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

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