Science of the Total Environment 648 (2019) 572–581

Contents lists available at ScienceDirect

Science of the Total Environment

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

Metagenomic assessment of the microbial community and methanogenic pathways in biosolids from a municipal wastewater treatment plant in Medellín, Colombia

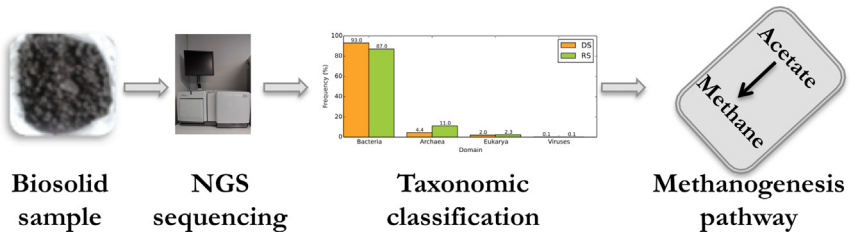
Katherine Bedoya [a,b], Oscar Coltell-Simon [c,d], Felipe Cabarcas [b,e], Juan F. Alzate [a,b,*]

[a] Grupo de Parasitología, Facultad de Medicina, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín, Colombia
[b] Centro Nacional de Secuenciación Genómica-CNSG, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín, Colombia
[c] Department of Computer Languages and Systems, School of Technology and Experimental Sciences, Universitat Jaume I, Castellón, Spain
[d] CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Madrid, Spain
[e] Grupo SISTEMIC, Ingeniería Electrónica, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín, Colombia

HIGHLIGHTS

- >800 bacterial OTUs within the biosolids of WWTP San Fernando -Colombia
- *Pseudomonas* and *Coprothermobacter* dominance alternates between dry and rainy season
- Half metagenome DNA sequences cannot be classified using alignment-based methods
- Methanogenesis in WWTP San Fernando-Colombia is performed mainly by *Methanosaeta*

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 March 2018
Received in revised form 8 August 2018
Accepted 8 August 2018
Available online 11 August 2018

Editor: Frederic Coulon

Keywords:
Biosolid
Wastewater
Metagenomics
Methanogenic pathway
Microbiota

ABSTRACT

Abundance and diversity of microbial communities in biosolids are variable and poorly studied in the tropics, and it is known that rainfall is one of the events that could affect the phylogenetic and functional microbial structure. In the present study, using NGS technics, we studied the microbial diversity as well as the methanogenesis pathway in one of the largest WWTP in Colombia. Besides, we sampled and analyzed biosolids from rainy season and dry season.

Phylogenetic classification showed a predominance of bacteria in both samples and difference in the dominant groups depending on the rainfall season. Whereas *Pseudomonas* was the dominant bacteria in the dry season, *Coprothermobacter* was in the rainy season. Archaea abundance was higher in the rainy season (11.5%) doubling dry season proportion. The bioreactor biogas production and total solids content showed similar results between rainy and dry season at the sampling dates. The most abundant Archaea related with methanogenesis was *Methanosaeta*, which is a methanogenic microorganism that exclusively uses acetate to produce methane. Moreover, annotation of the methanogenic pathway in the metagenome showed abundance in genes encoding Acetyl-CoA synthetases (ACSS), an enzyme that catalyzes acetate activation. Our results suggest that the microbial diversity was stable among the two time points tested, rainy season and dry season; and, although there were changes in the microbial abundance of dominant bacterial species, anaerobic digester performance is not affected.

© 2018 Elsevier B.V. All rights reserved.

1. Introduction

Municipal wastewater treatment plants (WWTP) produce large amounts of sludge as a by-product, which can be recycled in land

* Corresponding author at: Grupo de Parasitología, Facultad de Medicina, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín, Colombia.
E-mail address: jfernando.alzate@udea.edu.co (J.F. Alzate).

https://doi.org/10.1016/j.scitotenv.2018.08.119
0048-9697/© 2018 Elsevier B.V. All rights reserved.

application after complementary stabilization (Kacprzak et al., 2017). Anaerobic digestion has been commonly used in municipal sewage sludge treatment because it removes organic pollutants, pathogens, and transforms organic matter in an organic residue while it produces methane (Coelho et al., 2011; Narihiro and Sekiguchi, 2007; Abdelgadir et al., 2014). This biological process is mediated by a heterogeneous microbial community, which accomplishes four main metabolic steps; hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Angelidaki and Batstone, 2011). Within the hydrolysis step, high molecular weight biomolecules such as lipids, polysaccharides, proteins and nucleic acids are metabolized into monomers, e.g., glucose, amino acids, and fatty acids. These are converted into short-chain fatty acids, alcohols, H_2, ammonia, and carbon dioxide by acidogenic bacteria in the second step (Abdelgadir et al., 2014). At the third stage, acetogenic bacteria such as *Syntropomonas*, *Syntrophobacter*, and *Cloacamonas* produces acetic acid and hydrogen that are subsequently used by methanogenic archaea for methane production at the last step (Supaphol et al., 2011; Appels et al., 2008; Ali Shah et al., 2014).

There are three methanogenic pathways: i) Acetoclastic or acetotrophic; performed by *Metanosarcinales*, were methyl groups are transferred to acetyl-CoA activated acetate molecules. ii) Hydrogenotrophic; CO_2 is reduced to methane using H_2 and formate as major electron donors; *Methanothermobacter* and *Methanosarcina* are key hydrogenotrophic genera. iii) Methylotrophic; the substrate is methanol, methylated amines, and methylated sulfides, and it is performed by the orders Methanosarcinales, Methanomassiliicoccales, and Methanobacteriales (Ali Shah et al., 2014; Guo et al., 2015).

Previous studies have shown changes in the microbial community structure of wastewater treatment plants due to environmental conditions such as seasons and rainfall (Appels et al., 2008; Ali Shah et al., 2014). Another important feature that will affect the microbial community structure and populations is the geographical localization of the WWTP (Zhang et al., 2012). Nevertheless, ecological studies have shown functionally redundant and resilient organisms in anaerobic reactors which generate stability in the bioreactor performance following disturbances such as feeding rate (Werner et al., 2011).

Despite the central role of microbial communities in anaerobic digestion in WWTP, fundamental aspects related to the ecology and taxonomy of the organisms that participate in this process are limited (Treu et al., 2016). Analysis of microbial communities in anaerobic reactors traditionally has been based on molecular tools such as denaturing gradient gel electrophoresis (DGGE), fluorescent in situ hybridization (FISH), and 16S rRNA clone libraries in bacterial plasmids (Ferrera and Sánchez, 2016). However, these approaches are not able to elucidate the whole complexity of the genetic and functional diversity in microbial structure (Ju et al., 2014). Notwithstanding high-throughput sequencing technologies offer an effective method to characterize the phylogenetic composition and metabolic profiling in environmental samples, few studies have been made in activated sludge and biosolid samples using this sequencing method (Zhang et al., 2012; Kröber et al., 2009; Rivière et al., 2009).

The microbial composition in biosolid and sewage sludge depends on several aspects such as infectious diseases prevalence in contributing population, geographical localization, the presence of hospitals and factories in the area and seasonal variations and few studies have been carried to characterize its microbiological load throughout the world (Zhang et al., 2017; Meerbergen et al., 2017; Shchegolkova et al., 2016; Ju et al., 2014; Sidhu and Toze, 2009).

This study aimed to characterize the microbial community in biosolids produced in a wastewater treatment plant from Colombia, the first study of this kind in tropical America. Furthermore, we analyze the biosolid microbial load at two time-points where low (dry season) or high (rainy season) rainfall precipitation was measured. To gain insights into the microbiota profile of this byproduct, a combination of metagenomics as well and 16S-amplicons metataxonomic approaches were performed. Additionally, genes related with methanogenesis pathway were annotated.

2. Materials and methods

2.1. Operational performance of the anaerobic digester

The temperature of the anaerobic digester was 35 ± 1 °C. Biogas production was 336.2 m^3/h in DS and 269 m^3/h in RS. Total solids measured in biosolids were 40.5% and 35.2%, and volatile solids measured were 34.5% and 40.6%, in DS and RS, respectively.

2.2. Sampling and DNA extraction

Two biosolid samples were collected from municipal Waste Water Treatment Plant (WWTP) San Fernando (Operated by the company EPM) in Itagüí, Colombia, one of them in the rainy season (9.1 mm/h precipitation, average maximum temperature 27.8 °C, average minimum temperature 17.1 °C, August 2013) and other in the dry season (1.9 mm/h precipitation, average maximum temperature 28 °C, average minimum temperature 17.4 °C, February 2012). This WWTP services a population of approximately 500.000 people and receives an influent flow of 1.8m^3/s of domestic, hospital and industrial wastewater. Organic pollutants are removed using an activated sludge system treatment and secondary settling tank. Primary sludge treatment consists of anaerobic mesophilic digestion followed by dewatering process (centrifugation). Dewatered biosolids (about 500 *g*) were collected and transferred to the laboratory in refrigeration. DNA extraction was performed using Powermax® Soil DNA Isolation Kit (MO BIO Laboratories) according to the manufacturer's instructions. DNA was quantified using Picogreen Fluorescent Assay and DNA quality was evaluated by gel electrophoresis (1% agarose).

2.3. DNA library construction and pyrosequencing

High-Throughput Pyrosequencing was conducted using Roche 454 FLX + platform (Roche, USA). DNA was prepared following the manufacturer's instructions for whole genome shotgun libraries. Briefly, DNA fragmentation was made by nebulization, different rapid library (RL) adapters were attached to the DNA fragments of each DNA sample (RS and DS) after fragment end repair protocol. Finally, emulsion-based clonal amplification (emPCR) was performed and beads-carrying the amplified fragments were enriched and quantified. Sequencing was performed in a 454 instrument at the Centro Nacional de Secuenciación Genómica-CNSG, Universidad de Antioquia, Medellín, Colombia. Two full PTPs were used for each library, RS or DS, in independent runs (a total of 4).

2.4. Bioinformatic analysis

Raw reads were filtered using an in-house script applied to remove low quality and short sequences. All filtered reads were assembled using Newbler Denovo Assembly software v2.9 with parameters of minimum overlap ml = 60 nucleotides and minimum percentage of identities mi = 95 (Margulies et al., 2006). Contigs were classified and annotated with Meta Genome Rapid Annotation using Subsystem Technology (MG-RAST) server v3. MG-RAST provides protein similarities analysis, including both functional and taxonomic classification by BLAST similarity search in databases such as M5nr (Glass and Meyer, 2011). Taxonomic assignment of unassembled sequences was made using Metagenome Analyzer (MEGAN) software v5.11.3 that bases its taxonomic classification on the NCBI taxonomy (Huson et al., 2011). The program uses lowest common ancestor (LCA) algorithm and takes the results of a BLAST comparison. Sequences were aligned using

Download English Version:

https://daneshyari.com/en/article/8858198

Download Persian Version:

https://daneshyari.com/article/8858198

Daneshyari.com