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16S rRNA gene profiling of planktonic and biofilm microbial populations in the Gulf of Guinea using Illumina NGS

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ABSTRACT

16S rRNA gene profiling using a pipeline involving the Greengenes database revealed that bacterial populations in innermost (proximal to the steel surface) and outer regions of biofilms on carbon steel exposed 3 m below the surface at an offshore site in the Gulf of Guinea differed from one another and from seawater. There was a preponderance of gammaproteobacterial sequences, representing organisms known for hydrocarbon degradation. Total DNA from the innermost layer was 1500 times that recovered from the outermost. Stramenopiles (diatom) sequences were prevalent in the former. Rhodobacteriaceae, key biofilm formers, comprised 14.9% and 4.22% OTUs of inner and outer layers, respectively. Photosynthetic anaerobic sulfur oxidizer sequences were also prominent in the biofilms. Analysis of data using a different pipeline with Silva111 allowed detection of 0.3–0.4% SRB in the biofilms. The high abundance of aerobic micro-algal sequences in inner biofilm suggests they are initial colonizers of carbon steel surfaces in a marine environment. This is the first time that the microbial population of the strongly attached inner layer of the biofilm on steel has been differentiated from the outer, readily removed layer. The accepted scraping removal method is obviously inadequate and the resulting microbial analysis does not offer complete information on the biofilm community structure.

1. Introduction

The introduction of Illumina high throughput DNA sequencing in the early 21st century has allowed the generation of a huge database of microbial genomic sequences and detailed investigations of microbial populations in previously studied and unstudied locations. One of the most investigated ecosystems has been the world's oceans. Comparisons of surface, subsurface and deeper seawater populations have been published (see, for example Friedline et al., 2012; Arumugam et al., 2013; Signori et al., 2014). There is considerable information on the microorganisms present in the open sea (for example, Yutin et al., 2007; Yooseph et al., 2010). The open seawater (planktonic) microbial populations and those accumulating on surfaces immersed in the sea are considerably different and this subject has been recently been reviewed by Dang and Lovell (2016), but there is no published data on Gulf of Guinea microbiomes.

Steel offshore oil rigs of various types are economically important structures that are prone to corrosion in aggressive seawater environments. The site under study is located some 113 km offshore in water of depth 1463 m. Despite recent observations that microbiologically influenced corrosion (MIC) in this area was very high (Lee et al., 2015), there has, as yet, been no investigation of the microbial populations in the area. The present study was based on the hypotheses that there would be differences in microbial populations between biofilms on the metal surfaces and the local seawater, and that bacteria with the potential for MIC could be present. To investigate this, a sequencing study of the site was initiated using the Illumina MiSeq system. Populations in the local seawater were compared with those attached to carbon steel coupons, including both those microbial cells removed from the

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surface by the traditional scraping method, and those remaining on the metal surface after scraping (denominated “outer” and “inner” biofilm, respectively). The corrosion associated with these coupons is presented and discussed in another document (Witt et al., 2016).

2. Materials and methods

2.1. Metal coupons and exposure

Mild steel coupons (0.5 × 3 × 1/16 inches) were manufactured with a 3/16 inch hole located 0.25 inches from one end. Twenty of these were attached to a synthetic fibre rope with plastic cable ties (Fig. 1).

In November 2014, coupons were exposed in the upper water column, 3 m below the surface of the sea, 113 km off the coast in the Gulf of Guinea. The pH of the water at sampling was 6.94, it contained <0.001 mg l^{-1} phosphate and total nitrate, 2960.3 mg l^{-1} sulfate and 28.45 mg l^{-1} ammonium ions (Witt et al., 2016). The system was originally designed to withstand exposure in the field for four to eight weeks (28–56 days). However, after a severe storm, all coupons were found to be missing from the rope and a second system was installed. After 5 weeks, only four coupons were found to remain attached to the rope. These coupons had suffered significant corrosion at all the edges; hence aggressive corrosion attack was proposed as the most likely cause of coupon loss. The 4 remaining coupons were collected for analysis under sterile conditions. Each coupon was allowed to dry in a laminar flow cabinet and then dipped into DNAzol® solution (Life Technologies, Carlsbad, CA, USA) to preserve DNA. DNAzol treated coupons were placed into sterile containers and shipped for analysis at the University of Oklahoma, where they were used for SEM and DNA analysis.

2.2. Seawater sampling

Seawater in the proximity of the coupons was collected, in a sterile manner, from the site and immediately sent to the Biocorrosion laboratory at the University of Oklahoma, where they were stored at −20 °C until analysis.

Fig. 1. Carbon steel coupon attached to the fiber rope prior to exposure, with protective plastic wrap.

2.3. Sample preparation for microbial analysis

Seven hundred ml of seawater were filtered through Nalgene Rapid-Flow sterile disposable polyethersulfone (PES) membrane filters, pore size 0.1 μm (Thermo Scientific, Waltham, MA, USA). The filter was transferred into a sterile 50 ml tube, 1 ml DNAzol® (Life Technologies, Carlsbad, CA, USA) added and the filter stored at −20 °C for 12 days prior to DNA extraction. Metal coupons with their attached biofilms were also stored at −20 °C, until DNA extraction.

2.4. DNA extraction, high-throughput sequencing and data processing

2.4.1. DNA extraction from seawater

Maxwell® 16 Tissue LEV Total RNA purification kit, with the Maxwell® 16 instrument, was used for DNA extraction, with slight modifications from the manufacturer's instructions. The filter was thawed, 1 ml nuclease-free water and 10 μl Proteinase K (Valencia, CA, USA) added, and the tube incubated at 55 °C for 1 h. Five hundred μl RLA and 500 μl RDB buffers were added. The filter was bead beaten for 2min using Lysing Matrix E beads (MP Biomedical, Santa Ana, CA, USA) and centrifuged at 6000 g for 5min. Aliquots of the supernatant were transferred into 2 Maxwell cartridges. Extraction was carried out using the DNA and FFPE/Cells programme in the Maxwell® 16 instrument. DNA was eluted in 100 μl nuclease-free water.

2.4.2. DNA extraction from biofilm

Biofilm on the coupon surface was removed using a sterile scalpel. Approx. 100 mg of biofilm was collected; this is referred to as the outer biofilm. A combination of the Power Soil DNA extraction kit (MO-BIO Laboratories, Carlsbad, CA) and the Maxwell® 16 method described above was used for DNA recovery.

After scraping off the outer biofilm, the entire coupon was subjected to DNA extraction using the larger tube of the PowerMax Soil DNA kit (MO-BIO Laboratories, Carlsbad, CA). The manufacturer's instructions were followed with the additional step of Proteinase K treatment. DNA was eluted in 5 ml of the eluent provided by the PowerMax Soil DNA kit.

DNA extracted from seawater and both types of biofilm was quantified using a Qubit 2.0 Fluorimeter with high sensitivity dsDNA reagents, according to the manufacturer's instructions (Invitrogen/Life Technologies, Carlsbad, CA, USA).

2.4.3. 16S rRNA gene amplicon library preparation

The bacterial 16SrRNA gene V4 region was amplified for high-throughput sequencing analysis using universal prokaryotic primers, 519F (5′ CAGCMGCCGCGGTAA 3′) and 785R (5′ TACNVGGGTATCTAATCC 3′) (Klindworth et al., 2013), with the forward primer modified to contain the M13 sequence on its 5′ end (M13-519F). 16S rRNA gene amplification took place in 50 μl PCR reactions containing 5–10 μL DNA, 25 μL of 2x Phusion master mix, 10 pmol of M13-519F and 785R primers. Amplification was performed in a Techne Prime thermal cycler, with an initial denaturation at 98 °C for 1 min, followed by 25 cycles of denaturation at 98 °C for 10 s, annealing at 52 °C for 20 s, extension at 72 °C for 10 s, and a final extension at 72 °C for 5 min. PCR products were visualized on 1% (w/v) agarose gels pre-stained with SYBRSafe (Invitrogen, Carlsbad, CA, USA) and the images were recorded using a Gel Logic 112 Imaging System and Molecular Imaging Software v5 (Carestream, WoodBridge, CT, USA).

Triplicate reactions of each PCR product were pooled in order to minimize PCR biases; 2 × 15 μl for each sample was run on a 1% (w/v) agarose gel and bands corresponding to the desired length