Environ Sci Pollut Res
DOI 10.1007/s11356-017-0078-z

RESEARCH ARTICLE

Prokaryotic community profiling of local algae wastewaters using advanced 16S rRNA gene sequencing

Alya Limayem[1,2,3] · **Andrew Micciche**[4] · **Bina Nayak**[5] · **Shyam Mohapatra**[2,3]

Received: 6 December 2016 / Accepted: 3 September 2017

Abstract Algae biomass-fed wastewaters are a promising source of lipid and bioenergy manufacture, revealing substantial end-product investment returns. However, wastewaters would contain lytic pathogens carrying drug resistance detrimental to algae yield and environmental safety. This study was conducted to simultaneously decipher through high-throughput advanced Illumina 16S ribosomal RNA (rRNA) gene sequencing, the cultivable and uncultivable bacterial community profile found in a single sample that was directly recovered from the local wastewater systems. Samples were collected from two previously documented sources including anaerobically digested (AD) municipal wastewater and swine wastewater with algae namely *Chlorella* spp. in addition to control samples, swine wastewater, and municipal wastewater without algae. Results indicated the presence of a significant level of *Bacteria* in all samples with an average of approximately 95.49% followed by *Archaea* 2.34%, in local wastewaters designed for algae cultivation. Taxonomic genus identification indicated the presence of *Calothrix*, *Pseudomonas*, and *Clostridium* as the most prevalent strains in both local municipal and swine wastewater samples containing algae with an average of 17.37, 12.19, and 7.84%, respectively. Interestingly, swine wastewater without algae displayed the lowest level of *Pseudomonas* strains < 0.1%. The abundance of some *Pseudomonas* species in wastewaters containing algae indicates potential coexistence between these strains and algae microenvironment, suggesting further investigations. This finding was particularly relevant for the earlier documented adverse effects of some nosocomial *Pseudomonas* strains on algae growth and their multidrug resistance potential, requiring the development of targeted bioremediation with regard to the beneficial flora.

Keywords High-throughput Illumina16S rRNA gene amplicon sequencing · Algae wastewaters · Lytic bacteria · Drug resistance · *Pseudomonas* · Bioinformatics · Profiling · Taxonomy

Responsible editor: Gerald Thouand

✉ Alya Limayem
alimayem@usf.edu

[1] Department of Graduate Studies and Pharmaceutical Sciences, University of South Florida, 4202 East Fowler Avenue, Tampa, FL 33620, USA

[2] Division of Translational Medicine, Center for Education in Nanobioengineering, University of South Florida, 4202 East Fowler Avenue, Tampa, FL, USA

[3] College of Pharmacy, University of South Florida, 4202 East Fowler Avenue, Tampa, FL, USA

[4] Department of Cell Biology, Microbiology and Molecular Biology, University of South Florida, 4202 East Fowler Avenue, Tampa, FL, USA

[5] Pinellas County Utilities, Water Quality Division, Largo, FL, USA

Introduction

Increasing concerns towards petroleum-based gasoline shortage and energy security with potential consequences on climate change have spurred researchers' interest in renewable bioenergy including algae biomass, which acts as a CO_2 sink (Aristidou & Penttilä, 2000; Von Sivers and Zacchi 1996; Goldemberg 2007). Currently, algal-derived biofuel is regaining interest from researchers as a practical source of sustainable energy primarily for biodiesel and fuel for aviation industries (Christi, 2007; Mata et al, 2010; Parmar et al. 2011). Life cycle assessment studies have recently demonstrated that algae bioenergy production is not sustainable unless wastewaters can be used as an

Published online: 23 October 2017

effective source of nutrients and water for algae cultivation, revealing a low-cost investment (Congressional Research Service (CRS); 2013, Davis et al, 2012; Lee et al. 2015; Woertz et al. 2009; Davis et al. 2014; Mandal and Mallick 2011).

Although some sources of wastewaters may convey a practical method of introducing vital nutrients to algae systems (Mulbry & Wilkie, 2001; Mulbry et al, 2008), they can also act as a delivery system for a substantial level of microorganisms that range from symbiotic to antagonist including some lytic pathogens (Unnithan et al. 2014; Wang et al. 2013). While synergism of some species among these microorganisms is useful to ecological balance and algae cultivation (Ferrel & Sarisky-Reed, 2010; United States Department of Agriculture, 2010), there is a substantial number of nosocomial pathogens carrying drug resistance that have been detected in wastewaters, requiring additional vigilance from algae cultivators (Slekovec et al. 2012). Aside from drug resistance, some strains from *Micrococcus*, *Pseudomonas*, and *Bacillus* genera have been reported as lytic microorganisms (Kim et al. 2007; Wang et al. 2013; Limayem et al. 2016), despite beneficial effects of some *Pseudomonas* species in providing algae with CO_2 (Praveen and Loh 2015). Additional controversy revealed that some nosocomial *Pseudomonas* strains with potential lytic effects on algae are found abundantly in wastewaters (Magalhães et al. 2016). These strains also have the ability to impede algae growth through secreting proteins, causing inhibitive effects on microalgae, *Chlorella vulgaris*, in addition to their multidrug resistance potential (Zhou et al. 2011; Wang et al, 2012; Wang et al. 2010a), thus requiring greater investigation of algae-wastewater bacterial profiling for the development of an optimal targeted nanoremediation. The available studies have evidenced numerous sources of microbes including *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* in wastewaters for algae cultivation (Su et al. 2011; Wang et al. 2010b; Wang et al. 2013; Day et al. 2012; Limayem et al. 2016; Pandey and Soupir 2011), suggesting an accurate bacterial screening of algae wastewater in each specific source in an attempt to design a specific treatment against resistant pathogens. Despite of some chemical wastewater treatments in addition to aerobic and anaerobic digestion (Shi et al. 2016; Pandey et al. 2015; Yan et al. 2016a, b), there has been identification of a considerable number of pathogens in wastewaters. Therefore, there is exigency to generate an accurate identification of bacterial community directly recovered from algae wastewaters. The objective of this study is to identify the prokaryotic community profiling and interaction in the available wastewater systems designed for algae cultivation to intervention. To this aim, samples were collected from existing wastewater bioreactors as a mean to characterize the microbial community structure differences in wastewater with and without algae. This effort was conducted to obtain a one-time snapshot of the bacterial community through a high-throughput Illumina gene sequencing with the understanding that even with identical temperature and sources, these systems could have varying community structures. Particular focus of this investigation was to elucidate the presence of specific pathogens namely nosocomial *Pseudomonas* spp., previously documented for both of their lytic effects on algae and unsafe multidrug resistance potential, requiring a targeted nanoremediation that should keep the ecological balance intact.

Materials and methods

Sample collection and preparation Wastewater samples with and without algae were kindly donated to our labs from the Environmental Engineering Building at the University of South Florida. Samples were collected in 25-mL centrifuge tubes including the anaerobically digested (AD) municipal wastewater and swine wastewater with algae namely *Chlorella* spp. in addition to control samples including swine wastewater and municipal wastewater without algae. Samples with algae (having an approximate concentration of 1400 mg/1 L batch) from a semi-continuously fed bioreactor were described extensively by Wang et al. (2016) and Arashiro et al. (2017). The same biomass was maintained for a single snapshot of bacterial community with and without *Chlorella* spp. The algal growth conditions were performed under natural illumination and a controlled temperature within a range of 25–32 °C along with a mixture of 2% CO_2/air (gas flow rate 0.5 L min^{-1}). The extracted DNA were quantified using QuantiFlour ds DNA System (Promega, Madison, WI) and sent for sequencing prior to 16S ribosomal RNA (rRNA) analysis. The purity and yield of the extracted DNA were checked using the ND-1000 NanoDrop spectrophotometer (Fisher Scientific, Pittsburgh, PA). Primers were selected to target the V3 and V4 regions of the 16S rRNA genes.

F primer: 5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG.

R primer: 5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC.

The primers incorporate Illumina adapter sequences, the 16S-specific sequence portion of primers adopted from Klindworth et al. (2013). PCR conditions for the 16S rRNA amplification are as follows: an initial denaturation of 95 °C for 3 min followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s and a final extension at 72 °C for 5 min.

High-throughput 16S rRNA sequencing

Overview

The genomes were sequenced on the Illumina MiSeq using 600 cycle V3 standard flow cell producing approximately

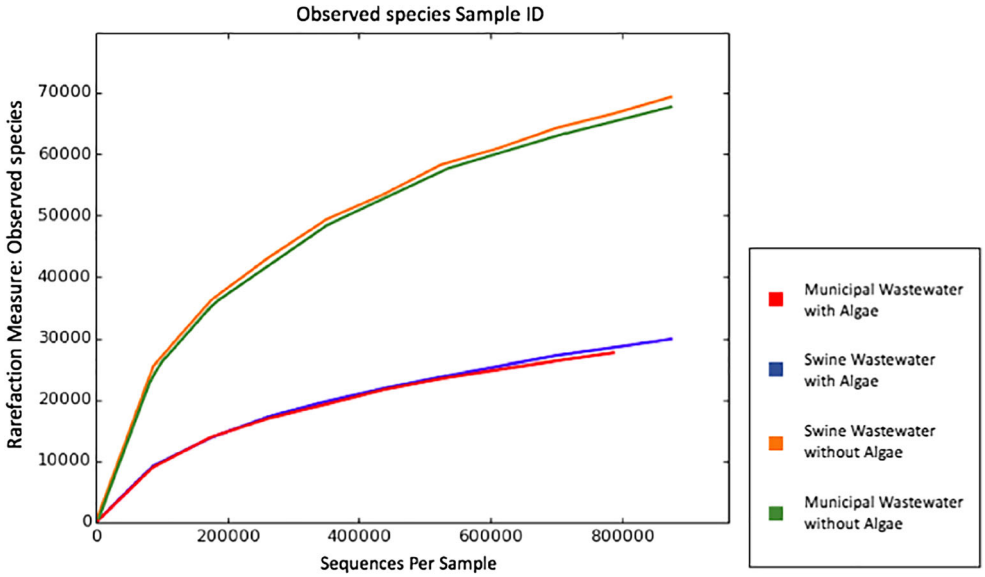

Fig. 1 Alpha refraction curve, observed OTUS

100,000 paired-end 2 × 300 base reads (Omega Bioservices, Norcross, GA). Result analyses were performed via Illumina's BaseSpace 16S rRNA application module Illumina-curated version of May 2013 Greengenes taxonomic database in parallel with the Ribosomal Database Project (RDP) for taxonomic classification.

Data analyses and classifications

Data analysis was performed by Trimmomatic trimming tool for paired-end sequencing data and quality control. Sequence reads that were less than an average quality of 25 in a 4-bp sliding window were truncated based on the Phred algorithm. Data formats and statistics of all the samples are described in Tables 6 and 7. Additionally, image data generated by Illumina MiSeq were transferred into raw reads through base calling software. It was therefore stored in fastq format including both biological sequences and their related quality scores.

The internal transcribed spacer (ITS) analysis was performed by QIIME pipeline. Sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity cutoff, and the relative abundance was calculated for OTUs in each sample. The sequence data were then classified by a native Bayesian classifier (Wang et al. 2007). The latter was originally designed for the RDP database; it was also adopted by Illumina in conjunction with the Greengenes database for taxonomic classification. The OTU sequences were then aligned for the Silva database to create a phylogenetic tree and an OTU table (Yilmaz et al. 2013), representing the abundance of OTU in each microbial sample. In addition to mapping, the community diversity (alpha diversity) was computed to generate rarefaction curves based on reference database Sliva119 (graphs of diversity vs. sequencing depth; Fig. 1; Quast et al. 2012). Ultimately, the estimation of the microbial communities' relationship was calculated to generate principal coordinate analysis (PCoA) plots and distance histograms representing a mathematic evaluation of the correlation among

Table 1 Kingdom-level identification of algae samples

Classification	Number of reads			
	Swine wastewater w/o algae	Swine wastewater w/ algae	Municipal wastewater w/o algae	Municipal wastewater w/ algae
Bacteria	1,481,600 (95.49%)	1,022,405 (97.94%)	1,378,243 (98.60%)	980,335 (98.12%)
Archaea	36,331 (2.34%)	15 (0%)	15 (0%)	15 (0%)
Unclassified at kingdom level	33,556 (2.16%)	21,522 (2.06%)	(2.23%)	18,809 (1.88%)
Viruses	8 (0%)	5 (0%)	9 (0%)	6 (0%)

Of the 1,551,495 reads of the Swine wastewater w/o algae, 95.49% was identified as bacteria, whereas 2.34% were identified as archaea, while only 8 reads were identified as viruses. Of the 1,043,947 reads of the Swine wastewater w/o algae, 97.94% was identified as bacteria, whereas only 15 reads were identified as archaea, while only 5 reads were identified as viruses

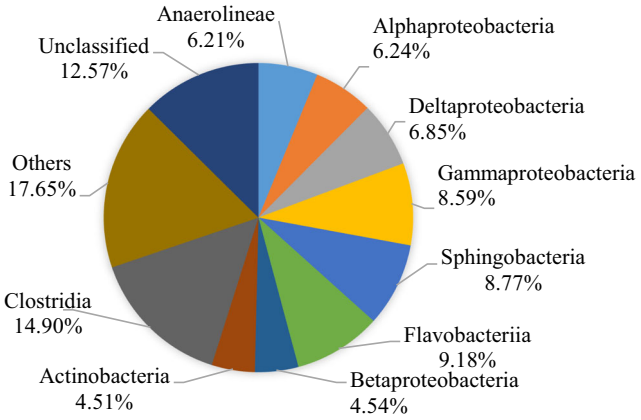

Fig. 2 Top 10 classes of bacteria found in local swine and municipal wastewaters with and without algae

the microbial communities (White et al. 1990). Below are the sequences of ITS-specific primers used for the Illumina high-throughput 16S rRNA sequencing:

ITS1 (White et al. 1990) 5′-TCCGTAGGTGAACC TGCGG-3′.

ITS4 (White et al. 1990) 5′-TCCTCCGCTTATTG ATATGC-3′.

Results and discussion

In all samples, as detailed in Table 1, swine wastewater without algae, swine wastewater with algae, and municipal wastewater with and without algae, bacteria represent more than 95% of the total reads (95.49, 97.94, 98.12, 98.60%, respectively). Archaea composed of 2.34% of the reads in the swine wastewater. An average of 2% of the reads was unclassified at the kingdom level (2.16, 2.06, 1.88, 2.33%). Viruses composed of less than 0.01% of reads. These results are typical of sequencing efforts using primers targeting the 16S rRNA gene amplicon. A steep portion of the rarefaction curve indicates that the sequencing volume is insufficient and the flat portion represents sufficient sequencing (Fig. 1).

Table 1 details the domain and kingdom level of the 16S rRNA analysis. Bacteria were predominantly identified, while Archaea were noted in the swine sample without algae but were noted only in minuscule amounts in samples with algae. Figure 2 details the top class identification indicating a wide diversity within each sample including *Clostridia*, *Alphaproteobacteria*, and *Sphingobacteria*, along with *Gammaproteobacteria*, which is the class encompassing *Pseudomonas* and particularly lytic *Pseudomonas* spp.

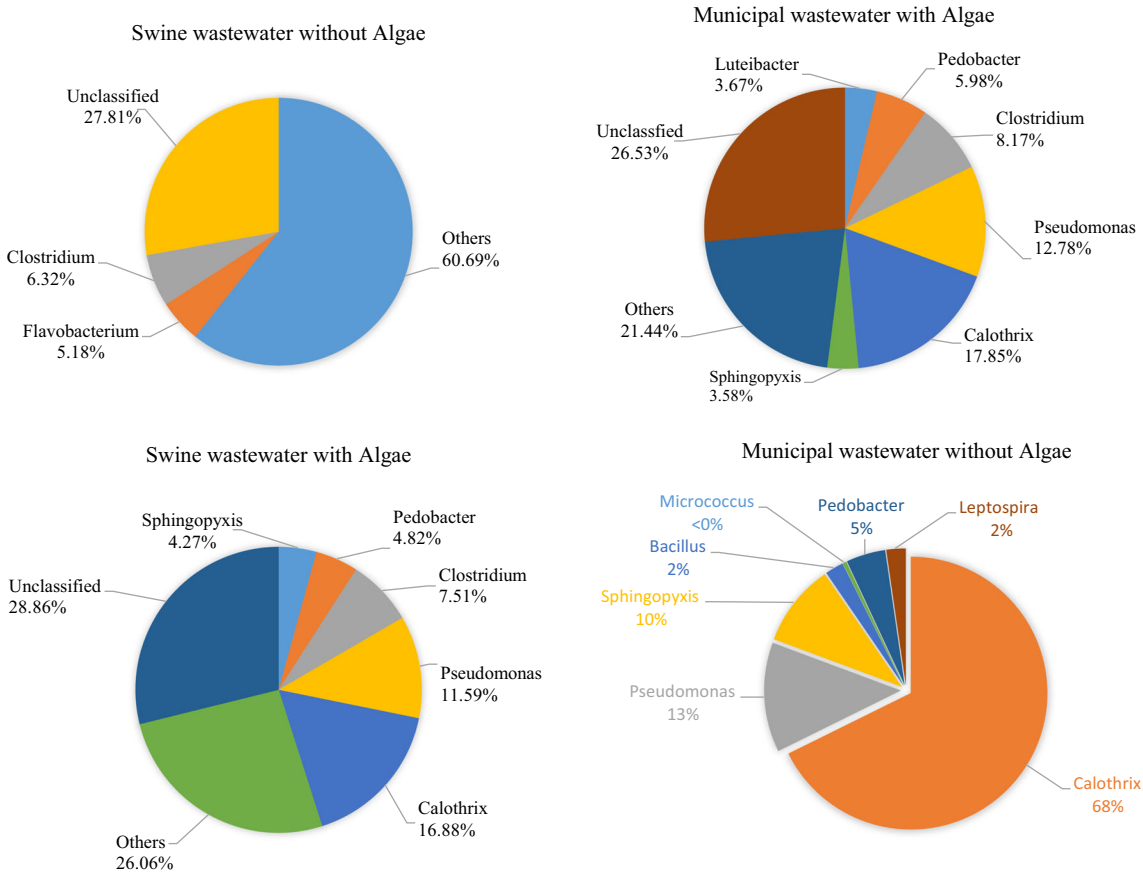

Fig. 3 Top 10 genera of bacteria identified in each type of sample: swine wastewater without algae, swine wastewater with algae, municipal wastewater with algae, and municipal wastewater without algae

Table 2 The most prevalent bacterial species in swine wastewater without algae samples

Genus	Species classification
Longilinea spp.	*L. arvoryzae* 1.93%
Methanosaeta spp.	*M. concilii* 1.88%
Bellilinea spp.	*B. caldifistulae* 1.79%
Pedobacter spp.	*P. kwangyangensis* 1.36%
Desulfonauticus autotrophicus spp.	*D. autotrophicus* 1.35%
Clostridium spp.	*C. cadaveris* 1.30%
Syntrophomonas spp.	*S. cellicola* 0.96%

The 16S rRNA identification of the swine wastewater without algae samples was unable to classify 920,223 (59.31%) of reads to the species level. *Longilinea arvoryzae* represented 1.93% of reads followed by *Methanosaeta concilii* (1.88%), *Bellilinea caldifistulae* (1.79%), *Pedobacter kwangyangensis* (1.36%), *Desulfonauticus autotrophicus* (1.35%), and *Clostridium cadaveris* (1.30%). *Syntrophomonas cellicola* represented less than 1% of the total number of reads (0.96%)

Genus identification, of which the largest is detailed in Fig. 3, indicates that *Pseudomonas*, *Calothrix*, cyanobacteria, and *Sphingopyxis*, a gram-negative Alphaproteobacteria, considered a top identified genus only in the swine wastewater with algae and municipal wastewater with algae samples. *Clostridium* species were identified among the top 7 genera in all four samples. *Pedobacter*, a known contaminant of soil and DNA kits and nuclease-free water, was also noted in all samples among the most prevalent genera (Salter et al. 2014). Across samples, *Escherichia*, *Yersinia*, *Streptococcus*, *Staphylococcus*, and *Enterococcus* were present but in low hits (< 100). Low presence of *Escherichia*, *Yersinia*, *Streptococcus*, *Staphylococcus*, and *Enterococcus* is most likely due to the treatment process on the wastewater prior to sampling. It is worth noting that only ~70% of the hits identified were done so at the genus level.

Table 3 The most prevalent bacterial species in swine wastewater with algae samples

Genus	Species classification
Calothrix spp.	*C. parietina* 16.88%
Pseudomonas spp.	*P. mendocina* 3.83% *P. xanthomarina* 1.06% *P. lundensis* 0.91%
Sphingopyxis spp.	*S. ginsengisoli* 3.42%
Pedobacter spp.	*P. africanus* 1.69%
Leptospira spp.	*L. meyeri* 1.17%

The 16S rRNA identification of the swine wastewater with algae samples. *Calothrix parietina* represented 16.88% of reads. Three of the top 8 identified species were from the genus *Pseudomonas*: *P. mendocina* (3.83%), *P. xanthomarina* (1.06%), and *P. lundensis* (0.91%). Also represented in the top 8 identified species was *Sphingopyxis ginsengisoli* (3.42%), *Pedobacter africanus* (1.69%), and *Leptospira meyeri* (1.17%)

Table 4 The most prevalent bacterial species in municipal wastewater without algae samples

Genus	Species classification
Calothrix spp.	*C. parietina* 18.64%
Pseudomonas spp.	*P. mendocina* 1.60% *P. xanthomarina* 0.92% *P. lundensis* 0.84% *P. aeruginosa* 0.22%
Sphingopyxis spp.	*S. ginsengisoli* 2.69%
Bacillus spp.	*B. pumilus* 0.34% *B. safensis* 0.26%
Micrococcus spp.	*M. luteus 0.12%*
Pedobacter spp.	*P. africanus* 1.28%
Leptospira spp.	*L. meyeri* 0.63%

The 16S rRNA identification of the municipal wastewater with algae samples. *Calothrix parietina* represented 18.64% of reads. Three of the top 8 identified species were from the genus *Pseudomonas*: *P. mendocina* (1.60%), *P. xanthomarina* (0.92%), *P. lundensis* (0.84%), and *P. aeruginosa* (0.22). Also represented in the top 8 identified species was *Sphingopyxis ginsengisoli* (2.69%), *Pedobacter africanus* (1.28%), and *Leptospira meyeri* (0.63%) with regard to *M. luteus* (0.12%) and *Bacillus pumilus* (0.34) and *B. Safensis* (0.26) species

The 16S rRNA identification of the swine wastewater without algae samples was as follows: *Longilinea arvoryzae* represented 1.93% of reads followed by *Methanosaeta concilii* (1.88%), *Bellilinea caldifistulae* (1.79%), *Pedobacter kwangyangensis* (1.36%), *Desulfonauticus autotrophicus* (1.35%), followed by *Clostridium cadaveris* (1.30%). *Syntrophomonas cellicola* represented less than 1% of the total number of reads (0.96%) (Table 2). For the swine wastewater with algae samples, three of the top 8 identified species were from the genus *Pseudomonas*: *P. mendocina* (3.83%),

Table 5 The most prevalent bacterial species in municipal wastewater with algae samples

Genus	Species classification
Calothrix spp.	*C. parietina* 17.84%
Pseudomonas spp.	*P. mendocina* 3.80% *P. xanthomarina* 1.29% *P. lundensis* 1.02%
Sphingopyxis spp.	*S. ginsengisoli* 2.87%
Pedobacter spp.	*P. africanus* 2.08%
Leptospira spp.	*L. meyeri* 0.95%

The 16S rRNA identification of the municipal wastewater with algae samples. *Calothrix parietina* represented 17.84% of reads. Three of the top 8 identified species were from the genus *Pseudomonas*: *P. mendocina* (3.80%), *P. xanthomarina* (1.29%), and *P. lundensis* (1.02%). Also represented in the top 8 identified species was *Sphingopyxis ginsengisoli* (2.87%), *Pedobacter africanus* (2.08%), and *Leptospira meyeri* (0.95%). All other 808 species identified represented the remaining 9.64% of total reads, and none exceeded 9541 reads

Table 6 The top 10 classes identified in each form of sample

Column number	Column name	Description
1	Sample	Sample name
2	Length	Average reads length
3	Reads	Number of reads
4	Bases	Number of bases
5	Q20 (%)	Percentage of sequences with < 1% sequence error
6	Q30 (%)	Percentage of sequences with < 0.1% sequence error
7	GC (%)	Percentage of base C+G content
8	*N* (ppm)	Percentage of undetermined bases per million bases

In the Swine wastewater without algae, nine classes exceeded 3.5%. Of the Swine wastewater w/o algae samples, 17% contained bacteria with less than a 3.5% abundance. *Clostridium* was present with over 3.5% abundance in all samples. Both samples with algae at the class level only varied by percentages of 1% and detailed the same classes of bacteria. The "Other" category in this pie chart is the sum of all classifications with less than 3.50% abundance

P. xanthomarina (1.06%), and *P. lundensis* (0.91%). Also represented in the top 8 identified species was *Sphingopyxis ginsengisoli* (3.42%), *Pedobacter africanus* (1.69%), and *Leptospira meyeri* (1.17%) (Table 3). However, the bacterial decipheration of the municipal wastewater with algae samples was as follows: *Calothrix parietina* represented 17.84% of reads. Three of the top 8 identified species were from the genus *Pseudomonas*: *P. mendocina* (3.80%), *P. xanthomarina* (1.29%), and *P. lundensis* (1.02%). Also represented in the top 8 identified species was *Sphingopyxis ginsengisoli* (2.87%), *Pedobacter africanus* (2.08%) followed by *Leptospira meyeri* (0.95%) and is shown in Tables 4 and 5.

At the species level, the number of reads identified in swine wastewater without algae was approximately two times higher than that of the reads identified in municipal and swine wastewaters with algae. Series of differences in bacterial makeup and numerous similarities between the algae samples give evidence for the concept that the addition of algae drastically changes the microbial environment. These similarities can be identified by comparing species data. The top 8 species identified are identical between the algae in municipal waste and in swine waste, with only minor variances in percent reads, but significantly different from the species identified in the swine wastewater samples without algae. This also holds true when viewing the top species identified at the genus level. These changes agree with our previous assertion that *Pseudomonas* spp. thrive in the presence of algae due to their high lytic activity. In addition to *Pseudomonas* spp., *C. parietina*, a blue-green algae, was found abundantly as the largest species identified, exceeding the number of reads of the top 7 identified species combined, in both types of samples with algae. Cyanobacteria have been implemented in algae bioreactors, and optimally grow in similar conditions as eukaryotic algae, but they may not produce the desired outputs of the bioreactor and instead utilize valuable nutrients designed for algal growth (Sheehan et al. 1998). As evidenced by the 16S rRNA analysis of the swine wastewater samples without algae, *C. parietina* can enter the bioreactors from this nutrient source in very low amounts. *C. parietina* has been reported to produce antimicrobial compounds which are more effective against gram-positive bacteria and some fungi (Issa 1999). Additionally, an inhibitory effect of the antibiotic produced

Table 7 The top 10 genera identified in each form of sample

Sample	Length	No. of reads	No. of bases	Q20 (%)	Q30 (%)	GC (%)	*N* (ppm)
Municipal wastewater with algae R1	300	999,165	299,818,095	98.60	64.56	54	209
Municipal wastewater with algae R2	299	999,165	299,071,257	88.10	2.33	54	985
Swine wastewater with algae R1	300	1,043,947	313,265,636	98.79	63.61	54	196
Swine wastewater with algae R2	299	1,043,947	312,478,938	88.25	3.35	55	961
Swine wastewater without algae R1	299	1,551,495	464,451,795	98.62	63.49	54	179
Swine wastewater without algae R2	299	1,551,495	464,117,293	88.56	4.70	54	938
Municipal wastewater without algae R1	299	1,100,120	300,615,220	98.20	64.222	54	212
Municipal wastewater without algae R2	300	1,100,120	301,130,402	98.60	3.33	54	980

In the Swine wastewater w/o algae, only two genera exceeded over a 3.5% abundance: *Flavobacterium* and *Clostridium*. Over 60% of the Swine wastewater w/o algae samples contained bacteria with less than a 3.5% abundance. *Clostridium* was present with over 3.5% abundance in all samples. *Sphingopyxis, Pedobacter, Calothrix,* and *Pseudomonas* were presented in abundance in the two algae samples, where the other category was in the 20%

by *C. parietina* on the growth rate and oxygen evolution of the green alga, *Chlorella fusca*, was observed. It is interesting to note that the seven most predominant species in both wastewater samples containing algae all belong to the gram-negative group. The pronounced inhibitory effect of *C. parietina* on gram-positive bacteria could potentially result in the proliferation of gram-negative bacteria thereby explaining their predominance in the samples. In the wastewater sample without algae, all except *C. cadaveris* belong to the gram-negative group (Tables 6 and 7).

Overall, the taxonomic genus decipheration indicated the presence of *Calothrix*, *Pseudomonas*, and *Clostridium* as the most abundant bacterial contaminants in both municipal and swine wastewater samples containing algae with an average of 17.37, 12.19, and 7.84%, respectively. Noticeably, swine wastewater without algae includes several bacterial genera except *Pseudomonas* strains < 0.1%. The existence of some *Pseudomonas* species in wastewaters containing algae indicates potential symbiosis between these strains and algae namely *C. vulgaris*. These results were particularly pertinent for the earlier documented lytic effects of some nosocomial *Pseudomonas* strains on wastewaters and their multidrug resistance potential, requiring further investigations on their interaction with algae for plausible development of targeted bioremediation inside bioreactors.

Conclusions

Several studies have reported a molecular screening of the bacterial consortia in algae-wastewater systems (Su et al. 2011; Kim et al. 2007; Wang et al. 2010a; Wang et al. 2013; Limayem et al. 2016). They also revealed the presence of microbial group demonstrating effects detrimental to algae growth. Aside from lytic bacteria, some studies revealed the existence of nosocomial drug resistance strains, namely *Pseudomonas* spp., in wastewaters that are also lytic to algae growth. However, there is a need to elucidate the microbial interaction with algae and the prevalence of pathogens in each specific microsystem in an effort to develop a targeted remedy. This research study revealed the presence of some strains of nosocomial *Pseudomonas*, emerging as an alternative fecal indicator and have been extensively found in wastewaters designed for algae-based products harboring high level of multidrug resistance beyond clinical settings. Although this study only scratched the surface of bacterial community structure profiles of wastewater systems with and without algae, further controlled experiments including the screening of nosocomial *Pseudomonas* strains for both of their lytic effects and drug resistance are warranted to ascertain that the observed bacterial community requires a targeted nanoremediation to keep beneficial synergism and the ecological balance safe.

Acknowledgements We thank the Office of Research and Innovations at the University of South Florida for their financial support through their interdisciplinary grant for the Department of Cell Biology, Microbiology, and Molecular Biology (CMMB) along with the College of Pharmacy and Internal Medicine. We also thank the Department of Environmental Engineering at the University of South Florida for providing us with algae-wastewater samples.

References

Arashiro LT, Rada-Ariza AM, Wang M, van der Steen P, Ergas SJ (2017) Modelling shortcut nitrogen removal from wastewater using an algal-bacterial consortium. Technology, Water Science & **in press**

Aristidou A, Penttilä M (2000) Metabolic engineering applications to renewable resource utilization. Curr Opin Biotechnol 11:187–198

Christi Y (2007) Biodiesel from microalgae. Biotechnol Adv 25:294–396

Congressional Research Service (CRS). Agricultural-based biofuels: overview and emerging issues. (2013) https://www.fas.org/sgp/crs/misc/R41282.pdf

Davis R, Fishman D, Frank ED, Wigmosta MS, Aden A, Coleman AM, Pienkos PT, Skaggs RJ, Venteris ER, Wang MQ (2012) Renewable diesel from algal lipids: an integrated baseline for cost, emissions, and resource potential from a harmonized model. ANL

Davis RE, Fishman DB, Frank ED, Johnson MC, Jones SB, Kinchin CM, Skaggs RL, Venteris ER, Wigmosta MS (2014) Integrated evaluation of cost, emissions, and resource potential for algal biofuels at the national scale. Environ Sci Technol 48(10):6035–6042

Day J, Thomas M, Achilles-Day UE, Leakey RJ (2012) Early detection of protozoan grazers in algal biofuel cultures. Bioresour Technol 114: 715–719

Ferrel J, Sarisky-Reed V (2010) National algal biofuels technology roadmap. U.S. Department of Energy (DOE). Office of Energy and Renewable Energy. OBP

Goldemberg J (2007) Ethanol for a sustainable energy future. Science 315(5813):808–810

Issa A (1999) Antibiotic production by the cyanobacteria *Oscillatoria angustissima* and *Calothrix parietina*. Environ Toxicol Pharmacol 8(1):33–37

Kim JD, Kim B, Lee CG (2007) Alga-lytic activity of *Pseudomonas fluorescens* against the red tide causing marine alga *Heterisugma akashiwo*. Biol Control 41(3):296–303

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res 41(1):e1

Lee E, Jalalizadeh M, Zhang Q (2015) Growth kinetic models for microalgae cultivation: a review. Algal Res 12:497–512

Limayem A, Gonzalez F, Micciche A, Haller E, Nayak B, Mohapatra SS (2016) Molecular identification and nanoremediation of microbial contaminants in algal systems using untreated wastewater. J Environ Sci Health Part B 51(12):868–872

Magalhães MJTL, Pontes G, Serra PT, Balieiro A, Castro D, Pieri FA, Crainey JL, Nogueira PA, Orlandi PP (2016) Multidrug resistant *Pseudomonas aeruginosa* survey in a stream receiving effluents from ineffective wastewater hospital plants. BMC Microbiol 16(1):193–200

Mandal S, Mallick N (2011) Waste utilization and biodiesel production by the green microalga *Scenedesmus obliquus*. Appl Environ Microbiol 77(1):374–377

Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: a review. Renew Sustainable Energy Rev 14(1):217–232

Mulbry WW, Wilkie AC (2001) Growth of benthic freshwater algae on dairy manures. J Appl Phycol 13(4):301–306

Mulbry W, Kondrad S, Buyer J (2008) Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. J Appl Phycol 20(6):1079–1085

Pandey PK, Soupir ML (2011) *Escherichia coli* inactivation kinetics in anaerobic digestion of dairy manure under moderate, mesophilic and thermophilic temperatures. AMB Express 1:18

Pandey PK, Biswas S, Vaddella VK, Soupir ML (2015) *Escherichia coli* persistence kinetics in dairy manure at moderate, mesophilic, and thermophilic temperatures under aerobic and anaerobic environments. Bioprocess Biosyst Eng 38(3):457–467

Parmar A, Singh NK, Pandey A, Gnansounou E, Madamwar D (2011) Cyanobacteria and microalgae: a positive prospect for biofuels. Bioresour Technol 102(22):10163–10172

Praveen P, Loh KC (2015) Photosynthetic aeration in biological wastewater treatment using immobilized microalgae-bacteria symbiosis. Appl Microbiol Biotechnol 99(23):10345–10354

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, ... & Glöckner FO (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, gks1219

Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Turner P, Parkhill J, Loman NJ, Walker AW (2014) Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol 12(1):87

Sheehan J, Dunahay T, Benemann J, Roessler P (1998) A look back at the U.S. Department of Energy's aquatic species program: biodiesel from algae. NREL, Golden, Colorado. NREL/TP-580-24190

Shi J, Pandey PK, Franz AK, Deng H, Jeannotte R (2016) *Chlorella vulgaris* production enhancement with supplementation of synthetic medium in dairy manure wastewater. AMB Express 6(1):15

Slekovec C, Plantin J, Cholley P, Thouverez M, Talon D, Bertrand X, Hocquet D (2012) Tracking down antibiotic-resistant *Pseudomonas aeruginosa* isolates in a wastewater network. PLoS One 7(12): E49300

Su Y, Mennerich A, Urban B (2011) Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal–bacterial culture. Water Res 45(11):3351–3358

United States Department of Agriculture (2010) A USDA regional roadmap to meeting the biofuels goals of the renewable fuels standard by 2022

Unnithan VV, Unc A, Smith GB (2014) Mini-review: a priori considerations for bacteria–algae interactions in algal biofuel systems receiving municipal wastewaters. Algal Res 4:35–40

Von Sivers M, Zacchi G (1996) Ethanol from lignocellulosics: a review of the economy. Bioresour Technol 56(2):131–140

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73(16):5261–5267

Wang L, Min M, Li Y, Chen P, Chen Y, Liu Y, & Ruan R (2010a). Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant. Applied biochemistry and biotechnology, 162(4):1174–1186.

Wang H, Liu L, Liu ZP, Qin S (2010b) Investigations of the characteristics and mode of action of an algalytic bacterium isolated from Tai Lake. J J Appl Phycol 22(4):473–478

Wang B, Yang X, Lu J, Zhou Y, Su J, Tian Y, Zhang J, Wang G, Zheng T (2012) A marine bacterium producing protein with algicidal activity against *Alexandrium tamarense*. Harmful Algae 13:83–88

Wang H, Zhang W, Chen L, Wang J, Liu T (2013) The contamination and control of biological pollutants in mass cultivation of microalgae. Bioresour Technol 128:745–750

Wang M, Lee E, Zhang Q, Ergas SJ (2016) Anaerobic co-digestion of swine manure and microalgae *Chlorella* sp.: experimental studies and energy analysis. BioEnergy Research 9(4):1204–1215

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18(1):315–322

Woertz I, Feffer A, Lundquist T, Nelson Y (2009) Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. J Environ Eng 135(11): 1115–1122

Yan C, Muñoz R, Zhu L, Wang Y (2016a) The effects of various LED (light emitting diode) lighting strategies on simultaneous biogas upgrading and biogas slurry nutrient reduction by using of microalgae *Chlorella sp*. Energy 106:554–561

Yan C, Zhu L, Wang Y (2016b) Photosynthetic CO2 uptake by microalgae for biogas upgrading and simultaneously biogas slurry decontamination by using of microalgae photobioreactor under various light wavelengths, light intensities, and photoperiods. Appl Energy 178:9–18

Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, ... & Glöckner FO (2013) The SILVA and "all-species living tree project (LTP)" taxonomic frameworks. Nucleic Acids Res, gkt1209

Zhou W, Qiao X, Sun J, Xing K, Tang X (2011) Ecological effect of z-qs01 strain on Chlorella vulgaris and its response to UV-B radiation stress. Procedia Environ Sci 11:741–748