Deep-Sea Research II 129 (2016) 350–359

Contents lists available at ScienceDirect

Deep-Sea Research II

journal homepage: www.elsevier.com/locate/dsr2

Impact of protists on a hydrocarbon-degrading bacterial community from deep-sea Gulf of Mexico sediments: A microcosm study

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ARTICLE INFO

Available online 26 January 2014

Keywords:
Gulf of Mexico
Deep-sea
Sediment
Hydrocarbons
Grazing
Protists

ABSTRACT

In spite of significant advancements towards understanding the dynamics of petroleum hydrocarbon degrading microbial consortia, the impacts (direct or indirect via grazing activities) of bacterivorous protists remain largely unknown. Microcosm experiments were used to examine whether protistan grazing affects the petroleum hydrocarbon degradation capacity of a deep-sea sediment microbial community from an active Gulf of Mexico cold seep. Differences in *n*-alkane content between native sediment microcosms and those treated with inhibitors of eukaryotes were assessed by comprehensive two-dimensional gas chromatography following 30–90 day incubations and analysis of shifts in microbial community composition using small subunit ribosomal RNA gene clone libraries. More biodegradation was observed in microcosms supplemented with eukaryotic inhibitors. SSU rRNA gene clone libraries from oil-amended treatments revealed an increase in the number of proteobacterial clones (particularly γ-proteobacteria) after spiking sediments with diesel oil. Bacterial community composition shifted, and degradation rates increased, in treatments where protists were inhibited, suggesting protists affect the hydrocarbon degrading capacity of microbial communities in sediments collected at this Gulf of Mexico site.

1. Introduction

Marine sediments can be locally impacted by hydrocarbon contaminants introduced into the environment through naturally occurring seeps or via anthropogenic activities (Bauer et al., 1988; Oros et al., 2007). Not only can petroleum products damage surrounding ecosystems, they also can constitute a carbon and energy source for diverse groups of hydrocarbon-degrading microorganisms present in water columns and sedimentary habitats (Slater et al., 2006).

The biodegradation of hydrocarbons is known to occur by cyanobacteria, fungi, and some algae (Dalby et al., 2008), however bacteria are currently considered by most to be the primary consumers of hydrocarbons in the environment (Atlas, 1981; Leahy and Colwell, 1990). Although present in hydrocarbon-containing environments, Archaea have not been observed to degrade hydrocarbons (Röling et al., 2004a). The molecular composition of petroleum varies with each deposit and can contain a complex mixture of different hydrocarbons derived from four classes: aromatics, asphaltenes, saturates, and resins. Although hydrocarbon-degrading bacteria are ubiquitous, with as many as 79 known genera (Dalby et al., 2008), the ability to fully degrade all of the compounds found in oil is thought to be beyond the capability of any single species. Microbial consortia preferentially degrade *n*-alkanes, branched-alkanes, and aromatics (Prince et al., 2007), however, due to their high carbon and low nutrient content, hydrocarbon degradation is typically limited by availability of nitrogen and phosphorus (Swannell et al., 1996). For this reason, fertilization has proven an effective treatment for stimulating hydrocarbon-degrader growth and consequently hydrocarbon degradation (Gertler et al., 2012; Röling et al., 2002).

The responses of marine bacterial communities to the presence of hydrocarbons has been well documented in a number of bioremediation studies of seawater and beach sand mesocosms (Gertler et al., 2012) and microcosms (Jung et al., 2010; Röling et al., 2002), as well as field studies of beach sands (Röling et al., 2004; Kostka et al., 2011). Although these studies generally agree that the oil-degrading microbial community size and composition is variable, site-specific members of a group of microbes collectively known as obligate hydrocarbonoclastic bacteria (OHCB) are

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http://dx.doi.org/10.1016/j.dsr2.2014.01.007

universally detected. Despite low natural abundances, genera of OHCB such as *Alcanivorax*, *Cycloclasticus*, and *Marinobacter* are known to rapidly proliferate and outcompete other indigenous bacteria upon introduction of petroleum into the environment (reviewed in Yakimov et al., 2007).

While significant evidence implicates OHCB as important environmental hydrocarbon degraders, much less is known about responses of the protistan community to petroleum in the environment. Furthermore, relatively little is known about the impact protists have on hydrocarbon degradation either directly or indirectly as a result of their grazing activities on hydrocarbon-degrading bacteria including members of OHCB. For instance, predation can be detrimental to bioremediation efforts if bacteria integral to the degradation process are removed due to predation (e.g., Gurijala and Alexander, 1990). The possibility that protists may make significant direct contributions to hydrocarbon degradation in certain environments has not been adequately tested. Much of what we know about protistan grazing comes from studies of planktonic bacteria (e.g., Sherr and Sherr, 2002). Aquatic bacterial communities are subject to population structure regulation by environmental factors that include nutrient availability, viral lysis, and predation by bacterivorous protists. Grazing, in particular, is thought to be one of the most important regulators of bacterial community composition and net production (Sherr and Sherr, 2002). Nutrient substrates released directly from heterotrophic protists or indirectly as a result of their predatory activities (sloppy feeding or digestive wastes) may stimulate accelerated growth of otherwise nutrient-limited bacteria, thus affecting the physiological condition of the community. Additionally, size or morphology-selective grazing can shift the taxonomic composition of bacterial communities to the benefit or detriment of individual species (reviewed in Hahn and Höfle, 2001). Although these processes are much less understood in sediments, recent evidence suggests that soil and sediment communities under grazing pressure may be similarly impacted (Murase et al., 2006), that bacterial communities under protozoan grazing pressure have lower abundances of bacteria, and grazing often stimulates the rate of decomposition of organic matter (Fenchel and Harrison, 1976). Studies of the interactions between protists, bacteria and organic contaminants in sedimentary environments are providing indications that protists can often have significant positive impacts on degradation processes, however the nature of these impacts appears to depend at least in part on the habitat and/or specific microorganisms investigated (e.g., Kinner et al., 2002; Kota et al., 1999; Mattison and Harayama, 2001; Mattison et al., 2005; Schmidt et al., 1992; Tso and Taghon, 2006). Among potential organic contaminants, petroleum hydrocarbons are of particular concern given recent increases in drilling and extraction activities over the past decade.

A previous microcosm study found that common planktonic eukaryotes, including *Paraphysomonas foraminifera*, limited bacterial growth through increased grazing in oil-polluted seawater (Dalby et al., 2008). However, in a hydrocarbon-rich natural environment where there is a higher likelihood of protist communities already adapted to hydrocarbon exposure, protist grazing activities may help maintain higher rates of bacterial hydrocarbon degradation by releasing needed nitrogen and phosphorus. To test this hypothesis specifically for deep marine sediments, we conducted a set of laboratory-based microcosm experiments using surface sediments from a Gulf of Mexico hydrocarbon seep site to determine whether the presence of indigenous protists (1) enhances the biodegradation of hydrocarbons performed by *in situ* microbes in these deep-sea hydrocarbon-seep sediments and (2) if protists alter the *in situ* bacterial community composition over a 12-week period. The degradation of fossil diesel spiked into these microcosms was monitored by gas chromatography. Changes in eukaryotic and bacterial *in situ* communities were analyzed by molecular methods.

2. Materials and methods

2.1. Sediment collection

Surface sediment samples were collected with a slurp gun attached to the HOV *Alvin* from the Rudyville site at the Mississippi Canyon Federal Lease Block 118 (MC118) long-term observatory in the Gulf of Mexico. MC118 is an active gas-hydrate and oil seep located on the continental slope of the northern Gulf of Mexico (28–51.114 N, 88–29.521 W) and is about 15 km from the source of the 2010 *Deepwater Horizon* oil spill. Soft, 5.5 °C sediments were retrieved using the HOV *Alvin* (Dive #4658) on December 1, 2010 from 900-m depth. Samples were mixed 50/50 with bottom seawater and the resulting slurry was stored at 4 °C during transport to the lab where experiments were initiated ten days later.

2.2. Microcosm experiments

Experiments were conducted to examine the effect of protistan grazing on bacterial community composition and hydrocarbon degradation under combinations of conditions including nutrient addition, oxic versus anoxic incubations, and addition of protist inhibitors (Table 1). Glass vials (60-ml volume) were used for all microcosms. Forty milliliters of the MC118 sediment/seawater slurry were dispensed into each of 42 sterile glass tubes and each tube was spiked with the addition of 25 microliters (~20 μg) of 100% fossil diesel fuel oil (WP 681 USEPA Standard Oil). This diesel fuel oil was selected because (1) microbial biodegradation of compounds present in this mixture is easily detected by gas chromatography and (2) it has a great capacity to mix into the water column (Reddy and Quinn, 2001) and sorb to sediments (Reddy et al., 2002; Peacock et al., 2007). Oxygen was removed from anaerobic treatments by nitrogen infusion of the slurry prior to capping, while aerobic incubations vials were loosely plugged with sterile cotton. All treatment combinations were conducted in triplicate. Oxic and anoxic control microcosms of sterilized (autoclaved) slurry (with or without nutrients added) were sacrificed on day 0 (6 tubes), 30 (oxic microcosms), and 90 (anoxic microcosms) to monitor for non-biological loss of hydrocarbons. Remaining tubes were divided into 8 experimental treatment groups based on combination of incubation conditions and additions each received (Table 1). At T_0 a sample of the sediment slurry

Table 1
Microcosm treatments used in this study.

Treatment	Sediment	Oil	Nutrients	Inhibitor	Oxygen
Oxic Control T_0 (+nut)	Sterile slurry	+	+	−	+
Oxic Control T_0	Sterile slurry	+	−	−	+
Oxic Control T_F (+nut)	Sterile slurry	+	+	−	+
Oxic Control T_F	Sterile slurry	+	−	−	+
Anoxic Control T_F (+nut)	Sterile slurry	+	+	−	−
Anoxic Control T_F	Sterile slurry	+	−	−	−
Oxic T_F (+ nut)[a]	Slurry	+	+	−	+
Oxic T_F (+inhib)	Slurry	+	−	+	+
Oxic T_F (+nut +inhib)[a]	Slurry	+	+	+	+
Oxic T_F	Slurry	+	−	−	+
Anoxic T_F (+nut)	Slurry	+	+	−	−
Anoxic T_F (+inhib)	Slurry	+	−	+	−
Anoxic T_F (+nut +inhib)	Slurry	+	+	+	−
Anoxic T_F	Slurry	+	−	−	−

[a] Treatments for which 18S rRNA gene clone libraries were analyzed in addition to a T_0 sample.