Lipids of oil-metabolizing microbes in the shallow subsurface as indirect indicators for offshore active petroleum systems

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Summary

Intact polar lipids (IPLs) are part of the sedimentary organic matter that can provide information about the community composition and metabolic activity of active near surface and deeper subsurface microbial ecosystems. IPLs consist of a core lipid chemically bonded to one or more polar headgroups. Both the polar headgroup and the core lipid can be structurally distinct and taxonomically diagnostic. Because of this, depending on the preservation state and origin of the detected lipid, these compounds can be further used to assess present and past environmental conditions of their habitat. Microbes in marine sediments metabolize organic matter from 1) the overlying water column, 2) terrestrial input, 3) other microorganisms living within the sediments, and 4) via the seepage of hydrocarbons migrating from deeper within a sedimentary basin. In the latter case, aerobic and anaerobic hydrocarbon oxidizing bacteria and archaea that use petroleum as a substrate for their metabolism may potentially provide better insights into the dynamics of the subseafloor biosphere, pathways for carbon cycling, and ultimately lead to novel proxies for the characterization of benthic seep activity. These proxies might then be used as indirect hydrocarbon indicators (IHIs) for de-risking offshore petroleum exploration. The advantage of using IPLs as an IHI is that the analysis is quick, cost effective, capable of being standardized, and can ideally help to further validate other IHI techniques, such as other bioassay methods that are currently being developed, e.g., using genomics. To test lipid signatures as potential IHIs for the presence of petroliferous hydrocarbons, surface and piston core sediments will be examined that were recently collected during expeditions on board the CCGS *Hudson* (2015 & 2016) and RV *Coriolis* (2017) from the oil and gas prone Scotian Shelf, Nova Scotia. This study is part of a Genome Canada funded Genomic Applications Partnership Program (GAPP) project that aims to integrate different bioassays and petroleum geochemistry to develop novel IHIs, which can better predict the potential of offshore petroleum in order to further reduce front-end exploration risks to oil and gas companies.

Introduction

Lipids are essential components of cellular membranes that contributes a significant portion of organic matter to Earth's soils and sediments (e.g., Langworthy et al., 1983; Killops and Killops, 2005). The structural configuration of membrane-building lipids is the physical basis for the lipid mono- and bi-layers that segregates the internal parts of the cell from the external environment. Membrane-building lipids are generally composed of a hydrophilic polar headgroup and a hydrophobic aliphatic tail (e.g., Madigan et al., 2003). When lipids are found in sediments with the polar headgroup still attached they are considered to be derived from living cells and are usually referred to as intact polar lipids (IPLs; Sturt et al., 2004). However, after a cell dies the polar headgroups are quickly degraded by enzymatic hydrolysis to yield a more stable core (or fossil) lipid (White et al., 1979; Harvey et al., 1986). While IPLs are used to investigate modern environments, core lipids can be preserved over geological timescales and are versatile and powerful tools for the reconstruction of paleoenvironments (Schouten et al., 2013).

The analysis of both IPLs and core lipids combines two levels of information: headgroup composition and core lipid characteristics. The polar headgroup, usually phosphate-, amino-, or sulfate-based and of glycosidic species, provides relevant taxonomic information (e.g., Schubotz et al., 2009; Popendorf et al., 2011) and may also be adapted to environmental conditions, for instance, in response to pH or temperature variations (Shimada et al., 2008) or nutrient limitation (Yoshinaga et al., 2015). Core lipids on the other hand, can also be indicative of taxonomy. For example, archaeal core lipids differ from bacterial and eukaryotic core lipids as they are composed of isoprenoidal carbon units bound to the glycerol backbone via ether bonds (e.g., Langworthy and Pond, 1986). Differences in chain length, degree of unsaturation or cyclization, and type of bonding between alkyl groups and the glycerol backbone may provide further information on taxonomy or environmental conditions (Schouten et al., 2013; Zhu, Yoshinaga, Peters et al., 2014).

In the past decade, research on IPLs has provided valuable information about microbial communities and carbon cycling. IPLs have been studied in oceans (Schubotz et al., 2009; Pitcher et al., 2011), wetlands (Moore et al., 2015), hot springs (Schubotz et al., 2013), hydrothermal systems (Gibson et al., 2013; Reeves et al., 2014), the deep subseafloor biosphere (Biddle et al., 2006; Lipp et al., 2008), and in oil and cold seeps (Rossel et al., 2011; Schubotz et al., 2011). These studies have largely been focused on documenting the presence, trends within, and diversity of IPLs in specific environments. However, the utilization of these lipids as indicators of other geochemical phenomena is largely unexplored. This is in contrast to the more fossil core lipids, which are extensively used to reconstruct environmental conditions and ecosystems. For instance, core lipids have been used to reconstruct sea-surface temperatures (Schouten et al., 2002), the input of soil organic matter (Hopmans et al., 2004), as well as soil pH and air temperatures (Weijers et al., 2007).

IPLs of oil-metabolizing microbes have scarcely been studied, but may have a great potential towards finding IHIs of seeps and underlying reservoirs. Schubotz et al. (2011) investigated biodegraded asphalt samples from the southern Gulf of Mexico and showed great varieties and elevated concentrations of bacterial and archaeal IPLs in oil-influenced shallow sediments that indicate microbial life and biomass production are stimulated by the presence of petroleum. Such IPLs in conjunction with genomic bioassays and petroleum geochemical data will likely reveal better insights into the dynamics of the subsurface biosphere and carbon cycling and will ultimately have the potential to lead to proxy development when detected. This prospect can have a great impact to predict the potential of offshore petroleum to further reduce front-end exploration risks to oil and gas companies, which is one of the aim of Genome Canada’s Genomic Applications Partnership Program (GAPP) project *Microbial genomics for de-risking offshore oil and gas exploration in Nova Scotia*. This collaborative project, led out of the University of Calgary’s Geomicrobiology Group is focusing on combining microbiology through genomics and lipids applications (University of Calgary and Saint Mary’s University) with petroleum geochemical analysis of sediments conducted by Applied Petroleum Technology (Canada) Ltd., to investigate novel IHIs for de-risking offshore petroleum exploration. Lipids analysis will provide an independent means of evaluating geochemical results and validating new genomic bioassay approaches for indirectly identifying active petroleum fed seeps along the Scotian shelf.

Methods

The oil and gas prone Scotian Shelf of Nova Scotia is targeted as a test area to investigate intact and core lipid signatures. Surface and piston core sediments have recently been collected during three cruises with the CCGS *Hudson* (2015 & 2016) and RV *Coriolis* (2017). For this study, IPLs and core lipids will be extracted from frozen samples using a modified Bligh and Dyer extraction technique in conjunction with ultrasonication and the addition of buffers to increase the recovery of IPLs (Sturt et al., 2004).

New analytical methods by Zhu et al. (2013) and Wörmer et al. (2013) that provide a robust and precise method for lipid analysis will be adopted whereby the obtained lipid extracts will be injected using an ultra high performance liquid chromatograph (UHPLC) coupled to mass spectrometer (MS) configured with reverse phase (RP) column separation and electrospray ionization (ESI). The advantages of UHPLC-RP-ESI-MS over conventional methods for lipid analysis of environmental samples that use normal phase (NP) separation columns and atmospheric pressure chemical ionization (APCI) sources are the broad analytical window, improved sensitivity and detection limit, high chromatographic resolution, softer ionization, and acid hydrolysis-free application. Furthermore, these methods provide the simultaneous analysis of core lipids and IPLs of a single measurement. The chemical structures of potentially novel lipids from oil-metabolizing microbes will be performed with high resolving MS, e.g., quadrupole time of flight (qTOF) MS or Orbitrap, which were already being used for the discovery of previously unresolved compounds in marine sediments (e.g., Zhu, Yoshinaga, Peters et al., 2014).

Examples

This project is currently at the beginning stages of development and no sediment samples have yet been extracted or analyzed. Initial method validation and lipid extraction in the organic geochemistry laboratory at Saint Mary's University are anticipated in October to November 2017.

Conclusions

As part of the GAPP program, we will be conducting a lipidomic survey of Scotian Shelf surface sediments and piston cores to evaluate if IPLs can be used as a taxonomic tool to identify oil-metabolizing microbes in the marine sediments. Together with genomic bioassays being developed in the University of Calgary's Geomicrobiology Group and petroleum geochemistry data produced by APT Canada Ltd. there is the potential to develop a novel time and cost effective IHI multi-pronged strategy for de-risking offshore petroleum exploration.

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