (Galvão et al., 2009). Accumulation of cyanotoxins by freshwater tilapia has been reported in previous studies (Deblois et al., 2008; Galvão et al., 2009). The potential for production of cyanotoxins among cyanobacteria from the present study is difficult to evaluate, since most of the OTUs belonged to uncultured cyanobacteria and the OTUs from toxic cyanobacteria detected (*Planktothrix*, *Phormidium*, *Aphanizomenon* and *Anabaena*) had low relative abundances (from 1.05% to 2.60% in some samples). Repeated/chronic exposure to low cyanotoxin levels remains a critical issue (Buratti et al., 2017). Considering that cyanobacteria are present in tilapia farms and taking into account that tilapia can accumulate cyanotoxins, periodic inspection of cyanotoxins in tilapia for human consumption is recommended as a safety measure.

5. Conclusions

Results from the study suggest that *Acinetobacter* and cyanobacteria are the main bacterial groups that may potentially pose health risks to humans in the tilapia farm. Monitoring of cyanotoxin production and a more detailed study of the virulence potential of acinetobacters (virulence factors, antibiotic resistance) is recommended for further studies, as well as more detailed studies on the possible seasonal variations of prevalence of these bacterial groups in tilapia farms.

Acknowledgements

This work was supported by the University of Jaén (Research Structure AGR230). We also acknowledge the Campus de Excelencia Internacional Agroalimentario CeiA3.**Conflict of interest**

The authors declare no conflict of interest.

References

Antunes, L.C.S., Visca, P., Towner, K.J., 2014. *Acinetobacter baumannii*: evolution of a global pathogen. Pathog. Dis. 71, 292–301.

Buratti, F.M., Manganelli, M., Vichi, S., Stefanelli, M., Scardala, S., Testai, E., Funari, E., 2017. Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. Arch. Toxicol. 91 (3), 1049–1130.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. USA 108 (1), 4516–4522.

Costerton, J.W., Stewart, P.S., Greenberg, E.P., 1999. Bacterial biofilms: a common cause of persistent infections. Science 284 (5418), 1318–1322.

Deblois, C.P., Aranda-Rodriguez, R., Giani, A., Bird, D.F., 2008. Microcystin accumulation in liver and muscle of tilapia in two large Brazilian hydroelectric reservoirs. Toxicon 51 (3), 435–448.

Donlan, R.M., Costerton, J.W., 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin. Microbiol. Rev. 15, 167–193.

Fan, L.M., Barry, K., Hu, G.D., Meng, S., Song, C., Wu, W., Chen, J.Z., Xu, P., 2016. Bacterioplankton community analysis in tilapia ponds by Illumina high-throughput sequencing. World J. Microbiol. Biotechnol. 32 (1), 10.

FAO, 2014. Fishery statistical collections. ⟨http://www.fao.org/fishery/statistics/global-aquaculture-production/en⟩.

FAO, 2017. National Aquaculture Sector Overview. Spain. ⟨http://www.fao.org/fishery/countrysector/naso_spain/en⟩.

Finegold, S.M., Vaisanen, M.-L., Molitoris, D.R., Tomzynski, T.J., Song, Y., Liu, C., Collins, M.D., Lawson, P.A., 2003. *Cetobacterium somerae* sp. nov. from human feces and emended description of the genus *Cetobacterium*. Syst. Appl. Microbiol. 26 (2), 177–181.

Galvão, J.A., Oetterer, M., Bittencourt-Oliveira, Mdo, C., Selma Gouvêa-Barros, S., Hiller, S., Erler, K., Luckas, B., Pinto, E., Kujbida, P., 2009. Saxitoxins accumulation by freshwater tilapia (*Oreochromis niloticus*) for human consumption. Toxicon 54 (6), 891–894.

Giatsis, C., Sipkema, D., Smidt, H., Heilig, H., Benvenuti, G., Verreth, J., Verdegem, M., 2015. The impact of rearing environment on the development of gut microbiota in tilapia larvae. Sci. Rep. 5, 18206.

Joly-Guillou, M.-L., 2005. Clinical impact and pathogenicity of *Acinetobacter*. Clin. Microbiol. Infect. 11, 868–873.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation ofgeneral 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucl. Acids Res. 41, e1.

Liu, H., Guo, X., Gooneratne, R., Lai, R., Zeng, C., Zhan, F., Wang, W., 2016. The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by

their trophic levels. Sci. Rep. 6, 24340.
Newaj-Fyzul, A., Mutani, A., Ramsubhag, A., Adesiyun, A., 2008. Prevalence of bacterial pathogens and their anti-microbial resistance in tilapia and their pond water in Trinidad. Zoon. Publ. Health 55 (4), 206–213.
Pakingking Jr., R., Palma, P., Usero, R., 2015. Quantitative and qualitative analyses of the bacterial microbiota of tilapia (*Oreochromis niloticus*) cultured in earthen ponds in the Philippines. World J. Microbiol. Biotechnol. 31 (2), 265–275.
Pech, Z.G.H., Chavez, C.M.R., Reynoso, F.L., 2017. Pathogenic bacteria in *Oreochromis niloticus* var. Stirling tilapia culture. Fish. Aqua. J. 8, 197.
Peleg, A.Y., Seifert, H., Paterson, D.L., 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin. Microbiol. Rev. 21, 538–582.
Pereira, R.P.A., Peplies, J., Brettar, I., Höfle, M.G., 2017. Development of a genus-specific next generation sequencing approach for sensitive and quantitative determination of the *Legionella* microbiome in freshwater systems. BMC Microbiol. 17, 79.
Rafael, D., 2008. Tilapia farming: a global review (1924–2004). Asia Life Sci. 17, 207–229.
Romero, J.L., Grande Burgos, M.J., Pérez-Pulido, R., Gálvez, A., Lucas, R., 2017. Resistance to antibiotics, biocides, preservatives and metals in bacteria isolated from seafoods: co-selection of strains resistant or tolerant to different classes of compounds. Front Microbiol. 8, 1650.
Standen, B.T., Rodiles, A., Peggs, D.L., Davies, S.J., Santos, G.A., Merrifield, D.L., 2015. Modulation of the intestinal microbiota and morphology of tilapia, *Oreochromis niloticus*, following the application of a multi-species probiotic. Appl. Microbiol. Biotechnol. 99 (20), 8403–8417.
Steenackers, H., Hermans, K., Vanderleyden, J., De Keersmaecker, S.C.J., 2012. Salmonella biofilms: an overview on occurrence, structure, regulation and eradication. Food Res. Int. 45, 502–531.
Tomaras, A.P., Dorsey, C.W., Edelmann, R.E., Actis, L.A., 2003. Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system. Microbiology 149, 3473–3484.
Tsuchiya, C., Sakata, T., Sugita, H., 2008. Novel ecological niche of *Cetobacterium somerae*, an anaerobic bacterium in the intestinal tracts of freshwater fish. Lett. Appl. Microbiol. 46 (1), 43–48.
Wong, D., Nielsen, T.B., Bonomo, R.A., Pantapalangkoor, P., Luna, B., Spellberg, B., 2017. Clinical and pathophysiological overview of *Acinetobacter* infections: a century of challenges. Clin. Microbiol. Rev. 30 (1), 409–447.
Yoon, E., Goussard, S., Touchon, M., Krizova, L., Cerqueira, G., Murphy, C., Lambert, T., Grillot-Courvalin, C., Nemec, A., Courvalin, P., 2014. Origin in *Acinetobacter guillouiae* and dissemination of the aminoglycoside-modifying enzyme Aph(3=)-VI. mBio 5 (5) (e01972-14).