Microbial Genomics for De-Risking Offshore Oil and Gas Exploration in Nova Scotia.
One-day Workshop, University of Calgary, January 30, 2018
Workshop Summary

Project Overview:

The Project's purpose is to develop, validate, and deploy different genomics-based bioassay tools for offshore prospecting on the Scotian Slope. The bioassays are focused on both bacteria that actively metabolise hydrocarbons in the seabed, as well as dormant bacteria (endospores of thermophiles) expelled from subsurface oil reservoirs via hydrocarbon seepage. Genomics results will be integrated with conventional geoscience information to map petroleum potential.

The project is organized around five primary activities:

(1) Piston coring programs undertaken during three successive summers to collect seabed samples in proximity to suspected oil seeps;

(2) Geochemical analysis to identify the presence of and characterize hydrocarbons in the samples, determine total organic carbon, and provide insights into the origins of the organic matter;

(3) Marine microbiology and genomics applied to the samples using different bioassay strategies to investigate the presence of different target groups of microorganisms;

(4) Integration, analysis and mapping to bring together geomicrobiology with the results of concurrent geoscience studies to generate GIS data packages and maps in a framework that will enable interested oil companies to undertake independent evaluations; and

(5) Evaluation of microbial lipids in the samples and integrate those results with the previous activities.

Workshop Objectives:

The primary goals of the workshop were:

(1) To inform participants about the current research project involving the development of new microbiology based tools, including the application of lipidomics, to reduce exploration risk;

(2) To provide an opportunity for several petroleum companies to present industry examples of their work with geomicrobiology for exploration risk reduction;

(3) To engage workshop participants in a discussion of how these tools can be further developed, field tested and commercialized to meet industry needs; and

(4) To explore opportunities for future collaboration as well as other areas where the development of genomics, lipidomics and geochemistry-based tools would be of benefit to the petroleum industry.

Session 1 – Current Research Project Overview

The session comprised four presentations to provide participants with an overview of the major components of the research project, the results achieved to date and the remaining work to be undertaken over the remainder of the project. These, as well as presentations from the next session, are available on the OERA (insert) and Genome Atlantic websites (insert – Note permission will be needed).

The Nova Scotia Department of Energy (NSDOE) positioned the project in the context of their ongoing geoscience research activities that began with the Play Fairway Analysis completed in 2011 and which have continued since then with the objective of reducing offshore exploration risk and maintaining industry exploration activity in the Nova Scotia offshore. With three cruises undertaken to collect piston cores and generate new data, this provided an opportunity to provide sample materials for conventional geochemical analysis as well as the potential development of new approaches to risk reduction through the application of geomicrobiology. NSDOE, together with the Geological Survey of Canada (GSC), undertook cruises in the summers of 2015, 2016 and 2017 over the Scotia Slope and Sydney Basin resulting in the collection of samples from piston cores and box cores in proximity to identified seeps, as well as surface slicks when possible. NSDOE also contracted external geochemical analysis, carried out by APT Canada, and is responsible for the ultimate integration of the results of the geomicrobiology, geochemical analyses and conventional geoscience work. Additional cruises are planned by NSDOE and GSC during the summer of 2018 using an AUV to better identify sites for future sampling, and in 2019 using an ROV for direct sampling at identified seep sites.

APT provided more detail on the coring cruise sample collection and presented the results of their geochemical analysis of the cores collected during each of the three cruises. They also commented on the relationship of their work to the geomicrobiology being undertaken by the University of Calgary. The piston core samples were used to determine if thermogenic hydrocarbons (gas or liquids) are present above background levels, and if from seepage, attempt to determine the nature of the source rock, its age and the maturity of the hydrocarbons in order to help define the petroleum system. One site (site 41) from the 2015 and 2016 cruises showed good evidence for thermogenic hydrocarbons from a seep, while several other sites showed possible geochemical evidence of thermogenic hydrocarbons that could have migrated from the subsurface. Only one site from the 2017 Sydney Basin cruise showed any evidence of possible petroleum seepage. Because in most instances there is some element of doubt in concluding there is petroleum seepage based solely on geochemical data, microbiological approaches can provide complementary data to confirm the presence of hydrocarbons from seepage.

The University of Calgary's work has focused on the possible use of aerobic and anaerobic seabed microbes in proximity to seeps, as well as dormant thermophilic spores, as tools for reducing exploration risk. Work using thermospores from arctic marine sediments has determined that thermospores have 16S rRNA gene database hits similar to microorganisms from oil fields as well as some hits similar to mid-ocean ridge systems. It has been hypothesized that thermophilic spores and cold-adapted oil-degrading bacteria have different distributions at seeps and hence could be of use for hydrocarbon prospecting in complementary assays. The coring cruises offshore Nova Scotia resulted in more than 400 samples for microbial analysis of bacterial community composition. Hydrocarbon positive sites, as determined by APT's geochemical analysis, show distinctly different profiles of sulfate concentrations by depth than hydrocarbon negative sites as a result of the presence of sulfate-reducing bacteria. The analysis of surface (14 sites) and subsurface samples (15) sites identified a relatively high abundance of *Atribacteria* in the surface samples from two of the three hydrocarbon positive sites, as well as one site that was hydrocarbon negative.

Another aspect of the University of Calgary work used 16S rRNA gene sequencing to look at the bacterial communities of 173 sediment cores from the Gulf of Mexico covering oil and gas positive sites, oil positive / gas negative sites, and oil and gas negative sites. Those oil and gas positive sites had a higher relative abundance of *Atribacteria* than both oil positive / gas negative and hydrocarbon negative sites. Further research is using metagenomics on samples from three sites to consider the functional role of uncultivated *Atribacteria.* There is preliminary evidence of hydrocarbon degradation genes in the metagenomes. These two projects have led to the preliminary conclusion that seep-associated *Atribacteria* in both the Nova Scotia offshore and the Gulf of Mexico may be associated with gas or oil and may be capable of hydrocarbon degradation. However further detailed analysis of genomic and geochemistry datasets is required.

A further research question being examined is whether spore-forming *Firmicutes* are prevalent in oil reservoirs. A review of approximately 75 studies using amplicon libraries from oil reservoirs determined that *Firmicutes* are indeed prevalent with *Caminicella* being a frequently detected genus. Another project is using DNA sequencing to characterize the microbial communities in 111 surface sediments from the Gulf of Mexico, heated to 50 ˚C, of which 71 are oil positive and the remainder oil negative. A total of 115 thermospores were detected in Eastern Gulf of Mexico surface sediments with a limited number showing strong correlation with the presence of oil. An analysis of some of the Scotian Slope sediments after heating detected seven lineages of thermospores, three of which are often found in oil reservoirs. Additional work is being undertaken to quantify spores in sediments using dipicolinic acid as an endospore-specific biomarker. An examination of samples from three Nova Scotia offshore sites identified higher spore counts in the hydrocarbon positive samples at the top of the core as well as deeper in the core. Collectively the work at the University of Calgary is demonstrating the potential of several mutually exclusive strategies for seep detection based on microbiological approaches, and hence for hydrocarbon prospecting.

Saint Mary's University in Halifax, Nova Scotia is undertaking complementary work using lipidomics, the large-scale study of the structure, pathways and networks of cellular lipids in biological and geochemical systems. To date the work has been proof of concept and has focused on bacterial and archaeal intact polar lipids (IPLs) as well as fossil lipids, some of which can act as hydrocarbon biomarkers. A matrix matched reference material from estuary muds in the Bay of Fundy is being used as a comparison to samples derived from the 2015 and 2016 coring cruises on the Scotia Slope. Initial work on three samples has shown higher concentrations of total lipid extract at the two hydrocarbon positive sites compared to the hydrocarbon negative site examined. Other findings to date have found that Scotian Shelf sediments have IPLs that are much less than in the Bay of Fundy reference standard, and both hydrocarbon positive and negative sites have archaeal IPLs while the hydrocarbon positive sites contain what are likely to be bacterial IPLs. Current efforts are directed towards validation of the research strategy using 73 available samples from 8 hydrocarbon positive sites (thermogenic and biogenic) and three hydrocarbon negative sites. The intent is to develop a Scotian Shelf lipids library, determine microbial substrate utilization and compare the results to the University of Calgary's work.

Session 2 – Industry Examples of Geomicrobiology for Exploration Risk Reduction

Presentations from Shell International Exploration and Production, and from Repsol Technology Centre highlighted work being undertaken by the upstream petroleum industry to develop and use microbiology as an exploration tool. Shell noted that naturally occurring oil seeps account for 600,000 tonnes of oil entering the marine environment annually, with 140,000 tonnes in the Gulf of Mexico alone. The detection and characterization of these seeps can provide invaluable information for hydrocarbon exploration. Work with samples from three sites in the Gulf of Mexico was described, one a hydrocarbon seep, the second a methane seep and the third a background site. Through analysis of the samples using 16S rRNA gene sequencing they found that the seep sediments had a ten-fold higher bacterial and archaeal cell counts than the non-seep sediments, abundance increased in the bottom section of the seep samples and there were more archaea than bacteria in contrast to the non-seep site. Further work using amplicons and metagenomics characterized the composition of the bacterial and archaeal communities. The final aspect involved contrasting the metabolic capacities across the three sites to identify differences. From the results of this work it was concluded that there are district microbial communities at seep sites; the methane seep was dominated by ANME-1 members oxidizing methane anaerobically while the hydrocarbon seep was dominated by *Chloflexi* and deltaproteobacterial lineages and exhibited potential for HC degradation; and the composition of seeping material was a strong determinant of seafloor microbial composition and function.

Repsol presented a case study of the application of microbial exploration techniques (MET) in the offshore noting that with the high cost, long timeframes and high degree of uncertainty for offshore exploration, any method that can reduce these can have a huge impact for the industry. Because seeps from oil reservoirs affect the composition of bacterial communities on the seafloor, there is the potential to use microbial anomalies as bioindicators for oil and gas. With advances in sequencing methods it is now possible to identify microorganisms more quickly, cheaper and precisely. The objective of Repsol's work was to evaluate microbial exploration techniques by comparing the results to well-established geochemistry approaches using 20 frozen piston core samples from a recent offshore geochemistry campaign. One exhibited a strong oil anomaly, two a gas anomaly, four a weak oil anomaly and the remaining thirteen were negatives. Through the application of DNA extraction and sequencing, they were able to determine the classification of microorganisms and infer functions they may carry out. Sites with oil and gas anomalies exhibited a higher abundance of particular taxa than hydrocarbon negative sites, whereas hydrocarbon negative sites had a higher abundance of certain other taxa compared to the sites with anomalies. This led to the conclusion that seepage from hydrocarbon reservoirs result in microbial anomalies in the seabed, and that such anomalies can be detected using genomics techniques and statistics.

Repsol's presentation then turned to the possible use of machine learning to classify piston cores based on their microbiome as an aid in detecting the presence of hydrocarbons. The use of microbiomic data presents a number of challenges and requires a relatively large number of samples (at least 50). Through the use of public data sets to generate a machine learning model that provides good accuracy on hydrocarbon negative samples (92%) and hydrocarbon positive samples (82.6%). The approach can be used to classify Repsol's samples with similarly high accuracy and with good agreement with geochemistry results. They have been able to generate a probability map solely based on microbiological data which points towards the "sweet spot" in an exploration block. They are currently training their machine learning models to distinguish between oil positive and gas positive samples.

Session 3 – Breakout Group Discussions:

Three questions were provided to breakout groups for discussion. The following is a summary of the main points that arose with respect to each of the three questions.

<u>Question #1:</u>

We have described the current research program and how we see the results being used. The results to date suggest that microbial genomic analysis is capable of identifying geologic anomalies associated with seeps.

- *Does this this work have the potential to have a significant impact on reducing the risk of exploring in a particular area or is it just interesting research?*

  Microbial genomic analysis does have the potential to reduce exploration risk when used in conjunction with more conventional geoscience tools to provide integrated results. The incremental cost is relatively low since it can be added to traditional coring and sample collection. In particular microbiological tools will add value if they can be demonstrated to have the ability to distinguish between oil and gas, determine the age of oil, differentiate between biogenic and thermogenic sources and identify the type of oil. The additional information generated would provide a new layer of data that can further inform industry exploration risk assessment, determination of resource potential and decision making.

- *Are the applications for offshore exploration of potential use for land-based exploration? Are there other potential uses within the upstream petroleum sector?*

  There is potential for use of the techniques in land-based exploration. However, the use of the technology on land requires robust case studies at this point to provide confidence in the results generated. Other potential uses of microbiological techniques identified were assessing the probability of biofilm development from upstream inputs, and the provision of baseline data for regulators and environmental assessments.

- *What are some of the primary barriers that need to be overcome in having these types of tools be accepted by industry as valid and useful?*

  Due to the immaturity of the microbiological techniques being developed, the lack of demonstrated reliability and the risk aversion of companies, greater confidence by industry in the technology is needed before acceptance. Further demonstration of proof of concept is required along with the development of a platform to enable quick, low cost turnaround of analytical results. These will contribute to the development of a business case for companies to invest in the use of microbiology as an exploration tool. At this point microbiological expertise is limited in petroleum companies and hence how the concepts are communicated and to whom is important to effectively advance interest in the approach.

Question #2:

To date the work being done is a collaboration involving academia, government, research organizations and a service company.

- *What needs to be done to get the industry (E&P companies, service companies, consultants) more engaged as collaborators in some of this work? Is there a minimum stage of technology development? How best to communicate results to garner interest?*

  In order for industry to become involved as a collaborator the technology should be at least at the proof of concept stage and there should be potential for quick turnaround time for analytical results. The importance of including seep samples from known reservoirs, as well as hydrocarbon negative samples, was identified as a way of providing control samples to give greater confidence in the results. The provision of sample materials by industry is an obvious area for collaboration. However, industry needs to see the potential value to them of collaboration, which in turn points to the importance of appropriate and effective communications through a variety of channels including case studies, workshops, conference presentations, publications and targeted meetings.

- *Would the development of genomic analysis tools be best explored under JIP or would companies consider this domain proprietary?*

  Because of the relatively early stage of the work on the use of genomics as an exploration tool, a "Joint Industry Partnership" (JIP) model could be an appropriate approach for industry involvement in some aspects of future work. Several existing JIP models were identified. Confidentiality is an issue that needs to be carefully managed within a JIP by agreeing up front on the level of data to be shared and protecting the confidentiality of proprietary data and technologies.

Question #3:

There are several factors influencing the adoption of new technologies by the oil & gas industry to support exploration.

- *What trends in global exploration support or limit the use of genomics applications for exploration?*

  The petroleum industry is relatively conservative which affects the speed of adoption of new technologies. The recent trend to lower crude oil prices coupled with greater exploration and production in deeper waters impacts company budgets and their deployment, which in turn influences the adoption of new technologies. In the case of genomics-based tools the lack of familiarity with the technology and clear evidence that it works, results in a lack of confidence and concerns about its cost. However, if new tools such as microbiology can help reduce exploration risk, address regulatory requirements or contribute to obtaining social license, they will be seen positively by industry. As identified previously communications with industry and having internal company champions are extremely important to gaining industry acceptance.

- *Are there shifts in the direction of the research that would make the use of microbiology as an exploration tool of more value or relevance?*

  Companies are inclined to spend on de-risking exploration in order to increase their level of confidence in an area. Current offshore exploration baseline data collection is mostly for identifying sediment features, obtaining cores and undertaking seismic surveys. Additional baseline data that adds value would be of interest to companies. Advances in automation such as the use of ROVs will enable site-specific sampling necessary for establishing the validity and reliability of genomics-based tools to increase industry confidence in their value as a means of reducing exploration risk. Additionally, advancements in geochemistry will shift direction and focus beyond current analytical approaches and complement new genomics tools. However, industry is looking for specifics and evidence that the genomic tool being developed will actually be a value-add for exploration.