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Characteristics of microbial community involved in early biofilms formation under the influence of wastewater treatment plant effluent

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ABSTRACT

Effluents from wastewater treatment plants (WWTPs) containing microorganisms and residual nutrients can influence the biofilm formation. Although the process and mechanism of bacterial biofilm formation have been well characterized, little is known about the characteristics and interaction of bacteria, archaea and eukaryotes in the early colonization, especially under the influence of WWTP effluent. The aim of this study was to characterize the important bacterial, archaeal and eukaryotic species in the early stage of biofilm formation downstream of the WWTP outlet. Water and biofilm samples were collected 24 and 48 hr after the deposition of bio-cords in the stream. Illumina Miseq sequencing of the 16S and 18S rDNA showed that, among the three domains, the bacterial biofilm community had the largest alpha and beta diversity. The early bacterial colonizers appeared to be "biofilm-specific", with only a few dominant operational taxonomic units (OTUs) shared between the biofilm and the ambient water environment. Alpha-proteobacteria and Ciliophora tended to dominate the bacterial and eukaryotic communities, respectively, of the early biofilm already at 24 hr, whereas archaea played only a minor role during the early stage of colonization. The network analysis showed that the three domains of microbial community connected highly during the early colonization and it might be a characteristic of the microbial communities in the biofilm formation process where co-occurrence relationships could drive coexistence and diversity maintenance within the microbial communities.

Introduction

Demands for better water protection have increased steadily worldwide, resulting in the treatment of ever larger amounts of wastewater through wastewater treatment plants (WWTPs) to reduce its pollutant and nutrient content. This has led to the discharge of large volumes of WWTP effluent into natural water bodies. The discharged WWTP effluent may contribute from 3% to as much as 100% of total stream flow (Marti et al., 2004; Merseburger et al., 2005), especially in headwater streams. Effluents from biological WWTPs typically contain dissolved organic matter (DOM), dissolved inorganic nitrogen, and microorganisms not retained during the treatment process (Brion and Billen, 2000). Thus, in the receiving lakes or streams, these effluents may alter both the water chemistry (Marti et al., 2004; Merbt et al., 2011) and the microbial community (Gücker et al., 2006; Wakelin et al., 2008). Microbes introduced into the receiving streams *via* effluent discharge are able to colonize

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and perturb the natural biofilms in streams (Brion and Billen, 2000; Cébron et al., 2003). Biofilms are highly metabolically active, complex structures of bacteria, archaea, and microbial eukaryotes growing on submerged substrata (Battin et al., 2003; Lock et al., 1984). Mußmann et al. (2013) reported that the nitrifier communities in biofilms downstream of a WWTP outlet were similar to those of active sludges. Merbt et al. (2015) also demonstrated that the population size and composition of both epilithic biofilm ammonia-oxidizing archaea and ammonia-oxidizing bacteria were perturbed by the WWTP effluent. The composition and density of a biofilm, as well as its biota, are habitat-specific (Dahms et al., 2004; Wieczorek and Todd, 1998). Thus, variations in the structure and function of biofilms produced by microbial communities under the influence of WWTP effluents may significantly influence the ecological equilibrium of the aquatic food web and the nutrient cycling (Norf et al., 2009; H. Xu et al. 2012).

Bacteria and microbial eukaryotes are the primary components of a biofilm and both play vital roles in the functioning of its microbial food webs and in its activity (Gold et al., 2002; Norf et al., 2009). For example, protozoa, as trophic–functional consumers that include bacterivores, algivores, raptors, and non-selective feeders, transfer nutrients and maintain the equilibrium of bacteria and eukaryotes in addition to mediating energy fluxes (Fischer et al., 2002; Norf et al., 2009; Xu et al., 2014). Biofilms also contain archaea, such as the ammonia oxidizers and methanogens detected in a membrane treatment unit (Sauder et al., 2012; Zhang et al., 2012) as well as the haloarchaea found in biofilms that form under the extreme environmental conditions characterized by bioenergetic arsenic metabolism (Rascovan et al., 2016).

Biofilm formation is commonly considered to occur in four main stages: (1) microbial attachment to a surface, (2) microcolony formation, (3) biofilm maturation, and (4) detachment of the microbes, which may then colonize new sites (Crouzet et al., 2014). Maturation of the biofilm depends on the characteristics of its precursor (Goecke et al., 2010), which may either inhibit or facilitate colonization (Graham et al., 2007; Hughes et al., 2005; Wieczorek and Todd, 1998). Hence, identification of the biological factors critical to early biofilm formation is important to inhibit or facilitate biofilm formation. The microbial structure found in natural fresh water ecosystems can be expected to greatly differ under the influence of WWTP effluents, which contain high densities of viable bacteria, micro-eukaryotic organisms, and nutrients. However, there has been little exploration of the bacterial, eukaryotic, and archaeal components of the early stage biofilm in environments exposed to wastewater effluents.

In this study, we examined the influence of WWTP inputs on the abundance, distribution, and composition of the bacteria, archaea, and microbial eukaryotes found in biofilm forming in headwaters streams. Our results provide insights into the complex relationships between the microbial species inhabiting biofilms. They also enhance our understanding of the microbiology of biofilms exposed to WWTP effluents and therefore of the microbial species and interactions important in early biofilm formation. We hypothesized that the different members of the bacterial, eukaryotic, and archaeal domains present in WWTP effluents differ in their potential to colonize biofilms in the effluent-receiving streams.

1. Materials and methods

1.1. Site and sample collection

Bio-cords, a cord contact filtration material made of polypropylene fine fiber and composed of chenille with a specific surface area of 1.6 m^2/m and porosity of more than 99%, were placed in the headstream receiving effluent from the Caoqiao WWTP (31°31′30.8″N, 120°00′47.4″E) in Changzhou, China. Water and the biofilms on the bio-cords were collected at 24 and 48 h after the deposition of a new bio-cord (sample tags: 24 W, water sample at 24 hr; 24B, biofilm sample at 24 hr; 48 W, water sample at 48 hr; 48B, biofilm sample at 48 hr). At each sampling time point, three replicate samples each from water and bio-cords were collected, respectively. Ten centimeters of the bio-cords were cut and transferred to sterile plastic bags for microbial community analysis; water samples were collected in sterile bottles. All the samples were immediately placed on ice and then delivered to the laboratory, where they were stored at −20°C.

1.2. Characterization of the microbial community

High-throughput gene sequencing was used to identify and estimate changes in the relative abundances of hundreds of different types of bacteria, eukaryotes, and archaea. Samples of bio-cords were cut into smaller pieces under sterile conditions. For the water samples, 1 L was filtered through a 0.22-μm pore size filter to capture the bacteria. The bio-cord samples and the filters were stored at −20°C until used for DNA extraction. Q5

Genomic DNA was extracted from the samples using the FastDNA® spin kit for soil (MP Biomedicals, CA, USA) following the instructions of the manufacturer. DNA concentration and purity were determined micro-spectrophotometrically (NanoDrop® ND-1000, NanoDrop Technologies, Wilmington, DE, USA). Three replicate DNA extractions were combined into one sample for Illumina high-throughput sequencing (HTS) after genomic DNA was extracted. HTS were performed externally (Majorbio; Shanghai, China) using standard protocols on a MiSeq platform (Illumina, USA).

The resulting sequencing data were processed using the QIIME pipeline v 1.9.1 (Caporaso et al., 2010b). Archaeal, bacterial, and eukaryotic reads were distinguished based on differences in their PCR primers and then analyzed separately. The reads were then clustered into operational taxonomic units (OTUs) based on 97% similarity with UCLUST (Edgar, 2010). Representative sequences from each OTU were assigned a taxonomy using RDP Classifier (Wang et al., 2007), with a minimum support threshold of 80%, and aligned using the Greengenes reference database (version 13_8) (DeSantis et al., 2006) by PyNAST (Caporaso et al., 2010a).

Raw sequence data (FASTQ files) generated for this study have been deposited in NCBI's Sequence Read Archive under BioProject number PRJNA328070.

1.3. Microbial diversity and statistical analysis

Microbial diversity was measured by analyzing the alpha and beta diversities, as determined using the QIIME pipeline and based on the OTUs. For alpha diversity, Good's coverage, Q6

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