Science of the Total Environment 618 (2018) 165–173

Contents lists available at ScienceDirect

Science of the Total Environment

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

Exploring the links between groundwater quality and bacterial communities near oil and gas extraction activities

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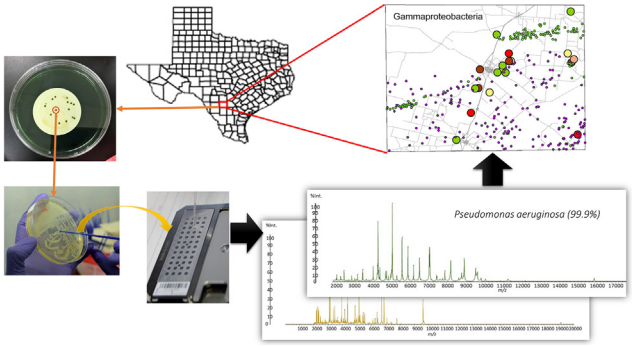
HIGHLIGHTS

- Stressful environments change bacterial communities.
- Groundwater samples located near agricultural and UD activities were collected.
- The bacteria present in contaminated groundwater were identified using MALDI-TOF MS.
- Mainly bacteria from the Phylum *Proteobacteria* were isolated.
- The bacterial communities varied significantly with the compositional differences.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 August 2017
Received in revised form 22 October 2017
Accepted 23 October 2017
Available online xxxx

Editor: D. Barcelo

Keywords:
MALDI-TOF MS
Unconventional drilling
Groundwater microbiome
Hydraulic fracturing
Biogeochemical cycles
Unconventional oil and gas

ABSTRACT

Bacterial communities in groundwater are very important as they maintain a balanced biogeochemical environment. When subjected to stressful environments, for example, due to anthropogenic contamination, bacterial communities and their dynamics change. Studying the responses of the groundwater microbiome in the face of environmental changes can add to our growing knowledge of microbial ecology, which can be utilized for the development of novel bioremediation strategies. High-throughput and simpler techniques that allow the real-time study of different microbiomes and their dynamics are necessary, especially when examining larger data sets. Matrix-assisted laser desorption-ionization (MALDI) time-of-flight mass spectrometry (TOF-MS) is a workhorse for the high-throughput identification of bacteria. In this work, groundwater samples were collected from a rural area in southern Texas, where agricultural activities and unconventional oil and gas development are the most prevalent anthropogenic activities. Bacterial communities were assessed using MALDI-TOF MS, with bacterial diversity and abundance being analyzed with the contexts of numerous organic and inorganic groundwater constituents. Mainly denitrifying and heterotrophic bacteria from the Phylum *Proteobacteria* were isolated. These microorganisms are able to either transform nitrate into gaseous forms of nitrogen or degrade organic compounds such as hydrocarbons. Overall, the bacterial communities varied significantly with respect to the compositional differences that were observed from the collected groundwater samples. Collectively, these data

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https://doi.org/10.1016/j.scitotenv.2017.10.264
0048-9697/

provide a baseline measurement of bacterial diversity in groundwater located near anthropogenic surface and subsurface activities.

1. Introduction

Microorganisms such as bacteria, viruses, and fungi are ubiquitous on earth (Horner-Devine et al., 2004). They are present in humans and animals, food, and the environment. These cells do not exist as individuals but interact and communicate with other cells and therefore act as a dynamically changing microbial community. Consequently, changes in their environment will eventually change their interactions and community (Blaser et al., 2016). According to Baas Becking's hypothesis, "everything is everywhere but the environment selects" (Fondi et al., 2016). Only specifically-adapted organisms will survive and proliferate in a particular environment. Therefore, understanding the factors that modulate diversity within a microbial ecosystem, such as physical (e.g. temperature) and chemical (e.g. nutrients) factors, is essential from a microbiological and ecological point of view (Blaser et al., 2016). Studying the responses of the microbiome to environmental changes can provide important knowledge for the development of new microbiological applications such as remediation of contaminated soil and water, and the search of novel biochemicals (Horner-Devine et al., 2004).

Several environmental studies have postulated that unconventional oil and gas development processes (UD), including hydraulic fracturing, may change the chemical composition of groundwater overlying hydrocarbon-rich petroliferous strata (Fontenot et al., 2013; Hildenbrand et al., 2017, 2016, 2015) and may affect the microbial communities that they support. Previous investigations have examined the impacts of hydraulic fracturing on surrounding environmental microbiomes in headwater stream ecosystems and surface waters (Fahrenfeld et al., 2016; Trexler et al., 2014). In both studies, the authors revealed that the microbial communities changed in response to altered conditions due to UD activities. In these works, DNA sequencing was used to characterize the water microbiome. High-throughput and simpler techniques that allow the real-time study of different microbiomes and their dynamics are necessary, especially when examining larger data sets and accounting for the costs and limited scope that are associated with more traditional techniques.

Mass spectrometry (MS), particularly matrix assisted laser desorption ionization - time of flight (MALDI-TOF), has already proved to be a workhorse for the identification of microorganisms (Basile and Mignon, 2016) and its application to environmental microbiology has increased significantly (Santos et al., 2016). MALDI-TOF MS allows the analysis of large biomolecules such as proteins by soft ionization meaning no fragmentation is induced. A matrix is overlaid on top of the sample to promote desorption and ionization of the analytes followed by acceleration in a vacuum through the application of an electric potential. The mass-to-charge ratio (m/z) will determine the time necessary to travel the flight tube and reach the detector (Dingle and Butler-Wu, 2013). A single colony can be used to obtain a protein profile that is unique for each microorganism thereby allowing its identification and/or differentiation (Freiwald and Sauer, 2009; Ghyselinck et al., 2011). In previous work, we demonstrated the ability of this technique to characterize the microbial ecology of groundwater located near UD activities. It was shown that the presence of high concentrations of hydrocarbon contaminants promotes the presence of pathogenic bacteria such as *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Stenotrophomonas maltophilia*, and *Bacillus cereus* (Martin et al., 2017; Santos et al., 2017).

In the work presented here, the connection between groundwater constituents and specific bacteria was evaluated using MALDI-TOF MS to further elucidate how environmental factors modulate the survival and proliferation of microorganisms in highly variable ecosystems. These data are some of the first to comprehensively characterize microbial communities in groundwater overlying oil and gas development, in a rural region engaged in various agricultural activities.

2. Materials and methods

2.1. Sample collection and analysis

Groundwater samples were collected from 19 water wells throughout Frio County, in southern Texas, overlying the Eagle Ford Shale. In situ measurements were performed using a YSI Professional Plus multiparametric probe and are presented in Table S1. To assess their degree of impairment, the chemical composition of groundwater samples was assessed by measuring volatile organic compounds (VOCs) using gas chromatography – mass spectrometry as described previously (Hildenbrand et al., 2016, 2015). Pertinent metal ions and anions were determined by inductively coupled plasma - mass spectrometry (ICP-MS) and ion chromatography (IC), as per EPA methods 200.7, 245.1, and 300A. Samples for the microbial analyses were collected in duplicate in 500 mL HDPE sterile sample bottles (Thermo Scientific™ Nalgene™) by filling bottles completely and leaving minimal headspace. Sterile deionized water was used as a transport blank to ensure no contamination, either chemical or biological, due to transportation of samples. Samples were stored on ice in coolers until they were processed.

2.2. Microbial analysis

Groundwater samples were filtered within 24 h of collection using the membrane filter technique. Volumes of 100 mL were filtered through sterile filter units coupled with sterile membrane filters of 0.22 μm pore size (EMD Millipore). All filtration was performed under aseptic techniques and in triplicate. Membranes were plated onto Nutrient agar (NA), m-Endo Agar LES, and Aeromonas Isolation Agar (Sigma-Aldrich, St. Louis, MO, USA). The spread plate technique was also performed and 0.1 mL of each water sample was spread in the agar plates. All plates were incubated at 25 °C for 24–48 h, except for the m-Endo agar media which was incubated at 37 °C. Bacteria quantification in water samples was performed according to Standard Methods for Water and Wastewater Analysis (APHA-AWWA-WPCF) (APHA et al., 1999) and results were expressed as colony forming units (CFU)/100 mL. The colonies were isolated in NA and incubated at 37 °C for 24 h. Pure cultures were preserved at −80 °C in Nutrient Broth (NB, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 15% (v/v) sterile glycerol (Amresco, Ohio, USA).

2.3. Microbial identification

2.3.1. MALDI-TOF MS

For the identification of microorganisms, two protein extraction methods were used according to the instrument manufacturer (Shimadzu Corporation, Kyoto, Japan): the Direct Smear *plus* Formic Acid method and the protein extraction method. The microbial colony or the protein extraction solution were placed on Fleximass™ DS disposable MALDI targets (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). A 40 mg/mL alpha-cyano-4-hydroxycinnamic acid matrix solution (CHCA, Sigma-Aldrich, St. Louis, MO, USA) in 33/33/33 acetonitrile/water/ethanol (J.T. Baker, Phillipsburg NJ, USA; Decon Labs, Inc., King of Prussia PA, USA) with 3% trifluoroacetic acid (Sigma-Aldrich,