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INSIGHTS ON PUTATIVE PATHOGEN REDUCTION FROM SWINE WASTEWATER TREATMENT PROCESSES THROUGH 16S rDNA PYROSEQUENCING ANALYSES

Mezzari, M.P.*[1]; Da Silva, M.L.B.[2]; Cantão, M.E.[2]; Ma, J. [3]; Nossa, C.W.[4]

[1]UNOESC, Biotechnology and Sciences Program, Videira, SC, 89560-000 Brazil
[2]EMBRAPA, BR153 Km 110 P.O. Box 21, Concórdia, SC, 89700-000 Brazil
[3]China University of Petroleum, 18 Fuxue Rd., Beijing, 102249, China
[4]Department of Ecology and Evolutionary Biology, Rice University, Houston, TX, USA.
**Email: melissa.mezzari@unoesc.edu.br*

SUMMARY: Pyrosequencing analyses of bacterial and archaeal 16S rDNA fragments were performed in effluent samples from two distinct field scale swine wastewater treatment plants (WWTP). A total of 83,333 and 63,636 sequence reads were obtained from Bacterial and Archaeal 16S rDNA, respectively. Bacterial communities were mostly associated with phylum Firmicutes, Bacteroidetes, Proteobacteria or Actinobacteria. Archaeal members of the phylum Eurychaeota were dominant in all samples evaluated. Different from Escherichia, and/ or Atopostipes, that are microorganisms conventionally utilized as indicators of fecal contamination, Bacteroidetes and/ or Prevotella were detected in most samples from the WWTP validating at some extent their usefulness as biomarker to predict environmental contamination by swine wastewaters. Satisfactory putative pathogen reduction was observed after the open pond natural attenuation lagoon but not after the air sparged nitrification/ denitrification followed by alkaline phosphorus precipitation treatment processes. Treated effluents from WWTPs could still contain pathogenic bacteria, thus presenting a potential threat to public health.
Keywords: bacterial biomarkers, pathogens, swine wastewater.

INTRODUCTION

Pathogens pose great risk to human health and traditional methods have been used to monitor total fecal coliforms, *Escherichia coli* and enterococci to indicate fecal contamination in aquatic environments. However, these methods are now considered obsolete since they cannot quantify, trace the sources of fecal contamination and cannot differentiate contamination between human or animal polllution. Molecular biology tools, which are based on identification of a host-specific gene marker or host-specific molecular fingerprint from test samples, have been used successfully to detect bacterial pathogens from contaminated environmental waters (Okabe et al., 2007; Jeong et al., 2011). Pyrosequencing is a high-throughput sequencing technology and a complementary molecular tool that can identify and quantifying fecal pollution sources in the environment by broadening microbial community detection, evaluating existing host-specific markers and enhancing the effectiveness of fecal source tracking (Jeong et al., 2011).

Molecular biomarkers developed to target swine fecal contamination are based on the 16S rRNA gene sequences of dominant Eubacteria or methanogenic Archaebacteria of the pig intestinal tract (Marti et al., 2010). Dominant groups of pig fecal Eubacteria, include Bacteroides-Prevotella, Eubacterium-Clostridiacea, Lactobacillus-Streptococcus, and to a lesser extent Bifidobacterium (Marti et al., 2010). These are bacteria that have restricted anaerobic enteric nature from warm-blooded animals and that are commonly used as indicators of environmental pollution by swine wastewaters. PCR-based methods targeting members of the Bacteroides-Prevotella group has been particularly well studied for analysis of community composition and diversity among fecal swine samples (Jeong et al., 2011). In the present study, Escherichia, Bacteroides, Prevotella (Okabe et al., 2007)

and Lactobacillus (Marti et al., 2010) were used as biomarkers to infer on the reduction of putative pathogens throughout two distinct swine wastewater treatment plants (WWTPs). An ancillary objective of this study focused on determining the efficacy of different treatment process on the reduction of putative pathogens based on the use of bacterial biomarkers.

MATERIAL AND METHODS

Bacterial and archaeal communities were sampled from different swine wastewater treatment plants: WWTP1 and WWTP2 (Figure 1). Samples from WWTP1 were collected from two anaerobic covered lagoons connected in series (designated as bio1 and 2, respectively) and the final effluent. Samples from WWTP2 were collected from the pen, the UASB reactor and the final effluent. WWTP 1 has been treating the effluent of approximately 3,000 confined animals from a full-scale commercial farm located in southern Brazil for the last 7 years. WWTP 2 has been treating effluent from approximately 2,850 confined swine at Brazilian Agricultural Research Corporation (EMBRAPA, Concórdia, Santa Catarina, Brazil) for the last 10 years.

Genomic DNA was extracted using MoBio UltraClean® kit for the liquid or PowerSoil® kit (Carlsbad, CA, US) for sediment samples according to the manufacturer's protocol. Pyrosequencing of the 16S rDNA region was performed with primers for bacterial 16S and for archaeal 16S according to Nossa et al. (2010). All 16S rDNA pyrosequencing reads were analyzed using the original standard flowgram format (SFF) output file from the sequencer in Mothur, version 1.32.1 following the 454 standard operating process (Schloss et al., 2009). Sequence alignment was performed in Mothur using SILVA database (release 111) clustered at 97% similarity as reference.

RESULTS AND DISCUSSION

Among the biomarkers tested, Escherichia was only detected in the pen floor sediment (Table 1). Thus, the usefulness of Escherichia as biomarker for water contamination by swine wastewater may be limited to infer on recent outbreaks on fecal contamination by fresh, untreated swine wastewaters. The relative abundance of bacterial biomarkers was highest at the pen floor (Table 1) because of its direct exposure to fecal contamination. Salmonella spp., which is commonly reported in swine wastewaters (Jeong et al., 2011; Viancelli et al., 2013), was not detected in any of the analyzed samples.

Due to tropical conditions the anaerobic biodigesters from both WWTPs have been operating without a heating source to stimulate thermophilic conditions. Under mesophilic conditions, these anaerobic biodigesters may not remove pathogens effectively (Viancelli et al., 2013). This could explain why bacteria pathogens were not completely removed during the anaerobic digestion processes in both WWTPs (Table 1). Bacteroidetes and Prevotella, were detected in higher abundances in effluent samples from Bio1 and Bio2 (at WWTP1) and UASB (at WWTP2) (Table 1). The relative abundance of putative pathogens was highest in the UASB effluent sample as compared to the anaerobic covered lagoons (i.e., Bio1 and Bio2). This could be related to UASB lower hydraulic retention times (HRT of 1 to 2 days) as compared to the anaerobic covered lagoons (30 – 35 days) which ultimately affects pathogens removal efficacy. The survival of bacteria pathogens throughout wastewater treatment systems is recognized, and their proliferation can be adversely exacerbated by the anaerobic system configuration and operational conditions (Topp et al., 2009).

The relative abundance of biomarkers in the final effluent from WWTP1 and WWTP2 were < 0.1% and 0.7%, respectively (Table 1). The natural attenuation lagoon at WWTP1 served not only as a polishing step to remove nutrients (N and P) (Table 1) but

also to effectively reduce pathogens. The mechanism of pathogen removal in natural attenuation lagoons was likely associated to effluent exposure to sunlight as natural source of bacterial inactivation by UV light (Vanotti et al., 2005). Regarding to pathogens reduction at WWTP2, it is plausible to assume that nitrification/ denitrification processes followed by the phosphorus precipitation step which requires high pH (pH of 9; Vanotti et al., 2005) were the main mechanisms of bacteria inactivation. Despite of the observed decrease in biomarkers relative abundance throughout the treatment processes, however, a final disinfection/ filtration step is still desirable to minimize or even eliminate concerns with infectious diseases due to proliferation of unwanted waterborne bacterial pathogens. This is particularly necessary when the treated effluent is considered for reuse (Viancelli et al., 2013) and needs to be handled to wash the pens.

CONCLUSIONS

Among the biomarkers tested to infer on putative pathogens (i.e., Escherichia, Lactobacillus, Bacteroides and/ or Prevotella), Bacteroides and Prevotella were less sensitive to secondary and tertiary treatment processes which validates its usefulness as indicators to better predict potential environmental contamination by swine wastewaters. Satisfactory pathogen reduction was attained after tertiary open pond natural attenuation lagoon treatment but not after the air sparged nitrification/ denitrification tank followed by alkaline phosphorus precipitation treatment. Thus, to minimize or even eliminate concerns on waterborne water pathogens that can have adverse implications on water reuse biosafety a final disinfection treatment process is desirable.

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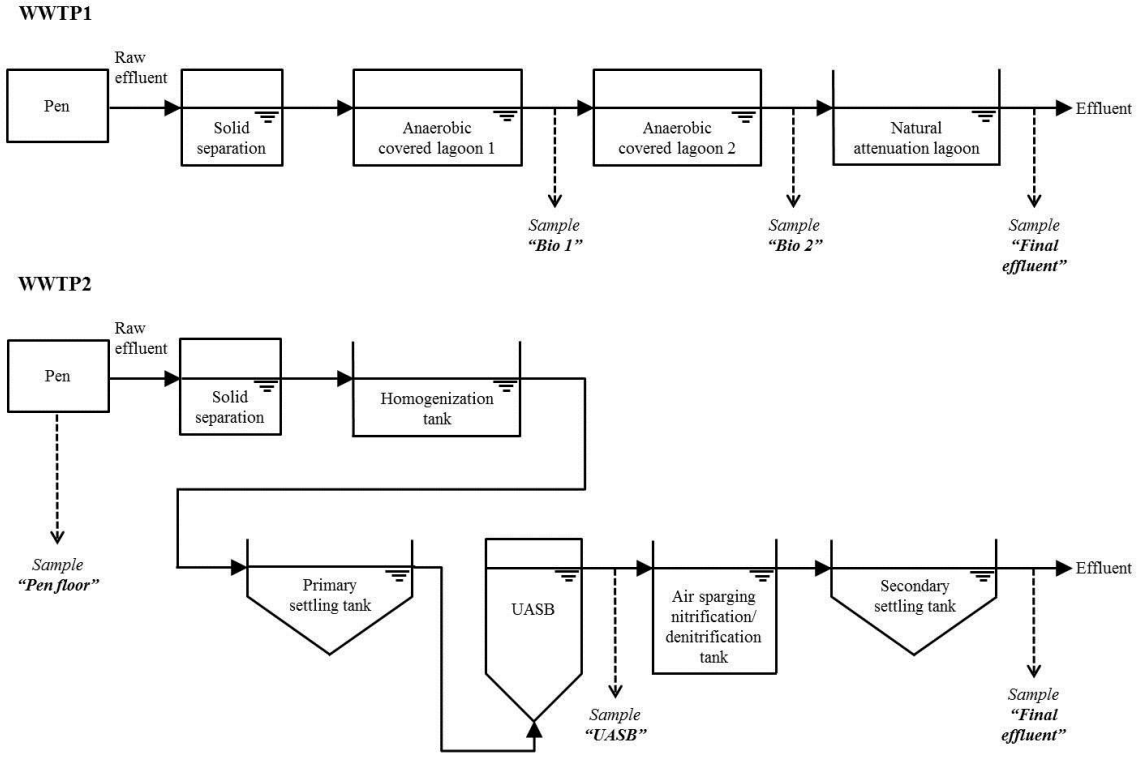

Figure 1. Schematic of the two independent WWTPs configurations indicating the sampling locations.

Table 1. 16S rDNA gene sequence reads obtained for selected bacterial genera used as biomarkers to infer on putative pathogen removal throughout the WWTPs. Data shown as percentage (%) of 83,333 16S rDNA total sequence reads.

BIOMARKERS	WWTP 1			WWTP 2		
	Bio1	Bio2	Final effluent	Pen floor	UASB	Final effluent
Prevotella	<0.1	0.2	<0.1	0.3	6.3	<0.1
Bacteroides	0.2	0.1	<0.1	1.5	2.9	0.7
Escherichia	<0.1	<0.1	<0.1	5.8	<0.1	<0.1
Lactobacillus	0.1	<0.1	<0.1	4.4	0.9	<0.1